Evidence of Widespread Ozone Damage to Vegetation in Europe (1990-2006)

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### Contents

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary</td>
<td>6</td>
</tr>
<tr>
<td>Sources of data</td>
<td>7</td>
</tr>
<tr>
<td>The ozone climate of Europe</td>
<td>7</td>
</tr>
<tr>
<td>Evidence of ozone damage to vegetation</td>
<td>7</td>
</tr>
<tr>
<td>Relationships between observed effects and areas predicted to be “at risk” of ozone damage</td>
<td>8</td>
</tr>
<tr>
<td>Challenges for the future</td>
<td>9</td>
</tr>
</tbody>
</table>

1. **Introduction**  
   - The role of the ICP Vegetation  
   - Rationale for this study  
   - Causes of ozone pollution  
   - The damaging effects of ozone pollution on vegetation  
   - Mapping the impacts of ozone in Europe  
   - Aims and structure of this report  

2. **Sources of data**  
   - Ozone concentrations in Europe  
   - EMEP ozone concentration and flux models  
   - Evidence of ozone effects from ICP Vegetation biomonitoring studies  
   - Evidence from filtered-air experiments  
   - Observations of visible injury symptoms in ambient air  

3. **European-scale evidence**  
   - Introduction  
   - Ozone at ICP Vegetation sites between June and August  
   - Visible injury in ambient air  
   - ICP Vegetation experiments  
   - EDU experiments  
   - Charcoal-filtered and non-filtered ozone exposure experiments  
   - Conclusions  

4. **Regional evidence of effects of ambient ozone**  
   - Introduction  
   - Northern Europe  
   - Atlantic Central Europe  
   - Continental Central Europe  
   - Eastern Mediterranean  
   - Western Mediterranean  
   - Conclusions  

5. **Evidence of ozone damage in areas identified by EMEP as high risk**  
   - Introduction  
   - Methods of comparison  
   - Sources of uncertainty  
   - Visible injury-based evidence  
   - Biomass- and yield-based evidence  
   - Is increasing AOT40 and/or AF3, gen associated with increasing damage?  
   - Conclusions  

6. **Overview, policy implications and future challenges**  
   - Overview  
   - Policy implications  
   - Challenges for the future  

**References**  
57
Summary

‘Evidence of Widespread Ozone Damage to Vegetation in Europe (1990 to 2006)’ provides a summary of the adverse effects of ozone on vegetation across Europe during the period 1990 to 2006 and compares these effects with modelled ozone concentration and flux data. Although many experimental studies have demonstrated that current ozone concentrations have the potential to impact on crops and (semi-)natural vegetation, until now there has been no comprehensive assessment of ozone effects in fields of crops and natural ecosystems. This report has established that effects of ambient ozone on crops and (semi-)natural vegetation are actually occurring in the field, with leaf injury and reductions in biomass or crop yield developing under ambient ozone conditions. Compared with ozone concentration maps, maps of stomatal fluxes of ozone were found to be better at predicting the occurrence of ozone damage to vegetation. This report is for scientists, policy makers and others with an interest in ozone pollution and its effects on crops and (semi-)natural vegetation.
Sources of data

Ozone concentrations and fluxes
In this report, we have used both ozone concentration data measured at the local scale at ICP Vegetation monitoring sites, and modelled ozone concentration and flux for 50 km x 50 km grid squares across Europe (supplied by EMEP MSC-WEST). Three ozone metrics have been used throughout: the 12h mean (an average of the ozone concentration between 8am and 8pm); AOT40 (the accumulation of hourly mean canopy height ozone concentrations above 40 ppb during daylight hours) and AFst3gen (a model of the cumulative flux of ozone into leaves of a generic crop which takes into account the influence of temperature, light and humidity on stomatal opening). We have assessed the evidence of ozone damage at AOT40s above the critical level for yield reduction (an AOT40 of 3 ppm h) and have compared effects with risk maps based on the ozone flux metric, AFst3gen.

Ozone damage to vegetation
The evidence of effects of ambient ozone on vegetation includes visible injury records, results from exposure studies in charcoal-filtered/non-filtered air, and the ICP Vegetation biomonitoring studies using ozone-sensitive and ozone-resistant biotypes of white clover. Data from peer-reviewed papers and conference proceedings as well as previously unpublished data from ozone research groups across Europe were collated to form a comprehensive database for use in this study.

The ozone climate of Europe
Current ozone concentrations vary greatly between regions and years owing to climatic variations, surface topography, land-use and local versus long-distance sources of ozone precursors and other pollutants. The highest ozone concentrations tend to occur in southern Europe, particularly in Italy, Greece, Slovenia, Spain and Switzerland. Moderate ozone concentrations are experienced in many other European countries, especially those in central Europe, with all countries of Europe experiencing periodic ozone episodes each year with several days of peak ozone concentrations exceeding 50 ppb and sometimes exceeding 90 ppb. Maps of ozone flux show that the climatic conditions are conducive to ozone uptake by vegetation across most of Europe, with moderate/high fluxes predicted for some areas such as southern Scandinavia where ozone concentrations are relatively low.

Evidence of ozone damage to vegetation
We have collated evidence of widespread effects of ambient ozone on vegetation across Europe, for example:

• Visible injury symptoms have been recorded on over 30 crop and 80 (semi-) natural vegetation species. There have been over 500 records of injury from a total of 16 countries, representing all regions of Europe, i.e. from northern as well as southern Europe. Visible injury has been recorded on (semi-)natural vegetation species including forbs and grasses in 14 countries, shrubs in 8 countries and crops in 12 countries. Crops that have shown visible injury symptoms attributed to ozone include maize, bean, potato, lettuce, and watermelon. In some cases, injury symptoms were extensive, e.g. in Greece, 100 % of the leaves in an onion field and 85% of the leaves in a watermelon field, were damaged by ozone in 1995 and 2004 respectively.
ICP Vegetation experiments have shown that ozone injury symptoms on well-watered clover plants occur throughout the period May to October, with symptoms being most severe in July and August. As part of the ICP Vegetation biomonitoring programme, visible injury symptoms on white clover have been recorded in all regions of Europe. The highest impacts have generally been found in Switzerland, Italy and Greece, although moderate impacts have also been found in central and northern Europe.

The biomass of the sensitive variety of white clover plants used in ICP Vegetation biomonitoring experiments was reduced with increasing ozone concentrations. The largest impacts of ozone on the biomass of clover plants were consistently found in southern Europe, particularly in Italy and Greece where biomass reductions of over 30% have been demonstrated in some years. Reductions in biomass of over 10% have been found in the Eastern Mediterranean, Western Mediterranean and Continental Central Europe regions, but such large reductions have not been recorded in either Northern Europe or Atlantic Central Europe.

Experiments using charcoal-filtered compared to non-filtered air have shown that effects occur in Northern Europe as well as in the Western Mediterranean regions. In some cases, effects of greater than 10% on biomass occur at concentrations below the current critical level for ozone. Species showing such effects from experiments of 2-4 months’ duration include Biserrula pelecinus, Briza maxima, Lolium rigidum, Trifolium glomeratum, Trifolium striatum and Trifolium cherleri (Spain, 2000), Solanum tuberosum (Sweden, 1999), Lycopersicon esculentum (Italy, 2004) and Cirsium dissectum (Netherlands, 1996).

Relationships between observed effects and areas predicted to be “at risk” of ozone damage

Ozone concentration (AOT40)-based risk maps

From the evidence presented in this report, maps of exceedance of the AOT40-based critical level for agricultural crops appear to be underestimating the potential for ozone damage in Europe. AOT40 worked best as a regional-scale indicator of damage, with both ozone injury score and biomass reduction linearly related to the mean EMEP modelled AOT40 for the 50 x 50 km grid squares that the ICP Vegetation sites represent. At this scale, a mean biomass reduction of greater than 10% occurred in Continental Central Europe and Eastern Mediterranean, where EMEP risk maps indicated that mean AOT40s were at or below the critical level. Furthermore, at the local scale, approximately one third of the ozone injury data points were in grid squares where the maps indicated that the critical level for yield reduction was not exceeded.
Ozone flux (AFst3gen)-based risk maps

This study has shown that although there is substantial evidence of adverse effects of ozone on crops and (semi-)natural vegetation in regions of high ozone concentration, there is also evidence of adverse effects where ozone concentrations are lower, but where ozone fluxes are relatively high, for example in north and north-west Europe.

Increasing stomatal flux was associated with increasing incidences of ozone injury, greater severity of ozone symptoms and increasing biomass reductions. Stomatal flux (AFst3gen) maps were better at predicting the widespread occurrence of ozone damage on vegetation than ozone concentration (AOT40) maps, which underestimated the impact of ozone across Europe. Effects including visible injury symptoms and biomass reductions, sometimes greater than 20%, have frequently been reported at ozone concentrations below the AOT40-based critical level. The analysis in this report has shown that there was either no or minimal impact of ozone in grid squares with ozone fluxes close to zero. All of the evidence of adverse effects was found in grid squares predicted to have AFst3gen values of at least 5 mmol m^-2, with virtually all damage being found in grid squares with an AFst3gen of at least 12 mmol m^-2. It is tentatively suggested that a flux threshold of approximately 12 mmol m^-2 is required for both injury and biomass effects, although some effects have been detected at fluxes predicted to be below this level.

Challenges for the future

This report has clearly indicated that current ozone concentrations are damaging vegetation in Europe. In the next decades, changing ozone profiles (decreasing peaks, increasing background) and changes in climate, including factors such as temperature, carbon dioxide concentration and precipitation that will modify the stomatal flux of ozone, will change the distribution and magnitude of effects of ozone across Europe. Thus, there is a need to further our knowledge of the damaging impacts of ozone on vegetation in Europe by:

- Spring and summer monitoring of ozone impacts in the field using an expanded network of sites, including sites in those areas of Europe for which little or no data currently exist
- Developing a long time-series of data for regionally representative sites to monitor the impacts of changing ozone profiles and climate
- Determining ozone flux-effect relationships for a wider range of crop species and for (semi-)natural vegetation ecosystems which take into account the modifying effect of global climate change
- Incorporating consideration of effects of soil moisture deficit as a key modifying factor of stomatal conductance into risk assessment maps for crops and (semi-)natural vegetation
- Developing the next generation of ozone risk maps which should include the modifying influence of climate change (e.g. temperature, increasing carbon dioxide concentration, changing precipitation patterns), and by collating suitable field-based data for evaluation of these new maps.
**Introduction**

**The role of the ICP Vegetation**

This synthesis of evidence for ozone effects on vegetation has been conducted by the ICP Vegetation, an International Cooperative Programme on the impacts of air pollution on vegetation. Reporting to the Working Group on Effects (WGE) of the Convention on Long-range Transboundary Air Pollution (LRTAP), the ICP Vegetation conducts experiments, analyses vegetation samples and develops models on impacts of ozone, heavy metals and nitrogen pollutants on crops and (semi-)natural vegetation in 33 countries of Europe plus the USA and Canada. ICP Vegetation-collated information on the impacts of ozone pollution is being used in the review of the 1999 Gothenburg Protocol to abate effects of acidification, eutrophication and ground-level ozone pollution (LRTAP Convention, 2007a). Contributions include dose-response functions for over 20 crops and 80 species of (semi-)natural vegetation, surveys of damage, data from exposure of ozone-sensitive and ozone-resistant biotypes to ambient ozone, and internationally-agreed methodology for mapping impacts and analysis of “stocks at risk” from ozone. Eighteen countries contribute to the ozone programme of the ICP Vegetation. Further information on the activities of the ICP Vegetation, including downloadable copies of the annual reports can be found at icpvegetation.ceh.ac.uk.

**Rationale for this study**

Quantification of the impacts of air pollution on vegetation in Europe has posed a significant challenge for European scientists over recent decades. Many experimental and modelling methods have been developed and explored, and impact assessment methodology is being regularly updated with developing scientific knowledge. In this report, we take stock of the evidence of damaging effects of one pollutant, ozone, on European crops and (semi-)natural vegetation by collating and synthesising data from field observations and ambient air experiments across Europe. Observations of damage are compared with maps predicting where the most damage is likely to have occurred during the years 1990 - 2006.
Causes of ozone pollution

Ozone is a naturally occurring chemical present in both the stratosphere (in the ‘ozone layer’, 10 - 40 km above the earth) and the troposphere (0 - 10 km above the earth). Natural sources of the precursors of ozone such as oxides of nitrogen (NOx, e.g. from soils, lightning and transport from the stratosphere) and non-methane volatile organic compounds (NMVOCs, e.g. from soils and vegetation), together with incursions of ozone from the stratosphere under certain meteorological conditions, ensure that there is always a background concentration of ozone in the troposphere. Additional tropospheric ozone is formed from complex photochemical reactions involving NOx, carbon monoxide (CO) and NMVOCs released due to anthropogenic emissions (especially from vehicle sources). These emissions have caused a steady rise in the background ozone concentration in Europe since the 1950s (NEGTEX, 2001). Superimposed on the background tropospheric ozone are ozone episodes where elevated ozone concentrations in excess of 60 ppb can last for several days (e.g. in the Snowdonia National Park, UK, 2003, Figure 1.1).

The Convention on Long-range Transboundary Air Pollution

Established in 1979, the Convention on Long-range Transboundary Air Pollution has provided a forum for addressing major air pollution problems through scientific collaboration and policy negotiation. The Convention, which has 51 Parties, aims to limit, reduce and where possible prevent air pollution. Eight internationally agreed Protocols have been established that commit Parties to targets for pollution control. The Parties meet annually at sessions of the Executive Body to review progress with ongoing work and to plan future activities including the development of new Protocols. There are three main subsidiary bodies for the Convention:

The Working Group on Effects provides information on the degree and geographical extent of impacts of major pollutants on human health and the environment through its six International Cooperative Programmes (including the ICP Vegetation) and the Task Force on Health.

The Cooperative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe (EMEP) provides scientific support to the Convention on atmospheric monitoring and modelling, emission inventories and projections and integrated assessment modelling.

The Working Group on Strategies and Review is the main negotiating body for the Convention, responsible for assessing scientific and technical activities, negotiating revisions to existing protocols and preparation of new ones and promoting exchange of technology.
Ozone damage to vegetation
Exposure experiments have shown that ozone causes:

- Visible injury, present as fine yellow/brown/red specks initially on the upper leaf surface that gradually coalesce to form large lesions
- Increased/precipitously die-back (senescence)
- Reductions in photosynthetic rate and changes in biomass partitioning resulting in less growth and reduced seed production
- Decreased ability to over-winter or survive natural stresses (e.g. drought, freezing)

Such episodes are usually associated with hot dry conditions and stable high pressure over large areas of Europe. For example, in July 2006, two significant ozone episodes occurred in Europe between 17 - 22 July and 25 - 28 July. During these episodes, ozone concentrations in excess of 90 ppb were experienced in countries such as the UK, Belgium, The Netherlands, France, Germany, Switzerland and Italy with the highest one hour value recorded being over 180 ppb in Italy (EEA, 2007). Ozone concentrations are usually highest in rural and upland areas downwind of major conurbations, where unlike in cities, fewer other pollutants are present to react with ozone to reduce the concentration.

