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Evidence of widespread effects of ozone on crops and (semi-)natural vegetation in Europe (1990 - 2006) in relation to AOT40 - and flux-based risk maps

Running title

Ozone effects on vegetation in Europe

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Ozone, crops, (semi-)natural vegetation, clover, ozone injury, biomass reduction, ambient air, AOT40, flux, risk assessment

Abstract

Records of effects of ambient ozone pollution on vegetation have been compiled for Europe for the years 1990 – 2006. Sources include scientific papers, conference proceedings, reports to research funders, records of confirmed ozone injury symptoms and an international biomonitoring experiment coordinated by the ICP Vegetation. The latter involved ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* L.) grown according to a common protocol and monitored for ozone injury and biomass differences in 17 European countries, from 1998 to 2006. Effects were separated into visible injury or growth/yield reduction. Of the 644 records of visible injury, 39% were for crops (27 species), 38.1 % were for (semi-)natural vegetation (95 species) and 22.9% were for shrubs (49 species). Due to inconsistencies in reporting effort from year to year it was not possible to determine geographical or temporal trends in the data. Nevertheless, this study has shown effects in ambient air in 18 European countries from Sweden in the north to Greece in the south. These effects data were superimposed on AOT40 (accumulated ozone concentrations over 40 ppb) and $POD3_{gen}$ (modelled accumulated stomatal flux over a threshold of 3 nmol m^{-2} s^{-1}) maps generated by the EMEP Eulerian model (50 km x 50 km grid) that were parameterised for a generic crop based on wheat and NC-S/NC-R white clover. Many effects were found in areas where the AOT40 (crops) was below the critical level of 3 ppm h. In contrast, the majority of effects were detected in grid squares where $POD3_{gen}$ (crops) were in the mid-high range (> 12 mmol m^{-2}). Overall, maps based on $POD3_{gen}$ provided better fit to the effects data than those based on AOT40, with the $POD3_{gen}$ model for clover fitting the clover effects data better than that for a generic crop.

Introduction

Concentrations of ozone in the troposphere have increased over recent decades (Vingarzan, 2004, Derwent *et al*., 2007), with health-based guidelines regularly exceeded across much of Europe (Meleux *et al*., 2007, EEA, 2009). In addition to health impacts (described in WHO, 2008), ozone is considered to be more damaging to vegetation than any other air pollutant (Ashmore, 2005). Experimental exposures at concentrations that can be experienced in Europe, especially in hot summers like 2003, have shown that crops and (semi-)natural vegetation could be damaged by reduced growth and seed production (e.g. Mills *et al*., 2007a, Hayes *et al*., 2007a, Booker *et al*., 2009), premature senescence (e.g.Tonneijck *et al*., 2004), reduced ability to over-winter (e.g. Hayes *et al*., 2006) and withstand stresses such as drought, (e.g. Wilkinson and Davies, 2009), and by producing visible injury symptoms such as chlorotic and bronze stippling of leaves (e.g. Manning *et al*., 2002). Earlier European surveys and biomonitoring experiments conducted during the 1980s and 1990s indicated that ambient ozone concentrations were sufficiently high to induce visible injury on over 20 crops growing in countries extending from Sweden in the north to Italy and Spain in southern Europe (Benton *et al*., 2000, Fumagalli *et al*., 2001). With northern hemispheric ozone concentrations predicted to continue to rise, at least for the next few decades (Royal Society, 2008), we undertook a study to review the evidence for effects of current ambient ozone on crops and (semi-)natural vegetation in Europe over the period 1990 – 2006, and related this evidence to maps being used by the LRTAP Convention[1] to indicate the areas of greatest risk of effects over these years. For one biomonitoring system (ozone-sensitive and –resistant white clover,

[1] Convention on Long-range Transboundary Air Pollution

described later) we also related biomass effects and visible injury to site- and year-specific modelled ozone parameters that describe both ozone concentration and ozone flux (uptake *via* the stomatal pores on the leaf surface). Thus, we provide here field-based biological validation of mapped European ozone risk assessment indices, information identified as urgently required by both Manning (2003) and Simpson *et al*. (2007).

The general increase in ozone concentrations in the Northern hemisphere in recent decades has been associated with a change in the profile of maximum seasonal and diurnal ozone concentrations. Owing to implementation of precursor emission controls, peak ozone concentrations in the USA and Western Europe have declined from 1980 to present, with the decline being less steep in recent years (Derwent *et al*., 2007, Vingarzan, 2004, Jenkin, 2008, Lefohn *et al*., 2008, Solberg *et al*., 2005). At the same time, background ozone concentrations have increased due to hemispheric transport of precursors from the developing areas of the world (NEGTAP, 2001, Dentener *et al*., 2006). Looking to the future, significant declines in background ozone in Europe will only be achievable if strict global controls on ozone precursor emissions are implemented (Royal Society, 2008, Dentener *et al*., 2006). A confounding factor in predicting future ozone climates is that higher temperatures and reduced cloudiness and precipitation as a result of climate change may increase summer peak and average ozone concentrations (Meleux *et al*., 2007). Current predictions are that by 2030, effects of ground level ozone pollution will have major implications for global food security with global yield losses due to ozone rising to 9 – 18% for wheat and 4 – 8% for rice under scenarios that allow for implementation of current legislation to control pollutant emissions (Van Dingenen *et al*., 2009). It has also recently emerged that ozone may reduce the ability of vegetation to absorb carbon dioxide resulting in even greater carbon dioxide concentrations in the atmosphere in the future, further increasing radiative forcing (Felzer *et al*., 2005, Sitch *et al*., 2007). Predictions for the future are complicated by enhanced temperature increasing ozone production over and above that associated with global increases in precursor emissions, and the ameliorating effect of increasing carbon dioxide concentration on ozone effects (by reducing stomatal uptake), see reviews by Booker *et al*., 2009, Feng and Kobayashi, 2009. Within the context of evaluating potential effects of ozone in the future, it is timely to synthesise data on the current evidence of effects of ozone in Europe on crops and (semi-)natural vegetation, and to draw attention to the urgent need to further reduce the emissions of ozone precursors on a global scale.