Models predict that, even with full compliance with the Gothenburg Protocol and European Union controls, 24h mean ozone concentrations are likely to continue to rise in Europe in the coming decades due in part to hemispheric transport of the precursors of ozone from developing areas of the world. Mean monthly 24h mean ozone concentrations for June, July and August are predicted to be greater than 45 ppb over most of Europe by 2100, and above 75 ppb in central and southern Europe (Sitch et al., 2007).

The damaging effects of ozone pollution on vegetation

The problem of keeping plants healthy in a polluted environment is not a new one. One of the earliest reports of air pollution damage to vegetation comes from the seventeenth century when gardeners found it very difficult to keep plants alive in smoke-engulfed London, UK (Treshow and Bell, 2002). Plants grew better during the English Civil War years in the middle of the 17th century, when trade reductions resulted in an improvement in air quality over London - “better than ever before” fruit yields were reported. About three centuries later, symptoms initially described as “weather fleck” appeared in the tobacco fields of the USA. Leaves were damaged by straw-coloured flecks making the tobacco unusable. Similar symptoms appeared in other crops during periods of warm humid weather. During the 1950s, ozone was identified as the causal agent, and was added to the list of vegetation-damaging air pollutants that by then also included sulphur dioxide, carbon monoxide, ammonia, fluorides and particulates. Now, some fifty years later, much more is known from controlled exposure experiments about the impacts of ozone pollution on sensitive species of crops, natural vegetation and trees (see text box).

For agricultural crops, there are economic consequences of ozone damage, with both yield quantity and quality being affected. The most sensitive crops include wheat, soybean, pulses and tomato, with potato, sugarbeet and rapeseed being moderately sensitive (Mills et al., 2007a). There are also implications for biodiversity in areas where high ozone exposure coincides with ecosystems of
high conservation value. Mills et al. (2007b) analysed published information on the responses of individual species to ozone and showed that grasslands (especially uplands, woodland fringes and dry grassland), heaths and wetlands contain a significant number of ozone-sensitive species and therefore are at potential risk of damage from ozone pollution. Complex communities can be relatively slow to respond due to natural buffering capacity against stresses, however, as shown by field exposure of a 30 year old sub-alpine pasture to ozone in Switzerland, cumulative damage can lead to significant biomass reduction over a five year timescale (Volk et al., 2006).

Of further concern are the indirect effects of ozone on radiative forcing of the climate. Models have predicted that the global land-carbon sink will be suppressed by ozone effects on plant productivity with a consequent further increase in atmospheric carbon dioxide concentration (Sitch et al., 2007). It is suggested that such indirect radiative forcing may contribute more to global warming than the direct effect on global warming of predicted increases in ozone pollution.

Mapping the impacts of ozone in Europe

One role of the ICP Vegetation in the last five years has been the overseeing of the revision of the critical levels for ozone. Critical levels are defined as “the atmospheric concentrations of pollutants in the atmosphere above which adverse effects on receptors, such as human beings, plants, ecosystems or materials may occur according to present knowledge” (LRTAP Convention, 2007b). For the negotiations for the Gothenburg Protocol, separate ozone critical levels were defined for crops, (semi-) natural vegetation and forest trees using the ozone parameter AOT40 (The sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb when the concentration exceeds 40 ppb during daylight hours, accumulated over a stated time period).

Two international ozone workshops have been held since the signing of the Gothenburg Protocol. The AOT40-based critical levels have been reviewed and there has been general agreement that a flux-based methodology provides a biologically stronger metric for ozone critical levels and risk assessment. Models have been developed that take into account the instantaneous impact of climate, soil conditions and plant growth stage on ozone flux, i.e. the uptake of ozone through the stomatal pores on the leaf surface (see text box on next page). As with AOT40, ozone impacts are most strongly related to the cumulative instantaneous fluxes above a threshold. The term AFstY (Accumulated stomatal flux above a threshold of Y nmol m^{-2} s^{-1}), is used to describe flux-based critical levels for two crops (wheat and potato) and provisionally for beech. The concentration-based and flux-based critical levels for crops and (semi-)natural vegetation are summarised in Table 1.1. Further information on the scientific bases and how to use these critical levels can be found in Pleijel et al., 2007, Mills et al., 2007a,b, and LRTAP Convention, 2007b.

During the last two years, a new method has been developed for modelling ozone fluxes to a generic crop, a generic deciduous tree and a generic Mediterranean tree (LRTAP Convention, 2007b). The models represent simplifications of the complete flux model that facilitate their use in integrated assessment and Europe-wide mapping of risk of effects. As described in Chapter 2, exceedances of ozone critical levels and generic flux-based risk maps are prepared by EMEP, an additional working group reporting to the Executive Body of the LRTAP Convention.

Table 1.1 Stomatal flux-based and AOT40-based critical levels of ozone for crops and (semi-)natural vegetation (from LRTAP Convention, 2007b).

| **Wheat** | An AFst6 of 1 mmol m^{-2} PLA, accumulated over either 970°C days, starting 270°C days before mid-anthesis (flowering) or 55 days starting 15 days before mid-anthesis
| **Potato** | An AFst6 of 5 mmol m^{-2} PLA accumulated over either 1130°C days starting at plant emergence or 70 days starting at plant emergence
| **Agricultural crops** | An AOT40 of 3 ppm h accumulated over 3 months
| **Horticultural crops** | An AOT40 of 6 ppm h accumulated over 3.5 months
| **Communities dominated by annuals** | An AOT40 of 3 ppm h accumulated over 3 months (or growing season, if shorter)
| **Communities dominated by perennials** | An AOT40 of 5 ppm h accumulated over 6 months
Modelling stomatal flux of ozone

Stomatal flux of ozone describes the movement of ozone from the outside of a leaf, through the stomatal pore and into the air spaces inside. It is modelled by predicting the transport of ozone through the stomatal pores per unit of leaf area at any moment in time. Stomatal uptake of ozone is determined by the influence of climatic factors (Vapour Pressure Deficit (VPD), temperature and light), soil factors (Soil Moisture Deficit (SMD)), ozone concentration and plant development stage (phenology) on the width of the stomatal pore. A generalised flux model has been included in the LRTAP Convention Modelling and Mapping Manual (available at http://www.icpmapping.org).

Terminology

\( F_s \) The instantaneous flux of ozone through the stomatal pores per unit projected leaf area (PLA) in nmol m\(^{-2}\) PLA s\(^{-1}\). \( F_s \) can be defined for any part of the plant, or the whole leaf area of the plant, but for this report, \( F_s \) refers specifically to the sunlit leaves at the top of the canopy. \( F_s \) is normally calculated from hourly mean values and is regarded here as the hourly mean flux of ozone through the stomata.

\( AF_{stY} \) The Accumulated flux above a flux threshold of \( Y \) nmol m\(^{-2}\) s\(^{-1}\), accumulated over a stated time period during daylight hours, units mmol m\(^{-2}\) PLA.

\( CLef \) The stomatal flux-based Critical Level of ozone, in mmol m\(^{-2}\) PLA, is the cumulative stomatal flux of ozone, \( AF_{stY} \), above which direct adverse effects may occur according to present knowledge.

Figure 1.2 provides an example of EMEP ozone risk maps plotted as AOT40 for wheat and \( AF_{st3gen} \) for a generic crop for the year 2003, a relatively high ozone year. This example illustrates some differences in predictions of 'high impact' areas using the two methods. The maps indicate that there is a greater gradient of AOT40 across Europe than there is for ozone flux. In particular, parts of some Mediterranean countries such as Spain have high AOT40 but relatively low ozone flux, whereas some parts of northern Europe e.g. southern Sweden have low AOT40 but relatively high ozone flux. These two approaches therefore identify different regions as having predicted high impacts of ambient ozone. In chapter 5 of this report, the collated evidence of impacts is compared with EMEP AOT40- and generic crop flux-based risk maps.

Aims of this report

The aims of this study have been:

- To identify areas where effects on (semi-)natural vegetation and crops have occurred in ambient ozone conditions in Europe over the timescale 1990 to 2006.
- To investigate whether observed effects are related to ozone concentration and ozone flux data.
- To investigate how well maps of exceedence of critical levels of ozone or generic fluxes have identified areas in which crops and (semi-)natural vegetation have been damaged in ambient ozone over recent years.

In the following chapters, the sources of ozone maps and evidence of damage are described (chapter 2) followed by a pan-European examination of the data (chapter 3). Chapter 4 provides examples of effects from five geographical regions of Europe and chapter 5 compares reported effects in ambient air with flux-based and AOT40-based maps of risk of damage. An overview, policy implications and further work are provided in chapter 6.
CHAPTER 2

Sources of Data

Ozone concentrations in Europe

Ozone concentrations across Europe are monitored using a variety of methodologies. In many countries (e.g. UK, Sweden, Slovenia), there are nationally coordinated networks of air quality measurement, including automatic ozone monitors. Data from more than 1000 automatic ozone monitoring stations across Europe are sent to EMEP for mapping purposes (Figure 2.1). In addition to automatic ozone monitors, many countries also use passive sampling methods such as diffusion tubes which provide mean concentrations over weekly to monthly time periods. Many participants of the ICP Vegetation operate one or more ozone monitors at or near to their institute or field stations to provide local data for use in the interpretation of effects. This data has been quality assured and collated by the Programme Centre for the ICP Vegetation.

Summary

Ozone concentrations and fluxes described in this report have been modelled for Europe on a 50 x 50 km grid by EMEP using the Eulerian model which simulates the emissions, transport, transformation and deposition of ozone. The large amount of data available to show where in Europe damaging effects of ozone on vegetation have/have not occurred has been collated and analysed. Sources include: ICP Vegetation biomonitoring experiments with white clover, sporadic visible injury surveys and comparison of impacts in charcoal-filtered compared to non-filtered air.
Evidence of ozone effects from ICP Vegetation biomonitoring studies

Biomonitoring using a chemical protectant against ozone damage

During the years 1994, 1995 and 1996 participants of the ICP Vegetation conducted studies in ambient air using the ozone-protectant ethylenediurea (EDU) at experimental sites and/or in commercial fields (Ball et al., 1998). Twelve sites from eight countries participated. For each species used in each study, half of the plants were treated with EDU and half with water. Plants were placed in ambient air and at the end of the exposure period (typically eight weeks), the two sets were compared for the extent of visible injury and/or biomass. Species tested in this way included subterranean clover (Trifolium subterraneum), bean (Phaseolus vulgaris), radish (Raphanus sativus), white clover (Trifolium repens), red clover (Trifolium pratense), tomato (Lycopersicon esculentum), soybean (Glycine max), watermelon (Citrullus lanatus) and tobacco (Nicotiana tabacum).

Ozone-sensitive (left) and ozone-resistant (right) white clover after exposure to ambient ozone for four weeks in Greece. Source: C. Saitanis

EMEP ozone concentration and flux models

The EMEP Eularian model maps ozone concentrations and fluxes on a 50 km x 50 km grid. Described in detail by Simpson et al. (2007), the EMEP model simulates the emissions, transport, transformation and removal of pollutants, and includes the calculation of ozone fluxes using the Deposition of Ozone and Stomatal Exchange (DOSE model, described in Simpson et al., 2003). AOT40 and generic crop flux maps have been supplied by EMEP for use in this study for the following years: 1990, and 1995 to 2004.

The accumulation period for the AOT40-based critical level for agricultural crops is three months, with the timing of the accumulation window reflecting the period of active growth of wheat and centred on anthesis (LRTAP Convention, 2007b). This provides a moving time interval to reflect the early growing seasons in southern Europe and later growing seasons in northern Europe. Default time periods have been provided for five geographical regions (Figure 2.2) and have been used here for regional analysis of impacts.

Evidence of ozone effects from ICP Vegetation biomonitoring studies

Biomonitoring using a chemical protectant against ozone damage

During the years 1994, 1995 and 1996 participants of the ICP Vegetation conducted studies in ambient air using the ozone-protectant ethylenediurea (EDU) at experimental sites and/or in commercial fields (Ball et al., 1998). Twelve sites from eight countries participated. For each species used in each study, half of the plants were treated with EDU and half with water. Plants were placed in ambient air and at the end of the exposure period (typically eight weeks), the two sets were compared for the extent of visible injury and/or biomass. Species tested in this way included subterranean clover (Trifolium subterraneum), bean (Phaseolus vulgaris), radish (Raphanus sativus), white clover (Trifolium repens), red clover (Trifolium pratense), tomato (Lycopersicon esculentum), soybean (Glycine max), watermelon (Citrullus lanatus) and tobacco (Nicotiana tabacum).

Ozone-sensitive (left) and ozone-resistant (right) white clover after exposure to ambient ozone for four weeks in Greece. Source: C. Saitanis
Biomonitoring with ozone-sensitive and ozone-resistant biotypes of white clover

The ICP Vegetation biomonitoring programme has involved exposure of an ozone sensitive biotype of white clover (Trifolium repens Regal, N C-S) to ambient air since 1996. Cuttings of clover were sent by the Programme Coordination Centre to participants across Europe, who established the plants according to a standard protocol. Ten to 25 plants of the N C-S clover were then exposed to ambient air conditions at these sites. The date for the start of the exposure varied between sites and between years, according to local growing seasons and experimental needs. The majority of sites began exposure of plants in May or June, and the last assessments were carried out in September or October. Plants were cut back to a height of 7cm every 28 days to allow new leaves to develop. At the time of these harvests, the plants were assessed for ozone-specific leaf injury using a common protocol. Injury was apparent in varying magnitudes ranging from pale cream stipple on the leaf surface to large necrotic patches with leaves severely damaged (Figure 2.3).

At some sites, ozone-resistant plants (N C-R) were also grown; the ratio of the biomass of N C-S to N C-R provides an indication of ambient ozone effects on growth at these sites. All data were checked for quality assurance prior to inclusion in the dataset.

There is scored injury data available from a total of 45 sites, representing 16 countries across Europe from 1998 to 2006 (Figure 2.4) and biomass ratio data is available from 1996 to 2006 for a total of 41 sites from 15 countries. However, each individual site did not necessarily perform the investigation every year resulting in only a few sites with a long time-series of data.
Evidence from filtered-air experiments

Although many research institutes within Europe have the capabilities to investigate the effects of ozone on vegetation experimentally, comparatively few use both charcoal-filtered (CF) and non-filtered (NF) air as treatments. Here these combinations of treatments have been used together, comparisons of responses provide valuable indications of the effects of ambient ozone. Data is available from sites in Sweden, Italy, The Netherlands, Austria, Switzerland, Belgium, Germany, Spain and Finland (Figure 2.5). Data from published papers and Conference Proceedings have been included in the database. In several cases, additional data has been provided by the authors of these papers to allow direct comparison of data between sites. Many crop and natural vegetation species have been investigated using CF and NF chambers and the effects observed have been diverse. Some studies investigated effects of ambient ozone on yield or growth-related parameters, although many other parameters have also been investigated. For the purposes of this report, data comparisons are made for biomass and yield effects only.

Observations of visible injury symptoms in ambient air

Visible injury symptoms attributed to ozone have been recorded in ambient air conditions across Europe in sporadic surveys conducted over the period 1990 - 2006. Symptoms have been observed on grasses, forbs, shrubs and crop species in experimental pots/plots, natural vegetation communities and commercial crops. In some countries/regions surveys of either natural vegetation or commercial crops have been performed intensively in specific years. Due to the sporadic nature of these surveys, it is not possible to investigate temporal trends in this dataset. In addition to records of visible injury from surveys, there are also many ad-hoc observations. Data has been collected from published sources and Conference Proceedings. Unpublished records from scientists, including those from the ICP Vegetation and ICP Forests, have also been included.

With such a diverse dataset, there was a need to apply quality assurance procedures to ensure comparability. Data of the highest quality originates from scientists that have collected plant material from species showing damage and exposed it to ozone experimentally to confirm the symptoms. Data has also been included if it has been collected by scientists who research ozone pollution and therefore are familiar with ‘typical’ ozone injury symptoms. Sometimes the scientist recording the observations classified the observation as ‘possibly due to ozone’, which may be due to ozone, but is a non-specific response and may be due to an alternative stress or a combination of stresses including ozone. This data has been collated for future use, but has not been included in the analyses presented here. Assessments of the amount of injury per leaf are subject to variation between researchers with underestimates of damage commonly happening (e.g. Bussotti et al., 2003a). To avoid such confounding factors for this study, only the presence of symptoms was recorded, rather than the extent. This study also does not take into account variability in the sensitivity to ozone of individuals within a wild population, as found for example in Centaurea jacea (Bungener et al., 2003).
CHAPTER 3

European-Scale Evidence

Introduction

The evidence for effects of ambient ozone on vegetation includes ozone concentration data, visible injury records, results from exposure experiments in charcoal-filtered/non-filtered air, and the ICP Vegetation biomonitoring studies. These data are considered on a regional basis in Chapter 4, and are compared to EMEP ozone concentration and flux data in Chapter 5. In this chapter, the evidence is investigated on a European scale to show which species are affected by ambient ozone, where in Europe these effects occur, and where the effects are largest.

Summary

This chapter demonstrates the extent of adverse effects of ozone on crops and (semi-) natural vegetation species across Europe. Over 500 incidences of visible injury symptoms have been recorded throughout Europe between 1990 and 2006 on 30 crop and over 80 (semi-) natural vegetation species. Effects on yield/biomass of (semi-) natural vegetation species and crops have been demonstrated on a variety of species and in several countries. In some cases, reductions in biomass/yield of over 10% have been recorded at ozone concentrations below the critical level. Results from ICP Vegetation biomonitoring studies show that the largest impacts of ozone are consistently found in Switzerland, Italy and Greece.
Ozone at ICP Vegetation sites between June and August

Ambient ozone concentrations are measured by many participants of the ICP Vegetation. These measurements typically coincide with the experimental period of the individual research groups. To illustrate differences between sites across regions, a comparison of three-month AOT40, accumulated over June to August (which coincides with the main experimental period for most sites) has been calculated for selected ICP Vegetation sites for 1999, 2000 and 2003 (Table 3.1). ICP Vegetation sites with high AOT40 over this period include Germany (Trier University), Italy (Naples and Isola Serafini), Slovenia (Iskrba and Ljubljana) and Switzerland (Cadenazzo).

The values shown in Table 3.1 also demonstrate the wide variation in ozone concentrations between years at many sites, for example in Belgium (Tervuren) and Germany (Trier University), the June-August AOT40 was four times higher in 2003 than in 2000. However this difference was smaller at some other sites e.g. Spain (Ebro Delta) and Sweden (Östad). The influence of using different time windows to accumulate AOT40 over three months in the different regions is also apparent, as over the three months June-August in 2003, Slovenia (Ljubljana) recorded a higher AOT40 than Germany (Trier University), but over the regionally classified three-month time window, this was reversed and Germany (Trier University) recorded a higher AOT40 than Slovenia (Ljubljana) (data not presented).

Data from selected ICP Vegetation sites in 2000 and 2003 shows that high ozone concentrations tend to occur in southern Europe, however, an individual site in southern Europe may not always have high ozone concentrations (Figure 3.1). For example, there was variation in the ozone concentrations in Eastern Spain, which may result from site-specific differences such as whether the site is urban, rural or coastal, local meteorological conditions, and altitude.
Visible injury in ambient air

Visible injury symptoms attributed to ozone pollution have been recorded in sixteen European countries, and between 1990 and 2006 the total number of records of visible injury symptoms exceeds 500. Records are from across Europe, i.e. from northern Europe as well as southern Europe. Visible injury symptoms on crop species have been recorded in twelve countries, symptoms on (semi-)natural vegetation species have been recorded in fourteen countries and symptoms on shrubs have been recorded in eight countries (Table 3.2). However, the absence of records of visible injury for a particular country does not mean that injury did not occur there.

<table>
<thead>
<tr>
<th>Site</th>
<th>1999</th>
<th>2000</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium (Tervuren)</td>
<td>4.80</td>
<td>2.13</td>
<td>8.11</td>
</tr>
<tr>
<td>Germany (TrierUniversity)</td>
<td>8.09</td>
<td>4.12</td>
<td>15.63</td>
</tr>
<tr>
<td>Italy (IsolaSerafini)</td>
<td>23.78</td>
<td>19.39</td>
<td>-</td>
</tr>
<tr>
<td>Italy (Naples)</td>
<td>16.33</td>
<td>19.86</td>
<td>13.70</td>
</tr>
<tr>
<td>Slovenia (Iskrba)</td>
<td>-</td>
<td>15.08</td>
<td>13.59</td>
</tr>
<tr>
<td>Slovenia (Ljubljana)</td>
<td>-</td>
<td>12.20</td>
<td>18.56</td>
</tr>
<tr>
<td>Spain (Ebro Delta)</td>
<td>8.97</td>
<td>2.78</td>
<td>3.99</td>
</tr>
<tr>
<td>Sweden (Ostad)</td>
<td>3.03</td>
<td>1.67</td>
<td>1.49</td>
</tr>
<tr>
<td>Switzerland (Cadenazzo)</td>
<td>19.22</td>
<td>-</td>
<td>29.46</td>
</tr>
<tr>
<td>UK (Bangor)</td>
<td>1.04</td>
<td>0.14</td>
<td>-</td>
</tr>
<tr>
<td>UK (Snowdon)</td>
<td>2.10</td>
<td>2.09</td>
<td>4.20</td>
</tr>
</tbody>
</table>

Table 3.1 AOT40, ppm h, at selected ICP Vegetation sites between June and August in 1999, 2000 and 2003. The percentage data capture, based on the number of days, is indicated in brackets.

Table 3.3 Crops that have shown ozone injury symptoms in the field, by country.

Table 3.2 Countries where visible injury has been recorded on crops, semi-natural vegetation and shrubs in Europe.
Visible injury symptoms attributed to ozone have been observed on 30 crop species, including bean, potato, onion, watermelon and grapevine. For some crops, the occurrence of visible leaf injury renders the crop unsellable, e.g. lettuce, whereas for other crops the visible injury does not directly affect the market value of the crop, but may be an indication that biochemical changes have occurred in the plants that may alter crop yield or quality. In Greece, Italy and Spain many crop species have shown ozone-injury symptoms, whereas for other countries there are records of ozone-injury symptoms for only one or two crops (Table 3.3). Similarly, some crops, e.g. bean, have had injury symptoms recorded in many countries (12) whereas others (e.g. chicory and soybean) have only had injury recorded in one or two countries. This is partly because not all crops are grown in all countries. In addition, ‘no records’ of injury on a particular crop in a particular country does not mean that injury did not occur.

Approximately 80 species of (semi-)natural vegetation have been recorded with symptoms attributed to ozone (Table 3.4). These include ‘stippling’ and those where the symptoms have subsequently been confirmed in exposure studies. A further 13 species have shown symptoms that may be due to ozone, although the symptoms were either non-specific or unconfirmed, and these records were not used in subsequent analyses (Table 3.5). Of the species with ‘typical ozone’ injury symptoms, a wide variety of plant families are included, from both forbs and grasses. There are several records for some species, e.g. Centaurea jacea (brown knapweed) and Epilobium angustifolium (fireweed/rosebay willowherb) but just a single record for others (data not presented).
Visible injury symptoms attributed to ozone have been recorded every year from 1990 to 2006. The number of records in each year is very variable (Figure 3.2), however much of this variation is because in some years more surveys of vegetation for visible injury symptoms occurred, rather than there necessarily being more symptoms in some years than others. Generally, there are more records per year from 2001 onwards than for earlier years. However, it is not possible to distinguish whether this is due to an increased occurrence of visible injury symptoms or whether there was increased effort in looking for (and identifying) symptoms.

The distribution of records of visible injury symptoms attributed to ozone shows that the observations of visible injury are widespread across Europe (Figure 3.3). The distribution is compared to EMEP maps of ozone concentration and flux in Chapter 5. Generally speaking, the records of ozone injury symptoms are clustered around research groups that work on effects of ozone on vegetation, which is probably because these researchers may actively look for symptoms around their institute and can recognise ozone injury symptoms when they occur.

The distribution of records of occurrence of visible injury symptoms shows that ozone injury can occur across the whole of Europe, although it is most commonly recorded in Continental Central Europe and the Western Mediterranean regions (Figure 3.4). Although many records are present as a result of surveys, these surveys only account for approximately one-quarter of the total records of visible injury, with the remaining records from ad-hoc observations.

ICP Vegetation experiments

Clover biomonitoring experiment - visible injury

Ozone injury on the ozone-sensitive NC-S variety of white clover used in the biomonitoring experiments of the ICP Vegetation has been detected at almost every site in every year, with the extent of injury reflecting the fluctuating ozone climate. Ozone injury was recorded as an injury score, where 1 = <1% of leaves affected, 2 = 1-5%, 3 = 5-25%, 4 = 25-50%, 5 = 50-90% and 6 = 100%. There was high year-to-year variation in the monthly injury scores, however, there was no consistency between the regions. For example in Atlantic Central Europe and Northern Europe in August, there were high visible injury scores in 2003, but this was not mirrored in the other regions. June 2005 had low injury scores in the Northern Europe and Western Mediterranean regions, but not in Continental Central Europe or Atlantic Central Europe.
Visible injury scores for the NC-S clover over June, July and August are illustrated per region for 1998 - 2006 (Figure 3.5). The component countries of each region were as defined in the Modelling and Mapping Manual for Critical Levels for Vegetation (LRTAP Convention 2007b) and the regional classification of countries is illustrated in Figure 2.2 of this report. Injury scores were usually highest for the Western Mediterranean (WM), with the exception of the unusually cool and wet summer of 2002. Injury scores were lowest in Northern Europe (NE) and Atlantic Central Europe (ACE) and were generally intermediate for Central Continental Europe (CCE). There was high year-to-year variation within each region, but no consistent trends. From the studies conducted so far, it has been shown that trends in impact reflect the spatial and temporal variation in ozone concentration, with no marked decline or increase evident.

When the three-month injury scores and AOT40 data were combined for all sites and years, there was increasing injury with increasing AOT40 (Figure 3.6). The response shown using this dataset is logarithmic, however, the scale used for assessing ozone injury is not linear and shows smaller differences between the highest percentages of ozone injured leaves. Although initially it appears that the different regions show different response relationships between AOT40 and ozone injury score, the different regions have different ozone exposures and therefore the linear relationship between AOT40 and visible injury score for each of the regions corresponds with a different portion of the overall logarithmic relationship (Figure 3.6b).

Visible injury to the NC-S variety has been detected across Europe. Switzerland, Italy and Greece generally show the highest impacts, however, moderate impacts of ambient ozone, in terms of injury score, can be found in central and northern Europe. The highest impacts in terms of visible injury were found in the months July and August, although large impacts also occurred in June (Figure 3.7a-d). More moderate impacts occurred in September. Only a few sites recorded development of ozone injury into October, but of these, some sites in some years have shown new ozone injury developing on the leaves of the clover during O ctob er, e.g. Spain (Ebro Delta) and Italy (Rome) in 2004. In both of these examples the daily maximum ozone concentrations were in the range 40-60 ppb, with these small peaks persisting for several days.

Ozone concentrations and visible injury impacts were variable between years and between the regions, however the clover biomonitoring dataset extends over many years so that overall trends can be examined. The combined dataset from 1998 to 2006 shows that in June and July, impacts of ambient ozone in Sweden were as high as those in Greece and Spain. Ozone concentrations, however, indicate that the highest impacts were predicted to be in southern Europe. These data therefore indicate that ozone concentration alone does not account for the impacts seen. It is likely that ozone injury development also shows a relationship with ozone flux.

Ozone injury on spinach.
Source: J Bender
Clover biomonitoring experiment - biomass ratio

Ozone sensitive (NC-S) and ozone resistant (NC-R) clover plants have been grown simultaneously at many ICP Vegetation sites. Plants were grown and exposed to ambient air according to a standard protocol. Plants were cut down to 7 cm above soil level every 28 days and the biomass of the two biotypes was compared. The ratio of biomass of the NC-S to NC-R clover was used to indicate the impact of ambient ozone on plant growth.

The response of NC-S and NC-R clover in charcoal-filtered/non-filtered air and in ambient air has been compared by Fagnano et al. (2004). In ambient air, the reduction in biomass of the NC-S biotype compared to NC-R was used. In the chamber experiment, the reduction in biomass of the NC-S biotype in non-filtered air compared to charcoal filtered air was investigated. The results showed that there was no significant difference in yield reduction using the two methods (23% in the ambient air experiment and 18% in the CF/NF experiment), demonstrating that the NC-S/NC-R biomass ratio is a good indicator of the biomass reduction caused by ambient ozone pollution. Biomass ratio and AOT40 data over three months were calculated from a range of ICP Vegetation sites from experiments over an 11 year period from a total of 24 sites. The three-month ratio of the biomass of NC-S compared to NC-R clover shows a decreasing ratio with increasing ozone exposure (Figure 3.8). There was a lot of scatter around the regression line of the biomass ratio compared to AOT40, however,
several sites have shown potential biomass reductions of 10% close to the current critical level of ozone of 3 ppm h, for example in Belgium (Terwuren, 1998) and Germany (Trier, 1999). A wide range of 3-month biomass ratios have been detected (Figure 3.9). Biomass ratios of less than 0.9 have been detected in the Eastern Mediterranean, Western Mediterranean and Continental Central Europe regions, but have not been recorded in either Northern Europe or Atlantic Central Europe. Biomass ratios of less than 0.7, indicating a large effect of ambient ozone on the clover plants, have been recorded in Greece and Italy.