A recent synthesis of published dose-response function data from field-based chamber experiments revealed that the most ozone-sensitive crops include wheat, soybean, pulses and tomato, with potato, sugar beet, rape and maize being moderately sensitive (Mills *et al*., 2007a). In a similar study for (semi-)natural vegetation, Mills *et al*. (2007b), compiled published dose-response functions for individual species and proposed that grasslands (especially uplands, dry grasslands and woodland fringes), heaths and wetlands are amongst the most ozone-sensitive habitats in Europe. Although these compilations of exposure-response data provide an indication of the relative sensitivity of different species, they do not provide actual evidence that current ozone climates are damaging vegetation in the open field without any confounding influence of an exposure chamber (as described in Heagle, 1989 and Sanders *et al*., 1991). Field-release studies with ozone are relatively few, and have provided mixed results. For example, Morgan *et al*. (2006) found that yield reductions for soybean were even larger than those predicted from open-top chamber experiments whilst Bassin *et al*. (2009) found that alpine vegetation was relatively resistant to ozone despite the sensitivity of the component species.

Several previously un-collated sources of evidence of visible ozone injury in ambient air in Europe exist in the literature including surveys of crops and (semi-)natural vegetation together with *ad hoc* observations. Examples include a survey of ozone injury symptoms on vegetation in an alpine valley (Bussotti *et al*., 2003b); surveys of symptoms on crops in Belgium, France, Spain and Switzerland (Benton *et al*., 2000) and surveys conducted in countries in the Carpathian mountain range area of central Europe (Manning *et al*., 2002). Researchers have also reported the presence of visible injury symptoms on crops grown in ambient air plots included as part of open-top chamber based ozone exposure experiments (e.g. De Temmerman *et al*., 2002). These types of data are useful sources of evidence of ozone effects in the field providing (as in the examples quoted), the assessors of ozone injury are well trained, follow a clearly defined protocol and preferably, that the ozone symptoms have been confirmed using ozone exposure experiments (Bussotti *et al*., 2006, Lorenz et al., 2008, Manning and Godzik, 2004).

Further evidence of ozone effects in ambient air derives from surveys conducted by placing a sentinel bioindicator species of known sensitivity to ozone at a series of sites in a local area and assessing the plants for injury symptoms at intervals thereafter. Ozone-sensitive tobacco (*Nicotiana tabacum* L. cv BELW3) has been used in several such surveys, particularly in Southern Europe (e.g. Spain, Ribas and Penuelas, 2003 and Italy, Nali *et al*., 2006). Over the last fifteen years, the participants of the ICP

Vegetation[2] have compiled evidence of ozone effects in ambient air by conducting a series of coordinated experiments across Europe with ozone-sensitive- (NC-S) and ozone-resistant (NC-R) biotypes of a commercial cultivar of white clover (*Trifolium repens* cv Regal) originally selected in Raleigh, North Carolina (Heagle *et al*., 1994). The NC-S strain develops ozone injury symptoms following an ambient ozone episode involving ozone concentrations of 50 – 60 ppb and higher, and after prolonged exposure to ozone has reduced above-ground growth whilst the NC-R biotype only responds to ozone at substantially higher concentrations. In addition to ozone injury data, this biomonitoring system also provides evidence of ambient ozone effects of ambient concentrations on biomass – an effect of potentially greater importance than visible injury.

During the last fifteen years, the ozone-effects research community in Europe has been establishing methods for determining the critical levels for ozone, above which effects on sensitive species can be expected. The overall aim has been to develop methods for mapping the areas of Europe where vegetation is at highest risk of ozone damage. The resulting maps are used within the LRTAP Convention as part of the negotiations for reductions in emissions of the precursors of ozone from the 51 signatories to the Convention (for details, see http://www.unece.org/env/lrtap/lrtap_h1.htm). Contributing countries agree to reductions by signing Convention Protocols, the most recent being the Gothenburg Protocol (1999) to abate acidification, eutrophication and ground-level ozone which is currently under review. The negotiations concerning ozone for the Gothenburg Protocol were based on exceedance of a concentration-based long-term critical level

[2] The International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops

of ozone for crops and (semi-)natural vegetation. This value, an AOT40[3] of 3 ppm h accumulated over three months was set at the Kuopio Workshop in 1996 (Kärenlampi and Skärby, 1996) and is still considered to be the lowest AOT40 at which significant yield or biomass loss due to ozone can be detected for agricultural crops and (semi-)natural vegetation dominated by annuals, according to current knowledge (LRTAP Convention, 2004). A critical level for visible injury has also been derived based on analysis of many ozone parameters in the days preceding ozone injury development on *Trifolium subterraneum* (Pihl-Karlsson *et al.,* 2004). The "short-term" critical level is a VPD-modified AOT30 ($AOT30_{VPD}$[4]) of 0.16 ppm.h accumulated over the eight days prior to injury development (LRTAP Convention, 2004).