If the individual datapoints are grouped per region to give regional mean values, there is a strong relationship between three-month AOT40 and NC-S/NC-R biomass ratio (Figure 3.10), with the regions with highest AOT40 showing the largest effects on the biomass ratio of clover.

The areas with the most consistent high impacts of ambient ozone on the three-month biomass ratio between 1996 and 2006 are illustrated (Figure 3.11). Median biomass ratios were used, although analysis using mean biomass ratios showed an identical pattern. Switzerland, Italy and Greece showed the highest ozone impacts (lowest biomass ratio), whereas the UK, Sweden, Finland and Slovenia showed the lowest impacts. Belgium, Germany, Netherlands, Spain and Austria showed intermediate effects.

**Canopy flux-effect relationship for white clover**

A model of stomatal conductance has been developed for clover, allowing comparisons of biomass ratios with fluxes of ozone to be made. The model was developed based on the responses of plants to 'real' ozone episodes in ambient conditions, in the wide range of climatic and pollution conditions experienced across Europe and without the confounding effect of any chamber-based ozone exposure system. Stomatal conductance measurements were made at nine ICP Vegetation sites during the period 1998 to 2001 for a range of climatic and ozone conditions. Initially a flux-effect model for biomass reduction was developed based on flux estimates to a single leaf, based on the Emberson et al. (2001) approach. This was subsequently scaled up to a whole canopy (Mills et al., 2006). The canopy stomatal conductance model was applied to selected data (that had quality assured hourly climate data to allow stomatal conductance to be calculated), using three-month biomass ratio and the corresponding climate and ozone data. The relationship between measured three-month NC-S/NC-R biomass ratio and ozone flux to the canopy showed an $r^2$ of 0.32. There was no improvement to the relationship when a threshold for canopy flux was incorporated. There was a stronger relationship between three-month AOT40 and NC-S/NC-R biomass ratio, using the same dataset ($r^2 = 0.53$, Figure 3.12).
EDU experiments

During the years 1994, 1995 and 1996 participants of the ICP Vegetation conducted studies in ambient air using the ozone-protectant ethylenediamine (EDU) at experimental sites and/or in commercial fields. For each individual study, half of the plants were treated with EDU and half with water. Plants were then left in ambient air and the two sets compared for the extent of visible injury and/or biomass. Species tested in this way included subterranean clover (Trifolium subterraneum), bean (Phaseolus vulgaris), radish (Raphanus sativus), white clover (Trifolium repens), red clover (Trifolium pratense), tomato (Lycopersicon esculentum), soybean (Glycine max), watermelon (Citrullus lanatus) and tobacco (Nicotiana tabacum). In many cases the occurrence of visible injury was reduced for plants treated with EDU. For example, ozone episodes in Sweden caused visible injury to some species (Trifolium subterraneum, Trifolium pratense, Trifolium repens), which was reduced by the use of EDU. In the Netherlands, EDU reduced visible injury on Phaseolus vulgaris in 1994, and on Trifolium subterraneum (cv Geraldton), between 1994-1996. EDU protected the plants almost completely from visible injury, whereas non-treated plants showed symptoms up to September (Tonneijck and van Dijk, 2002b).

EDU can also protect plants from decreases in biomass that would otherwise occur in ambient ozone conditions. Shoot biomass in Raphanus sativus (cv Cherry Belle) was higher in EDU treated plants than in non-treated plants in Sweden in 1990 (Pleijel et al., 1999b). In 1995 and 1996, the yield of green marketable pods and mature pods was reduced in non-EDU treated plants compared to those that had been treated with EDU (Tonneijck and van Dijk, 2002a). Similarly, in Catalonia (Spain) EDU provided partial protection against reductions in fruit yield, number of fruits and shoot biomass in 1994-1996 (Ribas and Penuelas, 2000).

A comprehensive analysis of the results of exposure of EDU and non-EDU treated Trifolium repens (L. cv Menna) at 12 sites, 9 countries in 1994, 1995 and 1996 showed that there was a decrease in the biomass ratio of non-EDU to EDU treated plants with increasing AOT40, however there was a lot of scatter in this relationship (Ball et al., 1998). There was no difference in the dose-response relationship in the different years (Figure 3.13).

Other evidence of impacts of ambient ozone on vegetation using EDU have also been reported. For example in August 1991, 25% visible leaf injury (stipple) was observed in vineyards in Frankonia (Germany). However, there was significantly reduced damage of plants treated with EDU (Vontiedemann and Herrmann, 1992).
Charcoal-filtered and non-filtered ozone exposure experiments

Comparisons of responses of plants in charcoal-filtered (CF) and non-filtered (NF) air can give indications of the impacts of ambient ozone pollution. A diverse range of species and response parameters have been investigated in this way, including crop and natural vegetation species. Many studies have investigated growth-related parameters, for example, reductions in growth (height increment) in non-filtered compared to charcoal-filtered air were recorded for carob and olive trees in Spain (16% and 12% reductions respectively) (Inclán et al., 1999). In some studies, only the extent of visible injury was compared between non-filtered and charcoal-filtered air. These data have been included in the 'records of observations of injury in ambient air' section, and therefore not included in this section to avoid duplication. These datasets included Bermejo et al. (2003), using data from Spain.

Some studies used charcoal-filtered compared to non-filtered air chambers. Others used charcoal-filtered or non-filtered air chambers compared to ambient air plots. It is recognised that the presence of a chamber will influence the response of the plants due to the different meteorological conditions within the chamber compared to outside (e.g. Vandermeiren et al., 2006). However, the data included in this database use a non-filtered chamber rather than an ambient air plot, therefore, although data from ambient air plots have been collated, these data have not been used in overall comparisons.

Using only the data which contain yield (or yield-type data, e.g. biomass), the mean and median yield loss per country was calculated, based on the difference between growth in charcoal-filtered and non-filtered air. Large yield reductions, based on growth in non-filtered compared to charcoal-filtered air can occur across Europe, particularly in Spain, Sweden and Italy (Table 3.6).

### Table 3.6 Yield reductions per country in non-filtered compared to charcoal-filtered air.

<table>
<thead>
<tr>
<th>Country</th>
<th>Years included</th>
<th>% yield loss (mean)</th>
<th>% yield loss (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>1994, 1995, 1996</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Belgium</td>
<td>2003, 2004</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Italy</td>
<td>2004</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1996, 2000</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Spain</td>
<td>2000</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1995, 1996</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 3.7 Mean and median yield reductions per region in non-filtered air compared to charcoal-filtered air, using only those data from experiments of two to four months duration.

<table>
<thead>
<tr>
<th>Region</th>
<th>% Yield reduction (mean)</th>
<th>% Yield reduction (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>NE</td>
<td>10.0</td>
<td>2.6</td>
</tr>
<tr>
<td>WM</td>
<td>10.5</td>
<td>14.0</td>
</tr>
</tbody>
</table>
The size of an individual response was not linearly related to the AOT40 at the site (Figure 3.14). This is because of the wide range in relative sensitivity to ozone of different species. Many of the studies exposed several species simultaneously (e.g. Gimeno et al., 2004, Franzaring et al., 2000) and although the ozone concentration may be the same (or very similar) for the species used, there can be large differences in the responses. The biomass changes in the study by Gimeno et al. (2004) ranged from -40% to +15%, and in the study by Franzaring et al. (2000), the biomass changes ranged from -14% to +9%. Comparisons of sensitivity to ozone of crops and (semi-)natural vegetation have been made (Mills et al., 2007a; Hayes et al., 2007). Species known to be sensitive to ozone (in terms of biomass/yield) include wheat, soybean, potato, whereas species thought to be insensitive to ozone include barley. Data from experiments in CF/NF air show large effects of ambient ozone on potato in Sweden in 1999, but smaller effects on wheat. The relative sensitivity to ozone of other species used in some of the studies is generally unknown. Unfortunately, there is not a common species or group of species used across the sites to determine whether yield/biomass reductions can be related to the ozone concentration.

The exposure periods of the different studies are of different lengths. The exposure length varied from 29 to 160 days, making it difficult to compare results from different experiments. Although a ‘daily AOT40’ could have been calculated for each study for comparison purposes, the differences in the lengths of the different experiments can also affect the magnitude of the effect seen due to the length of time that it takes for plants to grow.

The large diversity of lengths of experiments and parameters measured indicates that subsets of the data may be useful to make comparisons. Of the experiments with between 60 and 120 days duration (approximately 2 - 4 months), the AOT40 of the NF treatment ranges from 0 ppm h (Finland-Oulu, 1998) to 9.02 ppm h (Italy-Naples, 2004). Even using this subset of data, and only those that have a yield or equivalent parameter measured (e.g. biomass), a wide range of response size has been recorded (Figure 3.15), with the majority of recorded responses being a yield/biomass reduction in non-filtered air compared to charcoal-filtered air. The mean and median yield reductions in non-filtered air compared to charcoal-filtered air show that reductions in yield of approximately 10% were recorded in Northern Europe and the Western Mediterranean (Table 3.7). In Northern Europe the species used in the experiments include wheat and potato, which have been identified as sensitive to ozone, although during the exposure period for these experiments the critical level for ozone was not exceeded. In the Western Mediterranean a wide variety of species were tested, but the relative sensitivity to ozone of these species are mainly unknown.

Of the experiments that were of approximately 2-4 months duration, many significant effects were detected below the current critical level of 3 ppm h accumulated over three months. Species showing biomass reductions of greater than 10% in such experiments include Bissellula pellecensis, Briza maxima, Lolium rigidum, Trifolium glomeratum, Trifolium striatum and Trifoliumcherleri (Spain, 2000), Solanum tuberosum (Sweden, 1999), Lycopersicon esculentum (Italy, 2004) and Cirsium dissectum (Netherlands, 1996).

Conclusions

- Adverse effects of ozone on crop and natural vegetation species occur across Europe, with effects detected in northern as well as southern Europe.

- A wide variety of crop and natural vegetation species have been shown to be affected by ozone, with effects including visible injury and alterations in biomass found for over 100 species.

- In many cases the diversity of species used and responses measured, and differences in investigative effort between years make investigation of temporal and spatial trends difficult.

- The ICP Vegetation biomonitoring programme using white clover provides the longest time series of comparable data; no trends in effects were apparent over the timescale 1996 to 2006 due to the yearly fluctuating ozone concentrations.

- Generally, effects of ozone have been found to be more frequent/more severe in southern Europe, where the ozone concentrations are highest, however, significant impacts of ozone have also been demonstrated in northern Europe, where ozone concentrations are lower.
CHAPTER 4

Regional Evidence of Effects of Ambient Ozone

Summary

In this chapter, we summarise the evidence of adverse effects of ozone on crops and (semi-)natural vegetation at the regional scale. The ozone concentrations varied greatly between years, between regions and within countries. As a result, the impacts of ozone on vegetation varied greatly too. In general, leaf injury on white clover increased with increasing three-month AOT40 at ICP Vegetation biomonitoring sites. The responses of crops and (semi-)natural vegetation are species-specific, with both the nature and size of the response varying. The collated data clearly show that adverse effects such as ozone-induced leaf damage and reductions in biomass or crop yield occur at ozone concentrations currently experienced in Europe.
Introduction

In chapter 3, we described the evidence for effects of ambient ozone on vegetation at the European scale. In this chapter, we report in more detail on the evidence available at the regional scale. Evidence is presented for the following five regions, as defined in the Modelling and Mapping Manual (LRTAP Convention, 2007b) and shown in Figure 2.2: Northern Europe, Atlantic Central Europe, Continental Central Europe, Eastern Mediterranean and Western Mediterranean. Data on ozone concentrations (both AOT40 and 12-hour means) and visible injury are primarily available for sites that participated in the ICP Vegetation biomonitoring experiment with white clover. In addition, data is available from field surveys and exposure experiments in charcoal filtered and non-filtered air.

The sites that participated in the ICP Vegetation biomonitoring experiment often varied from year to year. Those sites for which ozone and visible injury data are shown here were chosen because of the completeness of data over a considerable timescale, rather than making any assumptions about whether or not the sites are representative for the region. In addition, the time window for calculated three-month AOT40 and assessment of visible leaf injury did not always coincide with that suggested for each region in the Modelling and Mapping Manual (LRTAP Convention, 2007b). One should bear in mind that the clover plants in the biomonitoring experiments were well-watered and therefore represent, similar to irrigated crops, a situation where they are at risk from high ozone stomatal fluxes due to high soil water availability.

Northern Europe

Ambient ozone concentrations and visible injury

In Sweden (Östad), the three-month AOT40 during June - August was very variable from year to year. With the exception of 1999, the critical level of 3 ppm h was not exceeded between 1996 and 2006. The 12-hour mean ozone concentration during June - August showed less variation (Figure 4.1). As the 12-hour mean ozone concentrations were sometimes close to 40 ppb, a modest rise in ozone concentrations could have an impact on the AOT40 if the critical threshold of 40 ppb is exceeded more frequently.

Visible injury has been observed in Sweden on species such as potato and clover (Trifolium). The majority of symptoms were observed in non-filtered air experimental plots or in experiments using EDU; non-EDU treated plants showed stippling whereas plants treated with EDU showed reduced or no stippling. Some stippling on leaves of Trifolium subterraneum and Trifolium pratense was also observed on a gradient away from a motorway (Pleijel et al., 1994a).

Figure 4.1 Three-month AOT40 and 12-hour mean ozone concentration for June-August in Sweden (Östad). Note: ozone concentrations recorded at 1 m.

Ozone injury on Trifolium subterraneum in Sweden. Source: H Pleijel
ICP Vegetation clover biomonitoring experiment

Visible injury on the ozone-sensitive variety of white clover has been observed every year in Sweden (Östad). The mean injury score for June - August shows large year-to-year variation (Figure 4.2). Although ozone-induced injury symptoms were frequently recorded, seldom more than 25% of leaves were affected at any one assessment. The mean injury score shows a good relationship ($r^2 = 0.77$) with the three-month AOT40, with increasing AOT40 resulting in increasing mean injury score. Almost every year visible injury was also recorded in September and was even observed on plants in October 2006, a year when the exposure to ambient ozone occurred for longer than usual. For Finland (Jokioinen) the mean injury score for June - August was only available for selected years and was generally much lower than in Sweden. In Northern Europe, the biomass ratio between the ozone-sensitive and ozone-resistant biotype of white clover was not significantly affected by ambient ozone concentrations.

Charcoal-filtered and non-filtered ozone exposure experiments

Reductions of approximately 20% in the yield of potato (cultivars 'Kardal' and 'Bintje'), for both tuber dry mass and number of tubers > 15mm, were found in non-filtered air compared to charcoal-filtered air in Sweden in 1999 (Pikki et al., 2004). Increased yellowing of leaves was also found and was more pronounced in the early maturing cultivar 'Bintje'. In this experiment, the AOT40 during 76 days of exposure was 1.38 ppm h in non-filtered air, which is equivalent to approximately 1.6 ppm h over three months. The considerable reduction in potato yield at such a low AOT40 in Sweden might well be explained by favourable climatic conditions and/or plant development during the summer months, allowing high stomatal ozone fluxes (see chapter 5).

Atlantic Central Europe

Ambient ozone concentrations and visible injury

As in Sweden (Östad), the three-month AOT40 during June - August was very variable in Belgium (Tervuren). The three-month AOT40 of 3 ppm h was exceeded in approximately 50% of the years, with the highest exceedences occurring in 2003 and 2006. The 12-hour mean ozone concentration during June - August shows a similar pattern, with the 12-hour mean ozone concentration being 40 ppb in 2006, a high ozone year (Figure 4.3). In Atlantic Central Europe, ozone episodes can also occur in early spring and late summer. For example, in 2003 an ozone episode peaking at 80 ppb occurred in April in Snowdonia, an upland region of North Wales, UK, and for several days in September ozone concentrations exceeded 60 ppb (Figure 1.1). The majority of visible injury symptoms in this region were observed in ambient air experiments on plants grown in pots. Injury records were primarily from Belgium and the UK. In a wetland in the UK, ozone-like symptoms were observed on Centaurea nigra and Eupatorium cannabinum following an ozone episode in August 2003 with maximum ozone concentrations up to 77 ppb.
Correlations between the degree of vegetation coverage of individual species and the three-month AOT40 for May - July in the previous year in South-Holland, the Netherlands, indicated that increased levels of ozone can be related to large changes (up to 40%) in vegetation coverage (Dueck et al., 2002). Whereas the coverage of species such as Phleum pratense, Aloepecurus pratensis, Phalaris arundinacea, Taraxacum officinale, Trifolium repens and Trifolium pratense was significantly reduced, the coverage of other species was not affected or even increased (e.g. Daucus carota, Festuca rubra, Lolium perenne) with increasing ozone, possibly due to changes in competitive ability.

ICP Vegetation clover biomonitoring experiment

Visible injury on the ozone-sensitive variety of white clover has been observed every year in Belgium and the UK. As in Sweden, the mean injury score for June - August was very variable between years in both countries, but seldom more than 25% of leaves were affected (Figure 4.4). In both the UK and Belgium high mean injury scores were found in 2003, a high ozone year, and in 2006 a high mean ozone injury score was observed in Belgium, in agreement with the high three-month AOT40. For Belgium (Tervuren), the mean injury score shows a good relationship ($r^2 = 0.74$) with the three-month AOT40, with increasing AOT40 resulting in increasing mean injury score. In Atlantic Central Europe, the biomass ratio between the ozone-sensitive and ozone-resistant biotype of white clover was not significantly affected by ambient ozone concentrations.

Charcoal-filtered and non-filtered ozone exposure experiments

In Belgium, the sugar yield of sugar beet (Beta vulgaris cv. Patriot) was significantly reduced in non-filtered air compared to charcoal-filtered air. The reduction was 8% and 4% at a five-month AOT40 of 6.5 and 2.9 ppm h in 2003 and 2004 respectively (De Temmerman et al., 2007). In the Netherlands, a variety of natural vegetation species were exposed to charcoal-filtered and non-filtered air between 2000 and 2002 (Tonneijck et al., 2004). The changes in shoot biomass varied with the number of years of exposure in the three-year experiment (Figure 4.5). In general, a decrease in shoot biomass was found for Agrostis capillaris and Plantago lanceolata and there was an increase in biomass for Lychnis flos-cuculi in non-filtered air compared to ‘charcoal-filtered air + 25 ppb ozone’. In the Dutch experiment, the AOT40 ranged from 0.56 - 0.93 ppm h in non-filtered air over an exposure period of 29 days early in the growing season each year.
Continental Central Europe

Ambient ozone concentrations and visible injury

The ozone concentrations within this region varied widely between sites, even within a relatively small spatial area. For example, in July 2000 the 28 day AOT40 at the close by German sites Deuselbach (rural, medium altitude), Trier City (urban, low altitude) and Trier University (suburban, low altitude) was 2.15, 0.27 and 1.47 ppm h respectively. The three-month AOT40 for June - August 2004 at ICP Vegetation sites in this region ranged from 1.86 in Poland (Poznán) to 16.69 ppm h in Switzerland (Cadenazzo), with intermediate values of 4.31 and 10.07 ppm h in Germany (Trier University) and Austria (Seibersdorf) respectively. At Trier University, the three-month AOT40 for June - August exceeded the critical level of 3 ppm h each year between 1999 and 2005, and in 2003 and 2005 the three-month AOT40 was higher than 10 ppm h (Figure 4.6). In this region, the mean three-month AOT40 was 8.31 ppm h between 1999 and 2005, with a standard error of 17%, which is considerably lower than the standard error between sites in individual years. At Trier University, the 12-hour mean ozone concentrations for June - August frequently exceeded 40 ppb.

Visible injury symptoms have been recorded across much of Continental Central Europe for a large number of species of forbs, grasses and shrubs. For example, injury was detected in a survey carried out in 2001 and 2002 in the Captharian mountains (Manning et al., 2002), there are many records from the Lattecaldo Tree Nursery in Switzerland (e.g. Skelly et al., 1999; Vanderheyden et al., 2001; http://www.ozone.wsl.ch) and there are records of injury from surveys conducted within the framework of ICP Forests (ICP Forests, 2003). There are occasional records of ozone-induced leaf injury symptoms on commercial crops such as Phaseolus vulgaris (J Bender, personal communication) and grapevine (Von Tiedemann and Herrmann, 1992) in Germany.

ICP Vegetation clover biomonitoring experiment

Visible injury on the ozone-sensitive variety of white clover has been observed in this region from May to October. However, ozone injury scores varied widely between sites. For example, in July 2002 the injury scores ranged from 1 (less than 1% of leaves affected) in Austria (Seibersorf) to 6 (100% of leaves affected) in Switzerland (Cadenazzo), and even the two sites in Germany had different injury scores (Figure 4.7). Whereas in other regions the highest injury scores were generally observed in the high ozone years 2003 and 2006, in Continental Central Europe the highest mean injury score for June - August was observed in 2001 and 2002. Maybe the stomata closed more due to the high temperatures and low humidity in 2003 and 2006 in this region. In Germany (Trier University), the mean injury score was well correlated ($r^2 = 0.67$) with the three-month AOT40 for June - August (Figure 4.8).

<table>
<thead>
<tr>
<th>Site</th>
<th>1999</th>
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<tr>
<td>Austria-Seibersdorf</td>
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<td>Germany-Hohenheim</td>
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<td>Switzerland-Cadenazzo</td>
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</table>

Figure 4.6 Three-month AOT40 and 12-hour mean ozone concentration for June - August in Germany (Trier University).

Figure 4.7 Mean leaf injury score for selected sites in Continental Central Europe in July 2002 (A) and for the whole region between 1998 and 2006 (B; 2006: only data for Poland).

Figure 4.8 Relationship between three-month AOT40 and mean leaf injury score for June - August in Germany (Trier University).
Charcoal-filtered and non-filtered ozone exposure experiments

In Germany, there was a greater percentage of underdeveloped pollen in Lolium perenne grown in ambient compared to charcoal filtered air (26.4% and 17.6% respectively) after exposure for 28 days in 1997 (Schoene et al., 2004). Although the yield of grapes (Welschriesling) was reduced by 5 - 8% in non-filtered compared to charcoal-filtered air for three consecutive years (1994 - 1996), with a carry-over effect of 9% into 1997, the differences were not statistically significant (Soja et al., 2004). A wide range in biomass responses was found in natural vegetation species exposed to non-filtered compared to charcoal-filtered air in Switzerland (Bungener et al., 1999b). Individual species were exposed for 160 days and the AOT40 was approximately 14 ppm h. Sensitive species include Arrhenaterum elatius, Carum carvi and Lotus corniculatus, which showed shoot biomass reductions of more than 10%, whilst Bromus erectus, Crepis biennis and Onobrychis sativa showed biomass increases of more than 10% in 'charcoal filtered air + ambient ozone' compared to charcoal-filtered air (Figure 4.9).

Eastern Mediterranean

Ambient ozone concentrations and visible injury

Ozone concentrations have been measured at a range of ICP Vegetation sites in Slovenia and Greece. In Slovenia (Ljubljana), the three-month AOT40 during June - August was very variable from year to year and ranged from 8.1 ppm h to 18.6 ppm h during 2003 to 2006 (Figure 4.10). The 12-hour mean ozone concentrations were close to 40 ppb during June - August 2004 to 2006, but were just over 50 ppb during 2003, a high ozone year. During April - October 2003, ozone concentrations in Ljubljana frequently exceeded 60 ppb, with several ozone episodes exceeding 80 ppb during June - August. In Greece, during June - August 2003 and 2004, the 12-hour mean ozone concentrations reached values up to 70 (Kalamata) and 53 (Athens) ppb respectively. In those years, ozone concentrations in Kalamata and Athens reached values up to 120 and 100 ppb respectively.

There are many records of ozone injury symptoms in the Eastern Mediterranean region, particularly from Greece. Ozone injury symptoms have been observed in a range of commercial crops including watermelon, chicory, onion and courgette (e.g. Velissariou 1996, 1999). In some cases, for example musk melon, onion and bean in 1995, the injury was 100%. Visible injury on up to 80% of lettuce leaves was also observed in commercial glasshouses with associated large crop losses; in this particular case, losses of €12,500 million occurred in one day in a one acre glasshouse (D Velissariou, personal communication). In 1998, an irrigated part of a field of chicory showed a greater extent of ozone injury than the non-irrigated part, which can be explained by enhanced stomatal ozone flux at high soil water availability.

Injury records from this region are mainly from crops; there are only a few records for trees and shrubs and no records of ozone injury on grasses or forbs. Some researchers have stated that there are few occurrences of visible injury on natural vegetation in this region, rather than that occurrences are under-recorded (Franc Batic˘, personal communication). It may well be that in this region ozone uptake is high in crops as they are often irrigated, whereas the majority of naturally occurring vegetation experiences hot, dry conditions during the highest ozone episodes, coinciding with low stomatal conductances and thus low stomatal ozone fluxes.
ICP Vegetation clover biomonitoring experiment

Visible injury on the ozone-sensitive variety of white clover has been observed from May to October. The extent of injury at individual sites and between years was very variable. The highest scores for the mean injury between June - August were recorded in 2002 and 2003 (Figure 4.11). The difference in biomass between the sensitive and resistant variety of white clover was large at some sites, e.g. in Greece (Athens) in 2006 (see photo chapter 2). Three-month biomass ratios of less than 0.9, indicating a 10% reduction in growth of the sensitive variety, have been recorded in both Greece and Slovenia. A three-month biomass ratio of less than 0.7, indicating a 30% reduction in growth of the sensitive variety, has been recorded in Greece (Kalamata) in 2003.

Charcoal-filtered and non-filtered ozone exposure experiments

There are no records from charcoal-filtered compared to non-filtered air exposure studies in the Eastern Mediterranean region.

Local variations in ozone impacts in Greece

(Source: D Velissariou)

Intense monitoring within small areas provides an indication of the local variations of ozone impacts. In Greece, biomonitoring with tobacco using the ozone-sensitive variety BelW3 was combined with ozone concentration measurements using diffusion tubes in Messinia and along the Parnis Mountain Range. Small plants of tobacco BelW3 were exposed for a short time of approximately one week at each site.

In Messinia the impact of ambient ozone on BelW3 varied across the area. The extent of injured leaves ranged from approximately 15 to 45% on average. Although the monitoring sites were at different altitudes (between 0 and 1300m), the extent of ozone injury was not related to altitude. It was suggested that a plume of ozone and precursors started in the north of the region and extended to the south, downwind of the pollution source, where the highest impacts were observed. In the Parnis Mountain range the highest mean ozone concentrations were detected in the North, where on average up to 53% of the leaves showed injury symptoms.

Ozone injury on BelW3 tobacco after 7 days of exposure (left) and diffusion tubes (above) along the Parnis mountain range in Greece.
Source: D Velissariou
Western Mediterranean

Ambient ozone concentrations and visible injury

In Italy (Naples), the three-month AOT40 for June - August was greater than 10 ppm h in each year that the ozone concentration was monitored. The 12-hour mean ozone concentrations were greater than 40 ppb in each year (Figure 4.12). Other Italian sites showed similar high ozone concentrations. Much more variability was shown between ICP Vegetation sites in Spain. In July 2000, the 28-day AOT40 for sites in Barcelona, the Ebro Delta and Valencia was 5.38, 0.19 and 0.73 ppm h respectively.

A wide range of commercial crops have shown visible injury symptoms in Italy and Spain, including watermelon, potato, peanut, soybean, tobacco, onion and tomato. In Italy, seventeen varieties of wheat, thirty-eight varieties of onion and two varieties of soybean all showed visible injury symptoms (Faoro and Iriti, 2003). Several varieties of potato showed visible injury symptoms in Valencia, Spain, in 2006 (Calvo and Sanz, unpublished data).

ICP Vegetation clover biomonitoring experiment

As in other regions, the mean injury scores for June - August showed large year-to-year variation over the period 1998 to 2006 (Figure 4.13). This was related to the variation in ambient ozone concentration. For example, in 2002, a low ozone year for this region, less than 5% of the leaves showed visible injury symptoms, whereas in other years usually more than 25% of leaves were affected.

Wheat yield in central Italy (Source: A De Marco)

In central Italy ozone was monitored at 17 sites and yield data was obtained for winter wheat for the years 2000 to 2004. Ambient ozone showed the highest concentrations in the south-east of this region, and lower concentrations in the north-west. The AOT40 during April - June was between 4 and 19 ppm h. Wheat yield was highest in the north-east of the region and varied between 32 and 52 kg ha⁻¹. In this case study, no relationship was found between AOT40 and yield of winter wheat. A preliminary analysis showed that a better relationship was found when using a stomatal flux-based index rather than the AOT40, i.e. a reduction in yield with increasing ozone stomatal flux.

Figure 4.12 Three-month AOT40 and 12-hour mean ozone concentration for June - August in Italy (Naples).
In September, the proportion of leaves affected ranged from less than 1% in 1999 to 50 - 100% leaves affected in 2003. The years 2002, 2005 and 2006 showed much lower injury scores than the other years. In 2002 and 2006, the low injury scores were due to the fact that no injury was observed on white clover in Spain and Portugal respectively. In the Western Mediterranean, the biomass ratio between the sensitive and resistant variety of white clover declined significantly with an increase in three-month AOT40 (Figure 4.13). At many Italian sites the biomass ratio was less than 0.8, indicating a 20% reduction in growth of the sensitive variety. The lowest values were observed in Isola Serafini, with a biomass ratio of 0.53 and 0.58 at three-month AOT40s of 20.4 and 32.8 ppm h in 1999 and 1998 respectively.