Recent research for the LRTAP Convention has led to a new index being developed that has a stronger biological basis than AOT40. It models the flux of ozone from the exterior of the leaf through the stomatal pores to the sites of damage ("stomatal flux" or "flux") using algorithms describing the species-specific effects of temperature, Photosynthetic Photon Flux Density (PPFD), soil water potential, vapour pressure deficit (VPD) and plant growth stage on stomatal functioning (Emberson *et al*., 2000, Pleijel *et al*., 2007). To date, flux-based critical levels have been derived for wheat, potato and provisionally for beech and birch, and flux-based risk assessment methods have been developed for a generic crop and generic tree species for use in large-scale integrated assessment modelling (LRTAP Convention, 2004). The latter are simplified full flux models that do not take into account genetic variability in sensitivity and use only temperature, PPFD and VPD as factors modifying stomatal

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

[4] The sum of the differences between the hourly mean ozone concentration (in ppb) modified by VPD (using the function described in LRTAP Convention, 2004) and 30 ppb when the concentration exceeds 30 ppb during daylight hours.

conductance with the notation, $PODY_{gen}$ [5] and units mmol m^{-2}, where Y = 3 for the generic crop flux model used in this study. The threshold Y is similar in concept to "40" in AOT40 and represents an amount of ozone flux that can be tolerated (or detoxified) by the plant before negative effects begin to occur. The $POD3_{gen}$ (crop) model is based on the parameterisation for wheat described in Pleijel *et al.* (2007). Unfortunately, there is no generally-accepted $POD3_{gen}$ for clover yet, and in any case the environmental and pollution-exposure conditions of potted plants within the ICP programme differ from those that would apply to homogeneous canopies of clover, but we use here available knowledge to derive a flux estimate for this network. This new index, $POD3_{gen}$(Clover) is used alongside the more standard AOT40 and $POD3_{gen}$ (Crop) functions in this paper.

Risk maps produced using AOT40 and generic flux (to crops or trees) provide very different spatial patterns of ozone impacts (Simpson *et al.*, 2007, Karlsson *et al.,* 2009). For both metrics, ozone impacts are predicted for southern Europe where ozone concentrations are highest. However, risk maps based on generic flux to crops indicate effects in central and north-west Europe where lower ozone concentrations and AOT40 values below the critical level occur in climatic conditions (moderate temperatures, moist climates) that are conducive to relatively high ozone flux. In the current study, we superimpose maps of ozone effects detected in the field onto AOT40- and $POD3_{gen}$ flux-based risk maps (for crops and NCS/NCR white clover) to determine whether the more biologically meaningful flux approach is a better predictor of vegetation damage than AOT40. Ideally ozone risk maps should be generated from measurements of AOT40 and flux, however, measured ozone

[5] Accumulated flux above a flux threshold of Y nmol m^{-2} s^{-1}, accumulated over a defined time period during daylight hours, using the generic flux model. Note, this index was formerly known as $AF_{st}Y$.

concentrations are only available from ca. 130 sites in Europe (reported in EMEP/CCC reports available at www.emep.int such as Fjæraa and Hjellbrekke, 2008), and spatial distribution of measurements is not uniform with many parts of eastern and southern Europe poorly covered. For ozone fluxes, hardly any measured data exist, with just a few research sites providing values (e.g. Tuovinen *et al*., 2004, 2007). Thus, assessment of risk of effects to vegetation is dependant on modelling procedures that can provide a geographically broad-scale prediction of both the AOT40 and the $POD3_{gen}$ metrics. Within Europe, the model of the European Evaluation and Monitoring Programme (EMEP) plays a key role in developing air pollution control strategies for both the LRTAP Convention and the European Union (see Simpson *et al*., 2003 for details). The model results for AOT40 have been compared with observations in, for example, Fowler *et al*. (1999) and Simpson *et al*. (2003), and in general fair agreement ($R>0.7$) was obtained. Far fewer data are available to evaluate the model's flux predictions, but studies by, for example, Tuovinen *et al*. (2004) and Klingberg *et al*. (2008), provide support for the basic formulation and deposition rates. The uncertainties associated with the EMEP model are considered further in the Discussion.

In conducting this study, we set out to answer the following questions: How widespread is the occurrence of ozone injury and ozone-induced biomass reduction in Europe and which species of crops, (semi-)natural vegetation and shrubs are affected? Is there any evidence of temporal or spatial trends in ozone effects as a result of changing ozone profiles? How well do AOT40-based and flux-based maps predict the areas where ozone injury and/or biomass reductions have been detected in ambient air? This paper provides an overview of analysis of ozone effects data for the period

1990 – 2006; further details including the regional distributions of effects can be found in a report by Hayes *et al*. (2007b).

Materials and methods

Sources of ozone–effects data

Data were sourced from scientific papers and conference proceedings published up to 2007 (see Tables 1 - 3), national-scale reports to funders of research (listed in Tables 1-3 as unpublished), the ICP Vegetation database for the clover experiment (described below) and specialists that have published papers on ozone effects that had noted confirmed ozone injury symptoms in the field (listed in Tables 1 - 3 as unpublished). Each record of an effect was entered into a database together with the species name, location of species (pot, experimental plot, commercial field, natural vegetation), date of observation, nature and extent of effect (injury, biomass or yield), local ozone and climate data if available, grid reference and data source. Only data with a known grid reference were included in the database.