Charcoal-filtered and non-filtered ozone exposure experiments

A thirty-day exposure in Spain resulted in an 11% reduction in biomass of Trifolium subterraneum in non-filtered compared to charcoal-filtered air. The mean ozone concentrations were 8 and 34 ppb in charcoal-filtered and non-filtered air respectively (Sanz et al., 2005). A wide range of responses of natural vegetation species exposed individually to charcoal-filtered or non-filtered air in Spain was found by Gimeno et al. (2004) in 2000. Whereas some species (Biserula pelicinus, Briza maxima, Lolium rigidum, Trifolium cherleri and Trifolium striatum) showed more than a 20% decrease in shoot biomass in non-filtered compared to charcoal-filtered air, other species (Aegilops triuncialis and Trifolium subterraneum) showed an increase (Figure 4.14). Similar responses were observed for total plant biomass. The ozone exposure was between 43 and 76 days, with AOT40s between 0.09 and 0.82 ppm h. In 1996, olive trees were exposed to charcoal-filtered or non-filtered air for 300 days in Spain. The AOT40 during this period was approximately 12 ppm h in the non-filtered air and 0 ppm h in the charcoal-filtered air. Impacts of ozone included a 12% reduction in growth (height increment), however, no visible injury symptoms were observed (Inclán et al., 1999).

Conclusions

- Adverse effects of ozone on vegetation occur at concentrations currently experienced in Europe.
- Typical effects of ozone were leaf injury, changes in both crop yield and quality and changes in biomass of (semi-)natural vegetation. The responses were species-specific and could potentially result in changes in biodiversity of (semi-)natural vegetation.
- The ozone concentrations varied greatly between years, between regions and within countries and as a result the impacts of ozone varied greatly too.
- Highest three-month AOT40s were detected in Eastern (up to 19 ppm h) and Western Mediterranean (up to 33 ppm h) and parts of Continental Central Europe (up to 17 ppm h).
- Lowest three-month AOT40s were detected in Northern Europe (up to 3 ppm h) and Atlantic Central Europe (up to 11 ppm h). Even so, leaf injury and reductions in biomass or crop yield were detected, which indicates that high ozone stomatal fluxes may be occurring in these regions.
CHAPTER 5

Evidence of ozone damage in areas identified by EMEP as high risk

Introduction

During the last decade, considerable progress has been made with the methods used to identify areas at risk of damage from ozone pollution in Europe. Both concentration-based and flux-based critical levels have been established for crops, and a new risk assessment method involving estimating fluxes to a generic crop (AF_{st,3gen}) has been developed recently for use in integrated assessment and European-scale models (LRTAP Convention, 2007b). In this chapter, we compare AOT40-based and AF_{st,3gen}-based maps of risk for the years 1995 to 2004 with the evidence of damage presented in the previous chapters for these years. In so doing, we aim to answer the policy maker’s question:

“Is there any evidence that there is actual ozone damage in the areas identified by the EMEP model as having high flux/high AOT40?”

Summary

In this chapter, we have compared the evidence presented in Chapters 3 and 4 with EMEP concentration (AOT40) and generic crop flux (AF_{st,3gen}) maps for the years 1995 to 2004, inclusive. Overall, the AF_{st,3gen} maps were better at predicting the widespread occurrence of ozone effects than the AOT40 maps, as the latter tended to underestimate the geographical extent of damage. For both parameters, the areas with the highest values coincided with areas where the largest effects were detected. The generic flux approach worked particularly well in predicting ozone effects at sites in north and north-west Europe, where the AOT40 maps indicated that there should not have been any damage as the critical level had not been exceeded.
The EMEP maps used here have been prepared as far as possible according to the recommendations in the Modelling and Mapping Manual (LRTAP Convention, 2007b, Simpson and Emberson, 2006). For both AOT40 and AF₃₃₃₃₃₃, ozone concentrations have been modelled for a crop height of 1m and the three-month accumulation period has been centred on the date of anthesis which was first determined for each grid square using the following latitude model:

\[
\text{Mid-anthesis} = (2.57 \times \text{latitude}) + 40
\]

The generic flux model is similar in principle to the full flux model described for wheat in the Modelling and Mapping Manual, but has been simplified for application in large-scale models. The parameterisations for maximum and minimum stomatal conductance (\(g_{\text{max}}\) and \(g_{\text{min}}\) respectively), temperature (\(f_{\text{temp}}\)), light (\(f_{\text{light}}\)) and vapour pressure deficit (\(f_{\text{VPD}}\) and \(\sum f_{\text{VPD}}\)) are unchanged whilst those for phenology (\(f_{\text{phen}}\)), soil water potential (\(f_{\text{SWP}}\)) and ozone (\(f_{\text{O3}}\)) are set to one and therefore excluded from the calculation.

The two types of maps presented in this comparison are based on different concepts. The AOT40 maps have been prepared to indicate areas across Europe where the critical level for yield reduction in agricultural crops of 3 ppm h is exceeded, with increasing exceedance indicating increasing potential risk of yield loss. AF₃₃₃₃₃₃ maps provide estimates of the potential effective phytotoxic cumulative stomatal uptake and hence provide an indicator of risk of crop yield loss with a stronger biological basis than the AOT40 maps. No critical level has been defined for AF₃₃₃₃₃₃-based risk, thus, all areas where AF₃₃₃₃₃₃>0 indicate areas with a potential risk of crop yield loss.

The biomonitoring experiments of the ICP Vegetation, in which ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover were exposed to ambient ozone at a wide range of sites across Europe, provide the only comprehensive dataset for comparison with the EMEP risk maps. These provide biological effect data in the form of growth reduction in the sensitive biotype, together with assessments of the extent of ozone injury at sites from 12 countries. In this chapter, the biomass reduction data is compared with AOT40 maps to provide an indication of effects of critical level exceedance, and AF₃₃₃₃₃₃ maps to indicate if the areas predicted to have the highest fluxes have the highest effects. Although not as biologically significant as effects on growth

### Table 5.1 Summary of effects data used in the comparison with EMEP risk maps for the period 1995 to 2004.

<table>
<thead>
<tr>
<th>Injury records from surveys, ICP Vegetation and other biomonitoring studies</th>
<th>ICP Vegetation biomonitoring experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injury score</strong></td>
<td><strong>Biomass ratio</strong></td>
</tr>
<tr>
<td><strong>No of data points</strong></td>
<td>319</td>
</tr>
<tr>
<td><strong>Years included</strong></td>
<td>1995 to 2004</td>
</tr>
<tr>
<td><strong>Total no. of sites</strong></td>
<td>79</td>
</tr>
<tr>
<td><strong>Countries represented</strong></td>
<td>Austria, Belgium, France, Germany, Greece, Hungary, Italy, The Netherlands, Poland, The Russian Federation, Slovenia, Spain, Sweden, Switzerland, United Kingdom</td>
</tr>
</tbody>
</table>
and yield, the presence and extent of ozone injury on crops and (semi-)natural vegetation are also superimposed on AOT40 and \( AF_{3\text{gen}} \) maps as surrogate indicators of biological damage.

In the analysis described, we have not separately studied effects in relation to the critical levels for horticultural crops (an AOT40 of 6 ppm h, moving time period of 3.5 months) nor the short-term critical level for visible injury (a VPD-modified AOT30 of 0.16 ppm h, accumulated over the preceding eight days) as suitable maps were not available. Similarly, analysis presented cannot be directly applied to critical level exceedance for (semi-)natural vegetation dominated by annuals (a 3 month AOT40 of 3 ppm h) or perennials (a six-month AOT40 of 5 ppm h) because different timing and/or length of time intervals are recommended in the Modelling and Mapping Manual. Instead, we have compared all recorded effects of the ozone climate (1995 - 2004) on crops and (semi-)natural vegetation with crop-based risk maps.

### Methods of comparison

Two main sources of effects data have been used in this comparison: 319 records of ozone injury (surveys, observations, biomonitoring studies) and the ICP Vegetation clover experiments (52 data points for injury score, 57 datapoints for biomass effects) as summarised in Table 5.1. These data were collected from countries representing each of the five geographical regions of Europe. Records of ozone injury were made in each year of the ten years for which mapping data were available (see Chapter 3), whilst in the clover experiment biomass effects data started to be collected in 1996 and injury score data collection started in 1998. For ease of interpretation, the biomass data from the clover experiments is presented in this chapter as % biomass reduction, normalised for the 1.05 N C-S/N C-R biomass ratio at zero AOT40/\( AF_{3\text{gen}} \). In addition, data from a small number of filtered air versus unfiltered air open-top chamber experiments have also been compared with EMEP grid square values for AOT40 and \( AF_{3\text{gen}} \). Unfortunately, there was insufficient data available covering a three-month period to allow such reported effects to be mapped.

The range categories for the EMEP risk maps presented here have been chosen to provide a fair comparison between AOT40 and \( AF_{3\text{gen}} \) maps and have been re-drawn using data supplied by EMEP MSC-W. Each map has five categories for values exceeding zero, plus a zero category. The maximum whole number value modelled in any of the years included was divided by six to provide six evenly spaced categories; the two highest categories were merged to give a category of >12 ppm h for AOT40 and >24 mmol m\(^{-2}\) for \( AF_{3\text{gen}} \).

### Sources of uncertainty

When this study was initiated, comparison of EMEP risk maps with actual evidence of damage was assumed to be relatively straightforward. However, there are several sources of uncertainty associated with the analysis presented here that should be taken into account in the interpretation of results. These sources fall within two main areas: those associated with quantification of ozone effects and those related to mapping effects in relation to ozone concentration or flux.
Plant data

Firstly, there is some uncertainty in the interpretation of ozone injury symptoms. Those symptoms reported on species growing naturally in fields or grasslands have the highest level of uncertainty and have only been included here if the symptoms have been confirmed by ozone exposure experiments or have been identified by an ozone specialist. Symptoms on known indicator plants such as tobacco or clover are much more easily recognised and have more certainty attached. Experiments such as those reported here from the ICP Vegetation clover network have been performed according to a common protocol, with photographs provided to guide assessments. This, together with the use of broad range injury scores as far as is reasonably possible, prevents the problems of under-estimation of damage described by Bussotti et al. (2003a).

There is also some concern about the use of the NC-S/NC-R clover biomass system as an indicator in the widely varying climates of Europe. Originally selected in the southern-Europe-like climate of North Carolina, USA (Heagle et al., 1995), these biotypes have been extensively employed in many parts of the USA as bioindicators. To ensure that only data from healthy well-growing plants has been included in this analysis, the data sent to the ICP Vegetation Coordination Centre first underwent rigorous quality assurance checks (described in Mills et al., 2000). This resulted in exclusion of some data e.g. from northern Europe where the NC-R biotype appears to be particularly sensitive to downy mildew.

Mapping ozone concentration and flux

The second source of uncertainty is associated with the mapping process. Firstly, there are uncertainties associated with mapping AOT40 and AF_{st3gen} within the EMEP model. Both of these indices are sensitive to the characteristics of the frequency distribution of ozone concentrations (Tuovinen et al., 2007) with both showing increased sensitivity with increasing threshold. However, as lower ozone concentrations contribute to AF_{st3gen} than to AOT40, this parameter is less sensitive to threshold effects than AOT40 (LRTAP Convention, 2007b). Other sources of uncertainty associated with the simulation of the emissions, transport and deposition of ozone and its precursors are described in Simpson et al. (2003, 2007). In general, flux-based metrics show less spatial gradation across Europe than AOT40 (Simpson et al., 2007); within this analysis we determined which parameter best reflects the spatial pattern in effects.

Table 5.2  Mean start dates for ICP Vegetation NC-S/NC-R biomass data together with the mean start dates defined in the Modelling and Mapping Manual and the mean EMEP time intervals for the grid squares the ICP Vegetation sites represent within each region.

<table>
<thead>
<tr>
<th>Region</th>
<th>ICP Vegetation experiments (harvest 1, start of exposure period)</th>
<th>AOT40, Modelling and Mapping Manual defined</th>
<th>EMEP intervals based on the latitude function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Julian Date</td>
<td>Calendar Date</td>
<td>Julian Date</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>179</td>
<td>28th June</td>
<td>152</td>
</tr>
<tr>
<td>Atlantic Central Europe</td>
<td>164</td>
<td>13th June</td>
<td>121</td>
</tr>
<tr>
<td>Continental Central Europe</td>
<td>161</td>
<td>10th June</td>
<td>105</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>168</td>
<td>17th June</td>
<td>60</td>
</tr>
<tr>
<td>Western Mediterranean</td>
<td>163</td>
<td>12th June</td>
<td>91</td>
</tr>
</tbody>
</table>
Another cause for concern is the disparity between the earlier time periods mapped by EMEP using the latitude function, the Modelling and Mapping Manual AOT40 recommendations for the five regions, and the later three-month exposure periods used in the ICP Vegetation biomonitoring experiments which started in June (Table 5.2). In northern Europe, the three-month time intervals were only approximately one month apart, with the average start date for EMEP AOT40 and AFst3gen maps being 29th May AOT40, the Mapping Manual interval starting on 1st June, and the ICP Vegetation experiments starting on average on 28th June. For Atlantic Central Europe and Continental Central Europe, the EMEP three-month periods started on average five weeks earlier than those used in the ICP Vegetation experiments. The differences in time interval were largest for the Mediterranean region, where the Mapping Manual time intervals for AOT40 and EMEP maps were 2-3 months and nearly two months earlier than those used in the ICP Vegetation experiments, respectively.

These differences in time interval undoubtedly contribute to lower AOT40 values modelled for EMEP grid squares compared to those recorded at ICP Vegetation sites (see Chapters 3 and 4), since ozone concentrations are generally higher across Europe in June, July and August than in the spring (e.g. EEA, 2006).

**Use of modelled versus measured ozone data**

In addition to other factors influencing the local distribution of ozone within a grid square (e.g. altitude, topography, surface roughness, local emissions, land-use), a further contributing factor to the difference between modelled and measured AOT40 is the measurement height at ICP Vegetation sites. For all the ICP Vegetation data presented here, the median measurement height was 3m with the first and third quartile heights being 2.5 and 3.1m. However, the EMEP deposition module estimates ozone concentrations at the lower height of 1m, making concentrations approximately 7% lower relative to 3m (LRTAP Convention, 2007b). Since the ozone concentration at the majority of ICP Vegetation sites is frequently in the range 35 - 50 ppb, a reduction of 7% in ozone concentration would cause a disproportionately large reduction in AOT40 without an associated large decrease in biological effect (for further explanation see Pleijel et al., 1995 and Tuovinen, 2000). Thus, for the reasons described, the AOT40 values measured at ICP Vegetation sites as part of the clover experiment cannot be directly compared with the EMEP modelled AOT40 values for wheat.
The EMEP maps are designed to indicate risk of damage within a specified year or average of five years, and in this sense can be compared with data from the ICP Vegetation clover experiments and injury surveys conducted in that particular year or five years. However, we acknowledge that in the strictest sense, the differences in timing and ozone exposure mean that the damage data reported are not directly comparable with the risk maps produced according to the methods described in the Modelling and Mapping Manual. To our knowledge, no other sources of ambient air impacts data exist for the specified time intervals and thus the results presented here provide significant progress towards answering the policy maker’s question (see above). It also aims to fill the shortcoming identified by Simpson et al., 2007 in which the biologists have a clear preference for the AFstY metric, but there is an absence of large-scale data with which to verify the predicted maps.

**Visible injury-based evidence**

**Injury surveys and biomonitoring programmes**

Over 300 records of the presence of visible injury on crops and (semi-) natural vegetation were reported in the years 1995 to 2004 (Table 5.1). These records were made at 79 locations within 15 European countries. The dates of visible injury records were not always accurately reported; in some cases dates were provided, whilst in others the month or season (spring or summer) were recorded. For this analysis, injury-occurring episodes reported to be during the period April to September, inclusive, were compiled.

Injury was reported in each of the five geographical regions in 1995 (Figure 5.1), a relatively high ozone year in which there were ozone smog episodes involving concentrations exceeding 90 ppb covering much of central and western Europe in early May and from late June to late August (EEA, 1996). Injury was detected in locations in the UK and The Russian Federation, where the EMEP maps indicate that the AOT40-based critical level for growth reduction was not exceeded, but AFst3gen values were 10.7 and 9.8 mmol m$^{-2}$ respectively. Five or more incidences of ozone injury were reported in EMEP grid squares found in Belgium, The Netherlands, Greece and Switzerland where AOT40 values ranged from 3.0 to 10.6 ppm h and AFst3gen ranged from 8.3 to 15.8 mmol m$^{-2}$.

**Figure 5.2** All ozone injury records from the period 1995 to 1999 superimposed on the five year mean EMEP modelled (a) AOT40 and (b) AFst3gen for 1995 to 1999. Map data source: EMEP MSC-W.
The locations of all records of injury over the timescale 1995 - 1999 and 2000 - 2004 have been compared with mean AOT40 and AF$_{3p}$ over the same timescales (Figure 5.2 and 5.3 respectively). The two time periods differed in their ozone profiles, with more grid cells with the highest range of AOT40 values in 1995 - 1999, than in 2000 - 2004 but with a similar frequency distribution for AF$_{3p}$ (data not presented). Over both time periods, ozone injury was detected over a very wide area of Europe, with records only being absent from Ireland, northern UK and northern Scandinavia. Exceedance of the AOT40-based long-term critical level was not a good indicator of the likelihood of ozone injury being detected. However, the flux-based risk maps for both time periods indicated that, in general, AF$_{3p}$ values of 12 mmol m$^{-2}$ and more were associated with increased likelihood of ozone injury occurring. Analysis indicated that less than 5% of injury records were in grid squares with AF$_{3p}$ values below 12 mmol m$^{-2}$. Due to the sporadic nature of records of ozone injury, it is not possible to analyze the data further to determine whether the lower AOT40s but similar fluxes of 2000 - 2004 compared to 1995 - 1999 were associated with fewer incidences of injury.

### Severity of ozone injury on ICP Vegetation clover plants

The second surrogate for biomass/yield reduction considered in this analysis has been the assessments made at ICP Vegetation sites of the severity of ozone injury symptoms on ozone-sensitive (NC-S) white clover. The plants were well watered throughout the exposure period and thus could be considered to be representative of the irrigated/well watered crops the AF$_{3p}$ index was parameterized for. The three injury scores made over the three months from June to August were averaged per grid square for each year data exists within the time period 2000 - 2004, and compared with the average modelled AOT40 and AF$_{3p}$ for those five years (Figure 5.4). The highest five-year mean injury scores were consistently found in eastern Spain, Switzerland, Italy and Slovenia, with lower scores occurring at more northern sites. There were many sites where ozone injury was detected at modelled AOT40s below the critical level, whilst all sites with mean injury scores above 0.5 were associated with 50 x 50 km AF$_{3p}$ values in excess of 12 mmol m$^{-2}$.
At the regional scale, there was clear evidence of increasing AOT40 or AF3were3gen being associated with increasing leaf injury scores (Figure 5.5). For each metric, data points for Continental Central Europe and the Eastern Mediterranean fall close together in the middle of the graph, with the data point for the Western Mediterranean indicating the highest injury scores and AOT40/AF3were3gen values. It is interesting that for AOT40, the Northern European data point is to the left of that for Atlantic Central Europe, indicating a larger injury score than might have been expected, but falls much closer to the Continental Central Europe point when AF3were3gen is the dose metric. This implies that for Northern Europe, ozone flux is a better indicator of effect than AOT40.

Some of the most interesting detail is lost in the time averaged maps (Figure 5.4) and regional analysis presented (Figure 5.5). Thus, individual year EMEP grid square AOT40 and AF3were3gen values have been plotted against the June-August mean injury score for each year (Figure 5.6), for all 52 data points in the database (1998 - 2004). At several sites in grid squares indicated by EMEP to be below the AOT40-based critical level of 3 ppm h, injury was scored at 1 (first symptoms) or 2 (1-5% of leaves injured) and occasionally 4 (25 - 50% of leaves injured). Injury on 5% or more of the leaves (score 2 and above) was detected at all of the sites indicated to have an AOT40 above the critical level and therefore at risk of growth or yield reduction. With only one exception (a semi-urban site in Germany (Essen)), AF3were3gen values of greater than 1 were associated with ozone injury scores above 0.5, with AF3were3gen values of <1 being associated with only negligible injury. For almost all cases the threshold for injury scores above 1 seems to be an AF3were3gen of around 12 mmol m⁻². However, four data points representing grid squares representing two sites in Switzerland had higher injury scores of 3 - 5 (5 - 90% of leaves injured) than might have been expected from their relatively low AF3were3gen values of 5-9 mmol m⁻². Possibly the generic flux model is underestimating risk at these sites since modelled AOT40 values were higher than average at 1.9 to 5.9 ppm h and ICP Vegetation ozone measurements indicate AOT40 values in the range 5.3 to 23.8 ppm h for these data points.
Analysis of injury scores at the three sites with the lowest modelled AOT40s - UK (Bangor), UK (Ascot) and Sweden (Östad), provides further support for the predictive power of the EMEP generic flux risk maps (Table 5.3). Injury scores of one and above were associated with AF_{st3gen} values of ≥ 13 mmol m^{-2}, whereas an injury score of near zero was associated with zero AF_{st3gen}. In contrast, there was little relationship between AOT40 and ozone injury score at these two sites. For example, AOT40s of ca. 0.3 - 0.4 ppm h were associated with injury scores of 0, 1.2, 1.4 and 2.0. At these more northern sites, the threshold of 40 ppb may be too high, and lower thresholds such as 30 ppb as used in the short-term critical level (LRTAP Convention, 2007b) or 20 ppb as suggested by Pihl Karlsson et al. (2003) following analysis of national survey data using Trifolium subterraneum L. (Subterranean clover) as a bioindicator, might be more suitable. Furthermore, as suggested by Detemmerman et al. (2002) for potato, reduced cellular repair due to shorter nights may also contribute to the enhanced ozone sensitivity in clover grown in Sweden.

Biomass- and yield-based evidence

Biomass reduction in white clover at ICP Vegetation sites

As with ozone injury score, the highest five year mean biomass reductions were mainly found in southern Europe (Figure 5.7), with reductions of over 40% being found in Italy and Greece. Interestingly, biomass reductions of 8 to 20% were also found in Germany and The Netherlands in areas where five year mean AF_{st3gen} values were 16 - 18 mmol m^{-2} (Figure 5.7 b). In these same areas, the EMEP grid values for AOT40 were below the critical level of 3 ppm h. These patterns were more evident when the individual biomass reduction data for each individual year was plotted against the EMEP grid values for that year (Figure 5.8). Although the highest biomass reductions were generally associated with the highest predicted AOT40 values, there was no pattern in the data for AOT40 values below 4 ppm h, with biomass changes ranging from -10 to +20%. For AF_{st3gen}, the signal was somewhat more clear-cut. The lowest AF_{st3gen} values (<2 mmol m^{-2}) were all associated with <5% biomass

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Site</th>
<th>Mean injury score</th>
<th>AOT40 (ppm h)</th>
<th>AF_{st3gen} (mmol m^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>UK</td>
<td>Bangor</td>
<td>0.0</td>
<td>0.26</td>
<td>0.54</td>
</tr>
<tr>
<td>2002</td>
<td>UK</td>
<td>Bangor</td>
<td>0.0</td>
<td>0.36</td>
<td>0.78</td>
</tr>
<tr>
<td>2002</td>
<td>Sweden</td>
<td>Östad</td>
<td>1.1</td>
<td>0.63</td>
<td>16.91</td>
</tr>
<tr>
<td>2003</td>
<td>UK</td>
<td>Ascot</td>
<td>1.2</td>
<td>0.89</td>
<td>13.27</td>
</tr>
<tr>
<td>2004</td>
<td>UK</td>
<td>Ascot</td>
<td>1.2</td>
<td>0.31</td>
<td>13.14</td>
</tr>
<tr>
<td>2004</td>
<td>Sweden</td>
<td>Östad</td>
<td>1.4</td>
<td>0.11</td>
<td>13.27</td>
</tr>
<tr>
<td>2002</td>
<td>UK</td>
<td>Ascot</td>
<td>1.4</td>
<td>0.39</td>
<td>13.96</td>
</tr>
<tr>
<td>2000</td>
<td>Sweden</td>
<td>Östad</td>
<td>1.7</td>
<td>0.80</td>
<td>13.99</td>
</tr>
<tr>
<td>2003</td>
<td>Sweden</td>
<td>Östad</td>
<td>2.0</td>
<td>0.31</td>
<td>19.31</td>
</tr>
<tr>
<td>2001</td>
<td>Sweden</td>
<td>Östad</td>
<td>2.5</td>
<td>0.50</td>
<td>16.91</td>
</tr>
</tbody>
</table>
reduction whilst greater biomass reductions were only associated with $A_{F_{st3}}$ values in excess of ca. 12 mmol m$^{-2}$. Unfortunately, there were not any ICP Vegetation sites with $A_{F_{st3}}$ values between 2 and 13 mmol m$^{-2}$ and so it is not possible to identify whether or not there is a flux threshold falling between these values.

A complicating factor in this analysis is that the NC-R biotype sometimes grows less well in northern climates where it can be more susceptible to fungal diseases than the NC-S biotype. For example, for the three years for which the plant data from the Sweden (Östad) site passed quality assurance tests, the mean biomass reduction was $-12.8 \pm 4.8\%$, i.e. in each year, the NC-S plants grew better than the NC-R plants. If we omit this data from a regional analysis of the data presented in Figure 5.8, then there was a very clear linear relationship between AOT40 and biomass reduction ($r^2 = 0.97$, Figure 5.9a). Ozone effects and AOT40 were lowest in Atlantic Central Europe, similar intermediate values occurred in Continental Central Europe and the Eastern Mediterranean, and the highest values were found in the Western Mediterranean. Conducting the same analysis for $A_{F_{st3}}$ revealed an exponential relationship (Figure 5.9b), with a small increase in effect with large increase in $A_{F_{st3}}$ from Atlantic Central Europe to Continental Central Europe/Eastern Mediterranean, through to a large increase in effect with small increase in $A_{F_{st3}}$ for the Western Mediterranean.

Biomass and yield reduction in non-filtered (NF) versus filtered-air (CF) open-top chambers

As described in earlier chapters, very few research groups have included CF and NF treatments within their open-top chamber ozone exposure experiments. Even fewer groups have made such comparisons in the years for which EMEP AOT40 and $A_{F_{st3}}$ maps are available, and fewer still conducted experiments over a three month or similar time period. Indeed, only four sets of data are available that met these criteria (Table 5.4).
The percentage biomass reduction in NF- compared to CF-air for these four experiments ranged from 1.5% (The Netherlands) to 24% (Sweden), with the lowest effect being in a grid square predicted to have the lowest \( A_{F_{st}3_{gen}} \).

Is increasing AOT40 and/or \( A_{F_{st}3_{gen}} \) associated with increasing damage?

Throughout this study, we have found widespread evidence of ozone effects on vegetation, with effects occurring in almost all areas of Europe. Relationships with \( A_{F_{st}3_{gen}} \), and to a lesser extent, AOT40, were strongest when considered at the regional scale (Figures 5.5 and 5.9), or as five year averages, with relationships being weakest when local-scale effect data was compared with 50 x 50 km grid square values for an individual year. Given the uncertainties described earlier this is not surprising; the AOT40- and \( A_{F_{st}3_{gen}} \)-based maps were developed for regional scale assessment of areas of risk rather than for application at the local-scale (LRTAP Convention, 2007b).

![Graphs showing the relationship between biomass reduction and EMEP AOT40 and AFst3gen](image)

**Figure 5.9** The relationship between the biomass reduction in NC-S clover at ICP Vegetation sites and the EMEP risk map three month (a) AOT40 and (b) \( A_{F_{st}3_{gen}} \) for the regions the sites represent (data from 1998 to 2004).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Species</th>
<th>Country</th>
<th>Year of study</th>
<th>Duration (days)</th>
<th>% biomass/yield reduction</th>
<th>EMEP AOT40 (ppm h)</th>
<th>EMEP ( A_{F_{st}3_{gen}} ) (mmol m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gimeno et al., 2004</td>
<td>12 species of (semi-) natural vegetation</td>
<td>Spain</td>
<td>2000</td>
<td>65</td>
<td>10.5</td>
<td>0.86</td>
<td>17.3</td>
</tr>
<tr>
<td>Piikki et al., 2004</td>
<td>Potato (Solanum tuberosum)</td>
<td>Sweden</td>
<td>1999</td>
<td>76</td>
<td>24</td>
<td>0.78</td>
<td>14.6</td>
</tr>
<tr>
<td>Martino et al., 2006</td>
<td>Tomato (Lycopersicon esculentum)</td>
<td>Italy</td>
<td>2004</td>
<td>78</td>
<td>10.8</td>
<td>4.47</td>
<td>17.3</td>
</tr>
<tr>
<td>Franzaring, et al., 2000</td>
<td>4 species of (semi-) natural vegetation</td>
<td>Netherlands</td>
<td>1996</td>
<td>106</td>
<td>1.5</td>
<td>1.70</td>
<td>12.1</td>
</tr>
</tbody>
</table>

**Table 5.4** Reductions in growth/yield in NF versus CF open-top chamber studies in relation to EMEP AOT40 and \( A_{F_{st}3_{gen}} \) values for the grid squares the experiments were conducted in. Grid square data source: EMEP MSC-W.
Figure 5.11  EMEP modelled mean (a) AOT\textsubscript{40} and (b) AF\textsubscript{3\textsubscript{gen}} for 1995 to 2004, superimposed with the location of all sites where an effect of ozone has been detected during those ten years. Map data source: EMEP MSC-W.
Of the effect parameters described here, scientists place highest value on biomass or yield reduction data since ozone injury is not always sufficient to induce such a biologically significant effect. Nevertheless, the presence and/or extent of ozone injury has proved to be a reliable indicator of areas with relatively high ozone fluxes and was particularly useful for indicating effects in Northern Europe where medium fluxes were predicted despite the low AOT40 values. When plotted against each other for the clover biomonitoring system, there was an apparent offset whereby ozone injury scores of ca. 1-2 occurred without there being any effect on biomass (Figure 5.10). This suggests that the presence of ozone injury may be a good “early warning” of other more damaging ozone effects, should ozone fluxes rise further. Furthermore, the AF_{3gen} maps seem to be a good indicator of the potential for ozone injury, since injury was virtually always detected in grid squares with AF_{3gen} values of at least 12 mmol m^{-2}. The only exceptions were two sites in Switzerland where the EMEP Model may have underestimated AF_{3gen}.