Records of observations of visible-injury symptoms in ambient air

Records of visible injury symptoms attributed to ozone included data observed on grasses, forbs, shrubs and crop species growing in experimental pots or plots, natural vegetation communities and commercial crops from surveys conducted during spring and summer months over the period 1990 - 2006. The database includes records of visible injury from biomonitoring experiments conducted for the ICP Vegetation using nine species from 1994 - 1996 (see Benton *et al*., 2000 for details), and white clover conducted from 1996 – 2006 (see below for details). There are also records included from both unpublished data and *ad-hoc* observations supplied by ozone-specialists that participate in the ICP Vegetation. Overall, this part of the database contained 644 records, of which 70.6% were published in the scientific literature and 29.3% were unpublished data.

With such a diverse dataset, there was a need to apply quality assurance procedures to ensure the data were comparable. Records of the highest quality were supplied by scientists that have collected seeds or cuttings from species showing injury in the field, grown the species and exposed it to ozone experimentally to confirm the symptoms. Since such records only accounted for ca. 6% of the data available, records were also included if collected by scientists who research and publish papers on ozone pollution effects and therefore are familiar with 'typical' ozone injury symptoms and could confirm that the symptoms seen matched those published following ozone exposure of the same or related species. No symptoms described as 'possibly' due to ozone were included in the analysis presented here. Assessments of the amount of injury per leaf are subject to variation between researchers with underestimates of damage commonly occurring (e.g. Bussotti *et al*., 2006). To avoid such confounding factors when comparing across survey types, ozone leaf injury data were only included if the presence, rather than the extent, of symptoms was recorded.

Evidence of ozone effects from ICP Vegetation biomonitoring studies with white clover

The ICP Vegetation biomonitoring programme has involved exposure of an ozone sensitive (NC-S) biotype of white clover (*Trifolium repens* L. cv. Regal) to ambient air since 1996. Cuttings of clover were sent by the Programme Coordination Centre at the Centre for Ecology and Hydrology, Bangor (UK) to participants across Europe, who established the plants according to a standard protocol (see Mills *et al*., 2000 for details). Twenty-eight days after establishing the cuttings, 10 to 25 plants of the NC-S clover were placed in 15 l pots and exposed to ambient air for four to six months at each site. 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 stipples on the leaf surface to large necrotic patches with leaves severely damaged. For comparison across sites, ozone injury was scored as the percentage of leaves visibly damaged by ozone using the following key: 1 = <1% of leaves affected, 2 = 1-5%, 3 = 5-25%, 4 = 25-50%, 5 = 50-90% and 6 = 90-100%. At some sites, ozone-resistant plants (NC-R) were also grown according to the same protocol; the ratio of the biomass of NC-S to NC-R (accumulated over harvests 2, 3 and 4 representing three months of growth) provided an indication of ambient ozone effects on growth at these sites. For those sites visited daily, the date of first appearance of ozone injury symptoms, defined as the date when 20% of the plants have one or more injured leaves, was recorded. All data were checked for quality assurance prior to inclusion in the dataset as described in Mills *et al*. (2000).

Scored injury data were available from a total of 45 sites, representing 16 countries across Europe from 1998 to 2006 and biomass ratio data were available from 1996 to

2006 for a total of 41 sites from 15 countries (Table 4). However, each individual site did not necessarily perform the investigation every year meaning there were very few sites with a long time-run of data.

Ozone risk maps

The EMEP Eulerian model maps ozone concentrations and fluxes on an (approx.) 50 km x 50 km grid. Described in detail by Simpson *et al*. (2003), 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 module (DO_3SE, described in Emberson *et al*., 2000, Simpson *et al*., 2003, 2007 and references therein). For use in this study, the EMEP model generated AOT40 values for crops (termed here AOT40 (crop)) and generic crop flux ($POD3_{gen}$ (crop)) maps using the methods described in the Modelling and Mapping Manual of the LRTAP Convention (LRTAP Convention, 2004) for the years 1995 through to 2004, inclusive. Parameterisation of the generic crop flux model is reproduced in Table 5. The accumulation period for AOT40 (crop) and $POD3_{gen}$ (crop) was three months, with the timing of the accumulation window reflecting the period of active growth of wheat and centred on anthesis (LRTAP Convention, 2004). This approach provided a moving time interval to reflect the early growing seasons in southern Europe and later growing seasons in northern Europe. The classification scales for the EMEP risk maps presented here have been chosen to provide a fair comparison between AOT40 and $POD3_{gen}$ maps. Each map has six categories for values exceeding zero, plus a zero category, with the maximum whole number value recorded in any of the years included being divided by six to provide the six evenly spaced categories.

For this study we have also developed a clover flux model that is specific to NC-S/NC-R clover ($POD3_{gen}$ (clover)) as used at the ICP biomonitoring network. The basic formulation is similar to that of other vegetation but uncertainties are introduced in calculating the ozone concentration at the canopy height because the clover plants in the ICP Vegetation network were in pots surrounded by short vegetation (grass species) – a complex situation for modelling. Thus, here we have assumed that the dominant vegetation is grassland rather than clover itself, with the ozone concentration gradients around the plants being driven more by the surroundings than by the characteristics of clover. On the other hand, the stomatal conductance of the clover itself still drives the uptake of O_3 into the plant. In an effort to account for this complex situation, we calculate the stomatal conductance of clover with clover-specific parameters, but make use of the O_3 gradients calculated for grassland to calculate the O_3 at canopy top. The parameterisations for this model are provided in Table 5, with the derivation and equations described in the Annex to this paper. Clover $POD3_{gen}$ and clover AOT40 maps have been generated for values accumulated over 84 days starting 15 June (the mean start date for ICP Vegetation clover experiments) to represent the three 28d growth periods. Grid square values for these maps are the average of five years data for 2000 – 2004.