AOT40-based risk maps were generally less successful in predicting the presence of ozone injury, with injury frequently being detected in grid squares below the critical level. The improved power of flux-based over concentration-based indices in predicting ozone injury has also been found by Pihl Karlsson et al. (2002) in analysis of clover bioindicator data from Sweden and was suggested by Klumpp et al. (2006) in analysis of ozone injury on Tobacco bioindicators in 12 European cities.

The highest reductions in biomass were detected in grid squares which fell within the two highest categories of AF_{3gen} values in the maps presented in Figure 5.7, whilst the lowest effects on biomass were associated with grid squares with minimal ozone flux. In contrast, the AOT40 maps did not successfully identify all areas where biomass reduction occurred as grid cells without critical level exceedance had biomass reduction.

To provide an overview of all the data considered in this chapter, Figure 5.11 illustrates the ten year mean AOT40 and AF_{3gen} for the period (1995 to 2004) overlaid with the location of all sites across Europe where any effect of ozone in ambient air has been detected (injury and/or biomass reduction). Nearly half of these locations fall in grid squares where the AOT40-based critical level for yield reduction was not exceeded, suggesting again that this indicator is not a completely reliable predictor of effect.

In contrast, over 95% of locations were in grid squares where the AF_{3gen} values were at least 12 mmol m^{-2} and 100% fell in areas indicated to be at risk of ozone damage by having AF_{3gen} values greater than zero. This leads to the overall conclusion that the flux-based index is indeed a better indicator of adverse effects of ozone in ambient air than the concentration-based index.

**Conclusions**

- AF_{3gen} maps were better at predicting the widespread occurrence of ozone damage on vegetation than AOT40 maps.
- Increasing AF_{3gen} is associated with increasing incidences of ozone injury and severity of injury symptoms as well as increasing biomass reduction.
- Maps identifying areas of exceedance of the AOT40-based critical level as being at risk of ozone damage (injury and biomass) are underestimating the impact of ozone across Europe as effects have been detected in areas with AOT40 values below the critical level.
- Although regarded as less biologically meaningful than biomass or yield effects, the presence of ozone injury provides an early indicator of damage - this is especially important in north- and north-west Europe where AOT40 values are relatively low and yet AF_{3gen} is relatively high in some years.
- It is tentatively suggested that a threshold AF_{3gen} of ca. 12 mmol m^{-2} is required for both injury and biomass effects, although some effects have been detected at fluxes below this level.
Overview, policy implications and future challenges

Overview

It has been known since the 1950s that ozone is a powerful oxidant that damages sensitive vegetation. Ozone exposure experiments conducted in the last three decades have shown that over 15 crops (Mills et al., 2007) and 40 species of (semi-)natural vegetation (Hayes et al., 2007) have the potential to respond to the ozone concentrations currently experienced in Europe during the spring and summer months. Several papers have been published which describe ozone effects in the open field that are identical to those found in ozone exposure experiments (e.g. Skelly et al., 1999, Fumagalli et al., 2001, Manning et al., 2002). In addition, participants in the ICP Vegetation have been using ozone-sensitive clover species as biomonitors of ozone effects since 1994. In this report, we have collated as far as possible, the published and unpublished evidence of ozone damage to vegetation growing in ambient air and analysed the data in relation to EMEP maps which predict those areas that are of greatest risk of ozone damage. Two methods of risk assessment are compared: the AOT 40-approach based on the ozone concentration in air above the canopy, and the generic flux-approach, a more biologically relevant method based on predicting the uptake of ozone through the stomatal pores on the leaf surface. In so doing, we believe we have provided answers to policy maker’s questions about evidence of actual ozone damage in the areas identified as being at risk of damage by the pollutant.
Evidence of ozone damage at the European scale

Visible injury symptoms attributed to ozone pollution have been recorded in sixteen European countries between 1990 and 2006. The total number of records exceeds 500, and includes injury on 30 crop species (e.g. bean, potato, maize, soybean, lettuce) and 80 species of (semi-)natural vegetation encompassing both forbs and grasses. The highest numbers of records were found in Spain, Italy, Belgium, Sweden, Greece and Poland. Unfortunately, it has not been possible to analyse spatial or temporal trends in this dataset as the locations where there were the most observations of injury tended to be within easy travelling distance of scientists specialising in ozone effects, rather than found in statistically designed surveys.

Some more detailed analysis was possible for the results of the ICP Vegetation biomonitoring experiments with white clover (1996 to 2006). Ozone leaf injury scores were generally highest at the sites with the highest AOT40. Across Europe, the ozone injury scores were highest in July and August, lower in June and September with a few sites recording injury in May and October. The biomass of the sensitive biotype of white clover (NC-S) was significantly reduced relative to that of the ozone-resistant biotype (NC-R) at a number of sites across Europe, especially those in central and southern Europe. NC-S biomass reduction for June-August was linearly related to AOT40 measured at the site ($r^2 = 0.45$ for all data and 0.81 for regional means), with the highest reductions at the sites with the highest AOT40s. Earlier experiments in 1994 to 1996, in which a protectant against ozone injury was applied to Trifolium subterraneum (subterranean clover) produced a similar linear relationship with AOT40 when compared with untreated plants.

In a few experiments, plants were exposed to sub-ambient ozone concentrations using open-top chambers ventilated with charcoal-filtered (CF) ambient air. Biomass reductions of greater than 10% (relative to the CF treatment) were recorded for plants grown in non-filtered (NF) air-ventilated open top chambers at sites in Sweden, The Netherlands, Spain and Italy.

Evidence of ozone damage at the regional scale

To analyse further the evidence for damaging effects of ozone pollution, the database was divided into the following five geographical regions: Northern Europe, Atlantic Central Europe, Continental Central Europe, Eastern Mediterranean and Western Mediterranean. In Northern Europe, represented by Sweden and Finland, an average of 5 - 25% of NC-S clover leaves were damaged by ozone in 1999 and 2006, whilst between 1 and 5% of leaves were damaged in all the years in between, except in 2005. The highest injury scores detected in Atlantic Central Europe on NC-S clover were in 2003 and 2006. Reductions in biomass in NF- compared to CF-ventilated open-top chambers were detected in Belgium and The Netherlands between 2000 and 2004. Ozone concentrations were higher in Continental Central Europe, with June - August AOT40s ranging 0.7 to 13.1 ppm h at ICP Vegetation sites in Austria, Germany, Poland and Switzerland. Interestingly, in this region the highest injury scores on NC-S clover were recorded in the more humid years of 2001 and 2002 rather than the drier “high ozone years” of 2003 and 2006, suggesting that ozone flux rather than concentration may be more important in determining the magnitude of ozone effect.
There were numerous records of ozone injury in Slovenia and Greece, representatives of the Eastern Mediterranean region, with farmers reporting severe value loss in salad crops due to foliar ozone injury rendering the crops un-sellable. In the clover biomass experiment, ambient ozone in Greece in 2003 reduced the biomass of NC-S clover by 30% relative to that of the resistant variety. The largest number of reports of the damaging effects of ozone were from the Western Mediterranean region where the 12h mean ozone concentration at ICP Vegetation sites in Italy and Spain was in excess of 40 ppb in each year from 1999 to 2006. Mean ozone injury scores on NC-S clover showed that over 25% of leaves were injured in this region by ozone in 1998 and 2003 with significant injury recorded in all other years. Biomass reductions in NC-S clover were frequently around 20% at many Italian sites, and were as high as 47 and 42% in 1998 and 1999 in Italy (Isola Serafini) when the AOT40 was 20.4 and 32.8 ppm h respectively.

Policy implications

From the onset, this report has set out to answer a series of questions raised by policy makers on the extent of ozone effects in areas identified by the mapping process as being at risk from ozone pollution. Here, we summarize the answers to those questions:

1. **Is there any evidence of temporal trends in ozone effects?**
   At the local scale, there was evidence of higher damage in years with higher ozone concentrations (e.g. 2003 and 2006) in regions of Europe where climatic conditions were conducive to high ozone fluxes. However, the timescale and density of data points were insufficient to allow any long-term trends related, for example, to the changing ozone profiles (lower peaks, increasing background), to be identified.
2. Is there evidence of ozone damage in areas of exceedance of the AOT40-based critical level?

Ozone effects were found in areas where the AOT40-based critical level was exceeded. AOT40 worked best as a regional-scale indicator of damage: both ozone injury score and biomass reduction were linearly related to the mean EMEP modelled AOT40 for the 50 x 50 km grid squares the ICP Vegetation sites represent ($r^2 = 0.84$ and 0.97 respectively).

3. Is there evidence of ozone damage in areas with AOT40s below the critical level?

Ozone damage was found in areas with AOT40s below the critical level. Thus, maps of exceedance of the AOT40-based critical level for agricultural crops (an AOT40 of 3 ppm h) appear to be underestimating the potential for ozone damage in Europe. For example, at the regional scale, the EMEP risk maps indicated that mean AOT40s were just below the critical level in grid squares representative of Continental Central Europe and Eastern Mediterranean and yet mean biomass reductions of greater than 10% were recorded in clover in these regions. Furthermore, the critical level for yield reduction did not protect against ozone injury, a response which in clover at least, occurs at lower AOT40s than the biomass response. When local evidence of ozone injury on crops and (semi-)natural vegetation was compared with EMEP AOT40 maps, up to one third of data points were in regions where the maps indicated that the critical level for yield reduction was not exceeded.

4. Does ozone damage occur in areas predicted by the flux-based method to be at risk from ozone effects?

The overriding concept of the generic flux maps is that they indicate risk of ozone damage wherever there is predicted to be any ozone flux to vegetation (i.e. where $AF_{st3gen} > 0$). In our analysis, ozone damage was found in grid squares predicted to have $AF_{st3gen}$ values of at least 6 mmol m$^{-2}$, with virtually all damage being found in grid squares with an $AF_{st3gen}$ of at least 12 mmol m$^{-2}$. Our analysis has shown quite clearly that there is either no or minimal impact of ozone in grid squares predicted to have an $AF_{st3gen}$ at/close to zero.

5. Is there evidence of more ozone damage in the areas with the highest fluxes?

The highest biomass reductions in NC-S white clover were found in grid squares predicted to have an $AF_{st3gen}$ of 18 or more mmol m$^{-2}$, with the highest reduction of nearly 50% being detected in the grid square having the highest predicted $AF_{st3gen}$ of those considered in this study (27.5 mmol m$^{-2}$). As with AOT40, $AF_{st3gen}$ worked best as a regional-scale indicator, with an exponential relationship between increasing $AF_{st3gen}$ and increasing effect ($r^2 = 0.96$ for injury and 0.78 for biomass reduction). Local-scale predictions were more susceptible to the uncertainties described in Chapter 5, causing some scatter in the relationship.

6. Is there evidence of ozone damage in areas with high flux, but low AOT40?

The dose metric, $AF_{st3gen}$ worked particularly well as an indicator of risk of damage in northern Europe and parts of Atlantic Central Europe. Injury was detected in these regions when $AF_{st3gen}$ values were predicted to be over ca. 12 mmol m$^{-2}$, but not when $AF_{st3gen}$ values were at/close to zero. According to the AOT40 maps, no injury or yield reduction would have been predicted at these sites because the values were too low (AOT40 was < 1 ppm h).
7. Overall, which maps (AOT40 or flux) best predicted areas with ozone damage?

For the reasons described above, maps of the generic flux to crops most accurately predicted the areas where there was evidence of ozone damage. Although AOT40 worked well at the regional scale, effects frequently occurred in areas predicted to be safe from ozone damage (i.e. areas where the AOT40 was below the critical level).

**Challenges for the future**

There is clear evidence that the ozone profiles over Europe are changing: peak concentrations are declining whilst the background ozone concentration is steadily increasing (NGETAP, 2001, Jonson et al., 2005). These changes, coupled with rising temperature, carbon dioxide and changes in precipitation mean that the AOT40-based and ozone flux-based maps for future decades are very likely to show changes in the distribution and magnitude of predicted effects of ozone across Europe (Simpson, et al., 2007). Thus, there is a need to further our knowledge of the damaging impacts of ozone on vegetation by:

- Spring and summer monitoring of ozone impacts in the field using an expanded network of sites, including sites in those areas of Europe for which little or no data currently exist.
- Developing a long time-series of data for regionally representative sites to monitor the impacts of changing ozone profiles and climate.
- Determining ozone flux-effect relationships for a wider range of crop species and for (semi-)natural vegetation ecosystems which take into account the modifying effect of global climate change.
- Incorporating consideration of effects of soil moisture deficit as a key modifying factor of stomatal conductance into risk assessment maps for crops and (semi-)natural vegetation.
- Developing the next generation of ozone risk maps which should include the modifying influence of climate change (e.g. temperature, increasing carbon dioxide concentration, changing precipitation patterns), and by collating suitable field-based data for evaluation of these new maps.
References

* indicates references that have been used in the databases created for this report.


‘Evidence of Widespread Ozone Damage to Vegetation in Europe (1990 to 2006)’ provides a summary of the adverse effects of ozone on vegetation across Europe during the period 1990 to 2006 and compares these effects with modelled ozone concentration and flux data. Although many experimental studies have demonstrated that current ozone concentrations have the potential to impact on crops and (semi-)natural vegetation, until now there has been no comprehensive assessment of ozone effects in fields of crops and natural ecosystems. This report has established that effects of ambient ozone on crops and (semi-)natural vegetation are actually occurring in the field, with leaf injury and reductions in biomass or crop yield developing under ambient ozone conditions. Compared with ozone concentration maps, maps of stomatal fluxes of ozone were found to be better at predicting the occurrence of ozone damage to vegetation. This report is for scientists, policy makers and others with an interest in ozone pollution and its effects on crops and (semi-)natural vegetation.

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