Comparison of ozone-effects data with EMEP modelled AOT40 and $POD3_{gen}$

Four sets of data were compared with EMEP modelled AOT40 and $POD3_{gen}$ maps and/or grid square values. Firstly, the 50 x 50 km grid squares where visible injury had been detected in published surveys and *ad hoc* observations and the ICP Vegetation biomonitoring experiments described here during the period 1995 -2004 were mapped with the ten year mean values for crop AOT40 and crop $POD3_{gen}$. For grid squares where an effect was detected in more than one year in the five-year periods, the presence of effect was only counted once. The second dataset was the mean injury score on white clover for the period June to August on NC-S white clover over the five-year period 2000 – 2004. This data were mapped against AOT40 (clover), AOT40 (crop), $POD3_{gen}$ (clover) and $POD3_{gen}$ (crop) for illustration but could not be used to produce dose-response functions due to the non-linearity in the injury score used in the plant-level assessments. The third dataset was for a sub-set of the clover biomonitoring sites where the first day of ozone injury was recorded. Clover AOT40, $AO30_{VPD}$ and $POD3_{gen}$ were calculated for the days from the start of the experiment and the eight days prior to ozone injury for use in an assessment of the short-term critical level for visible injury. Lastly, the fourth dataset used was the white clover biomass ratio (NC-S/NC-R) calculated from the total biomass for the months May, June and July, normalised for the 1.05 ratio recorded at zero AOT40 (Hayes *et al*., 2007b) and converted to percentage biomass reduction. The five-year mean values per site were mapped against the five-year mean crop and clover AOT40 and $POD3_{gen}$ (2000 – 2004). In addition, the site- and year-specific values for clover AOT40 (clover) and $POD3_{gen}$ (clover) using the site starting date were accumulated over 84 days and plotted against biomass ratio.

Statistical analysis

The datasets described were deemed too inconsistent for analysis of temporal and spatial trends as the number of sites surveyed for injury or included in the clover biomonitoring experiment varied from year to year and were not systematically selected for geographical representation. Response functions were fitted to the biomass data by non-linear regression within Minitab V.15. Where applicable, data are presented visually for four regions of Europe Northern Europe (NE), Atlantic Central Europe (ACE), Continental Central Europe (CCE), Mediterranean (Med), with countries included in each region as listed in LRTAP Convention (2004). In Tables 1-3, the Mediterranean region is split into eastern and western Mediterranean.

Results

Visible injury surveys, including injury occurrence on experimental ambient air plots

Over the years 1990 – 2006, ozone injury was detected in 16 countries of Europe representing each of the five geographical regions studied (Tables 1 - 3 and Figures 1-3). Records of injury were particularly common from Italy, Spain, and Switzerland, but were also common for more northern countries such as Belgium and Sweden. Overall, ozone injury was most commonly reported in central and Mediterranean Europe with more than 200 published records available for each of these regions, with fewer records reported in ACE and NE. There were large year-to-year differences in the number of published and unpublished records reported across Europe (Figure 2). Since this inconsistency may well have reflected the sporadic nature of surveys rather than fluctuations in ozone climate, it was not possible to statistically analyse this dataset for any geographical or temporal trends. Instead, the focus of the analysis of the visible injury dataset has been the overall geographical spread of sites where injury occurred together with the range of species injured in each region, based on the combined records for the period 1990 - 2006.

Of the 644 records of visible injury over the period 1990 – 2006, 39% were for crops, 38.1 % were for forbs and grasses, and 22.9% were for shrubs. Overall, 27 crop species exhibited visible injury including agricultural crops such as maize, potato, wheat, durum wheat and soybean, and horticultural crops such as lettuce, chicory, radish, courgette and onion (Table 1). These effects were detected in 14 countries representing each of the five geographical regions. Ten or more crops were injured in Greece, Italy and Spain and it is also of note that ozone injury was detected on six crop species in Sweden (NE).

Ninety-five species of grasses and forbs exhibited typical ozone injury in ambient air at sites across Europe (Table 2). Overall, the vast majority of the species injured were forbs, with injury being more difficult to identify on grasses and only reported for 8 species. Species from the same genus were injured at sites in several countries. For example, *Centaurea jacea* was injured in Poland, Switzerland and Italy, *Centaurea nigra* was injured in Italy, UK and Ukraine, *Centaurea paniculata* was injured in Switzerland and *Centaurea scabiosa* was injured in France and the Ukraine. In addition to the *Trifolium* spp. used in the ICP Vegetation biomonitoring programme, other examples of genus' that were well represented within the database were *Rubus* spp, with injury recorded on five species growing in France, Switzerland, Italy and Spain and *Epilobium* spp. with three species injured in France, Spain and Italy.

Ozone injury was reported for 49 species of shrubs growing in France, Italy, Poland, Spain and Switzerland (Table 3). Records for *Viburnum* spp. were the most widespread with injury occurring on four species at sites in France, Italy, Spain and Switzerland. *Rosa canina* was injured in three countries (Italy, Spain and Switzerland) whilst many other species were injured in two countries such as *Lonicera caprifolium*, *Robinia pseudoacacia* and *Sambucus racemosa*.

The geographical distribution of locations in Europe where visible injury was detected and published in the scientific literature over the period 1995 – 2004 is shown in Figure 3 superimposed on the 10 year average for AOT40 (crop) and $POD3_{gen}$ (crop). There was a clear north-south increase for AOT40 (crop), with the highest modelled values being found in Italy, whilst $POD3_{gen}$ (crop) was > 18 mmol m^{-2} across a large region of Europe covering central and southern areas and spreading northwards into southern UK and Scandinavia. Overall, 62% of injury locations were in grid squares with AOT40 (crop) values below the critical level of 3 ppm h to protect crops against effects on biomass and yield suggesting that some effects (even if not on biomass and yield) can occur well below this value (Figure 3). No obvious threshold value was apparent but most grid squares (29%) were in the category 2 - 3 ppm h for AOT40 (crop). In contrast, only 9% of the injury locations were found within grid squares with an $POD3_{gen}$ (crop) of < 12 mmol m^{-2}, with 7%, 27% and 47.3% of locations falling in grid square categories 12 - 18, 18 – 24 and 24 – 30 mmol m^{-2} respectively. The proportion of grid squares with injury falling within the three highest categories was 22% for AOT40 (crop) and 56% for $POD3_{gen}$ (crop). From Figure 3b, a $POD3_{gen}$ (crop) of 12-18 mmol m^{-2} can be tentatively interpreted as a threshold for likely occurrence of ozone injury. Unfortunately, published records of the absence of ozone symptoms are extremely rare making it impossible from this evidence to confirm this threshold.

Visible injury occurrence in the ICP Vegetation white clover biomonitoring experiments.

Ozone injury occurred at almost all sites in the years included in this study (1998 – 2004), with only 5 of the 52 site/year combinations recording an absence of injury. A sub-set of sites (28 data points) recorded the first date of ozone injury on NC-S white clover. For the 8 days prior to injury appearance or for all days since day 0 of the experiment, the EMEP modelled AOT40 (clover) provided little evidence for a threshold AOT40 value (Figure 4a). For both time periods, the cumulative frequency of percentage of data points increased rapidly with increasing AOT40 (clover), with 42.9% and 35.7% of data being for AOT40s below 0.1 ppm h (for 8 days and day 0 to first day of injury, respectively). A similar pattern existed for $AOT30_{VPD}$ (clover) (Figure 4b) with 4 sites recording ozone injury at grid square $AOT30_{VPD}$ of below the critical level of 0.16 ppm h (Italy-Isola Serafini 1999, Italy-Rome 2004, Belgium-Tervuren 2004, UK-Ascot 2004). In contrast, there was stronger evidence of a threshold for $POD3_{gen}$ (clover) of ca. 3-4 mmol m^{-2} with 3.5% of data points falling below 2 mmol m^{-2}, 7.2% falling between 2 and 3 mmol m^{-2}, and 30.2% falling between 3 and 4 mmol m^{-2} for $POD3_{gen}$ (clover) values accumulated for 8 days prior to injury (Figure 4c).

The five-year mean injury score values were superimposed on crop and clover AOT40 and $POD3_{gen}$ maps to illustrate the geographical distribution (Figure 5). The AOT40 maps for the two time periods showed similar patterns but AOT40 (clover) values were larger than AOT40 (crop) values. Site-specific AOT40 (clover) values, determined for 84 days from 15 June were higher than AOT40 (crop) values (clover AOT40 (clover) = 1.72 * AOT40 (crop), $r^2 = 0.75$, figure not presented) reflecting the higher ozone concentrations in the later months of the clover experiment (mean start dates were 33, 35 and 54 days later for ACE, CCE and Med respectively). $POD3_{gen}$

(clover) values were also higher than $POD3_{gen}$ (crop) values with the region of medium - high fluxes (> 24 mmol m^{-2}) covering larger areas of Europe stretching as far north as southern UK and southern Scandinavia. Overall, the highest injury scores on white clover were detected in central and southern Europe, but not all of these sites coincided with the highest mean crop or clover AOT40 values (Figure 5). In contrast, the injury score data showed a closer correlation with $POD3_{gen}$ with the highest injury scores being associated with medium to high fluxes. For example, all sites with a mean score >1 had an $POD3_{gen}$ (clover) of > 36 mmol m^{-2}. The corresponding value for AOT40 was an AOT40 (clover) of 1 ppm h.

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Effects on biomass in the ICP Vegetation white clover biomonitoring experiment

Data from 10 countries contributed to this analysis, with the mean normalised % biomass reduction being greatest in Italy (33.3 %, n=10) and Greece (30%, n= 2), with no effects being consistently detected in the UK (Table 4). For other countries there was a wide range in % biomass reduction reflecting year to year variation in AOT40 (clover) and $POD3_{gen}$ (clover), with maximum reductions being as high as 25.2 %, 20.8%, and 24.8 % for Austria, Germany and Spain respectively. The mean biomass reduction per site is shown in Figure 6 for crop and clover AOT40 and $POD3_{gen}$ averaged over the period 2000 – 04. For both AOT40 (crop) and AOT40 (clover), mean biomass reductions of > 10% were detected in areas where the modelled AOT40 was below the critical level of 3 ppm h (Figure 6). By comparison, such higher mean effects were found in the grid squares with medium – high fluxes.

Site- and year-specific grid values for AOT40 (clover) and AOT40 (crop) were calculated from the EMEP model outputs and plotted against percentage biomass reduction (Figure 7). Not surprisingly there was a lot of scatter in this data (discussed later). Separate plots of the data for sites with modelled AOT40 values that exceeded the critical level of 3 ppm h revealed linear relationships that were significant for AOT40(clover) ($r^2 = 0.58$, $p < 0.001$) where data from CCE and MED contributed, but not for AOT40 (crop) ($r^2=0.28$, $p=0.179$) where only data from MED had an AOT40 of > 3 ppm h. However, the relationship for AOT40 (clover) seems to be being driven by the large number of points with an AOT40 of 3 - 4 ppm h, with relatively few points for the higher AOT40 values. A similar approach was used for $POD3_{gen}$. For both Figure 8a and Figure 8b there is some indication of a threshold value, above which ozone effects start to occur consistently. For $POD3_{gen}$ (clover), this threshold is ca. 40 mmol m^{-2}; a separate plot of the sub-set of data for $POD3_{gen} > 40$ shows a strong linear relationship ($r^2 = 0.58$, $p < 0.001$), with data points from ACE and CCE well spread along the regression line. The relationship between $POD3_{gen}$ (crops) and clover biomass reduction was less strong for data points where $POD3_{gen}$ (crops) > 15 mmol m^{-2} especially for the higher fluxes, but was significant ($r^2= 0.21$, $p=0.002$).

For the 57 data points within the clover biomass dataset, the grid square values were compared for AOT40 (clover) and $POD3_{gen}$ (clover) (Figure 9). There was a strong polynomial relationship between the two parameters ($p = 0.93$) which could be broken down into region-specific linear relationships. At low AOT40s, $POD3_{gen}$ was higher for CCE than for ACE presumably indicating climatic conditions that were more conducive to ozone uptake. Between AOT40s of 0 and 3 ppm h, $POD3_{gen}$ for ACE increased more slowly for CCE and MED than for ACE; this slower rate of accumulation of $POD3_{gen}$ per unit AOT40 in CCE and MED continued at the higher AOT40s not found in ACE.

Discussion

This study has clearly indicated that many crops and (semi-)natural vegetation communities are responding to current ambient ozone in Europe. The most easily recognisable and most commonly reported effect has been the development of characteristic ozone injury on leaves. Such effects have been noted every year over the period 1990 – 2006, with over 170 species being reported as having developed ozone injury. Injury was reported in each of the five geographical regions of Europe, including in northern Europe where maps indicate relatively low AOT40. There is also evidence from the ICP Vegetation clover experiment that ambient ozone concentrations are sufficiently high at several locations in Europe to reduce the growth of an ozone-sensitive species. Injury and biomass effects were most prevalent in southern European countries, but were also found in central and northern Europe. The results presented here provide significant progress towards identifying field evidence of the improved performance of flux-based compared to concentration-based risk maps and provide some justification for the biologists' preference for flux-based approaches (as outlined by Simpson *et al.,* 2007). They also highlight the potential threat to vegetation from future increases in ozone pollution predicted for the near decades.

The ozone effects data presented here have been subjected to quality assurance procedures in order to reduce uncertainty. The highest potential source of uncertainty was in the field observations whereby scientists may have wrongly assigned visible injury caused by other stresses to ozone pollution. This was minimised by inclusion of observations that were either verified by ozone exposure experiments or recorded by experienced ozone-specialist scientists, the latter being the most consistent of 25 observers in a quality assurance trial described by Bussotti *et al*. (2006). Injury data from the ICP Vegetation clover experiments were more robust as a common protocol was followed using plant material originating from the same source. Photographs were provided to guide assessments and the use of a broad range of injury scores rather than % injury helped to prevent the problems of under-estimation of damage described by Bussotti *et al*. (2003a). The NC-S and NC-R biotypes were originally selected in the southern-Europe-like climate of North Carolina, USA (Heagle *et al.*, 1994), but have been extensively employed in many parts of the USA as bioindicators. In the current study, rigorous quality assurance checks (described in Mills *et al*., 2000) resulted in exclusion of about one-quarter of the data, including some from northern Europe where the NC-R biotype was sensitive to downy mildew in wetter summers.

Much of the evidence presented here is based on the occurrence of ozone injury on the leaves of sensitive species. Several authors have argued that visible injury is sometimes of little biological significance to the plant in that growth or seed production are not always reduced by ozone when injury symptoms are present (e.g. review by Bassin *et al.*, 2007). We tested this argument by comparing lists of species injured by ozone in ambient air with response functions we derived in earlier studies for yield and biomass effects that were based on ozone-exposure experiments (Hayes *et al.*, 2007a, Mills *et al.*, 2007a). Of the nine crop species with an AOT40 critical level of <= 5 ppm h (Mills *et al*., 2007a), all except cotton and turnip exhibited ozone injury in the surveys reported here. Similarly, 5 of the 8 species classified by Mills *et al*. (2007a) as moderately sensitive (maize, sugar beet, potato, tobacco and grapevine) were identified as showing ozone injury in the field whilst no visible effects were reported for the crops classified by Mills *et al*. (2007a) as resistant to ozone. Of the species of (semi-)natural vegetation exhibiting injury, 9 were reported by Hayes *et al.* (2007a) as having a relative sensitivity based on biomass effects of <0.9, and 6 had a relative sensitivity of 0.9 – 1. At the time of the Hayes *et al*. (2007a) study, there was no/insufficient biomass response data available with which to classify the other species that are reported here as developing ozone injury in the field. Although data from injury surveys cannot be directly compared with yield or biomass response data, there is thus clear evidence that those species known to respond negatively to ozone in experiments by either reduced growth or reduced seed production often develop ozone injury in the field in response to ambient ozone, whilst those known to be ozone insensitive have not been reported as showing such symptoms.

Before considering the link between measured effects and modelled ozone exposure it is important to consider the uncertainty associated with mapping AOT40 and $POD3_{gen}$ within the EMEP model. Both of these indices are sensitive to the characteristics of the frequency distribution of ozone concentrations (Tuovinen *et al*., 2007, 2009) with both showing increased sensitivity with increasing threshold. However, as lower ozone concentrations contribute more to $POD3_{gen}$ than to AOT40 (ca. 7 ppb for the $POD3_{gen}$ (clover) model included here), this parameter is less sensitive to threshold effects than AOT40 (LRTAP Convention, 2004, Tuovinen *et al*., 2007). Additional sources of uncertainty associated with the simulation of the emissions, transport and deposition of ozone and its precursors are described in Simpson *et al.* (2003a, 2003b, 2007). Because of the disparity between both the AOT40 (crop) and $POD3_{gen}$ (crop) accumulation periods in relation to the timing of the ICP Vegetation experiments (which usually start in June, 30 – 55 days after the latitude-derived $POD3_{gen}$ (crop) accumulation period starts, for further details, see Hayes *et al*., (2007b)), and the physical nature of the ICP network (potted plants surrounded by short vegetation), we devised for this study a generic clover flux model for the pot-based clover biomonitoring system (Annex 1). The higher g_{max}, lower f_{min} and higher T_{max} for $POD3_{gen}$ (clover) compared to $POD3_{gen}$ (crop), together with the higher ozone concentrations and warmer climate for the mid-June to mid-August period result in higher values for $POD3_{gen}$ (clover) than $POD3_{gen}$ (clover) for the same geographical region. A major uncertainty in the clover model is the calculation of canopy-height ozone concentration. This uncertainty is caused by a number of factors including the dense canopy and high stomatal conductance of the potted clover in relation to the surrounding grassland, and the experimental design with pots usually placed on grass and being well-spaced out, resulting in an heterogeneous surface roughness that is difficult to model. The method for calculating ozone concentration provided here for the clover model was thus a first attempt to account for such factors. It provides a consistent methodology across all sites, but it is impossible to assess any biases associated with the difficulty of modelling ozone concentration. In addition, the clover stomatal conductance parameterisation was generalized to provide one parameterisation for the 8 countries contributing data and for the two biotypes (NC-S and NC-R). This introduces some uncertainty into the analysis as within the gs datasets there was some evidence that plants showed variable acclimatisation to local conditions (e.g. in cooler climates the stomates show a tendency for closure at lower VPDs than in warmer climates, Mills *et al*., 2003). Unfortunately, the limited range of key environmental variables measured at individual sites did not allow for climate specific parameterisations to be developed; a generic "pan-European" flux modelling approach was selected enabling direct comparison with the crop maps shown.

At the farm scale, injury-causing ozone episodes can have a catastrophic effect, for example, one farmer in Greece lost a chicory crop worth Euro 15000 as a result of one ozone episode (Velissariou, *pers. comm.* and described in more detail in Hayes *et al.*, 2007b). Thus, there is a need to develop a method for assessing the risk of such catastrophic effects on a pan-European scale. Although the number of incidences of ozone injury per area strongly reflects the reporting effort, this study has nevertheless shown that ambient ozone over the period 1990 – 2006 induced visible injury on ozone sensitive species in many parts of Europe, including in northern Europe where ozone concentrations are generally lower than in central and southern Europe. Injury was detected in areas where there is a long history of relatively high ozone concentrations and some resistance to ozone has been detected in the field (e.g. *Centaurea jacea*, Bassin et al., 2004, *Plantago major*, Reiling and Davison, 1992). To fully understand the thresholds above which ozone injury occurs, more data is needed on the conditions that do not lead to ozone injury. In the absence of such data, we have analysed the data from the ICP Vegetation clover biomonitoring experiment by, for the first time, applying the EMEP model to short-time periods to determine whether exceedance of the modelled $AOT30_{VPD}$ critical level was associated with occurrence of visible injury. For 24 of the 28 data points this was found to be the case. However, a threshold was more evident for $POD3_{gen}$ (clover), with a rapid increase in incidences of ozone injury for values between 2 and 3 mmol m^{-2} for the 8 days prior to injury and from day 0 to injury. Using either index, the EMEP model could be used to predict for current and future ozone conditions the frequency of injury-causing episodes. Such maps and data could be used to assess the risk of damage to leafy vegetable crops, the economic value of which depends on the appearance of the leaf.

We tested here the efficacy of the AOT40-based critical level for crops using the clover biomass data and the EMEP modelled data for AOT40. Whether AOT40 was calculated for the crop growth period or the clover growth period, ca. 40% of sites had a > 10% biomass reduction in NC-S at modelled AOT40 values below the critical level of 3 ppm h. For both accumulation periods, a relatively small number of points for higher effects at high AOT40 values led to a significant linear relationship between AOT40 and % biomass reduction above the current critical level. Furthermore, there was widespread occurrence of visible injury in grid squares that were below the critical level for yield reduction. Although, as already discussed, the latter cannot necessarily be equated with an effect of biological significance to the plant, when taken with the biomass reduction data such widespread occurrence does tend to suggest that the AOT40-based critical level is insufficiently robust for predicting the damaging effects of ozone on vegetation in Europe. This approach to mapping risk could possibly be improved by (1) using a different cut-off value instead of 40 ppb as correlations between ozone injury and locally measured ozone concentrations were improved in both Spain and Sweden when 20 and 30 ppb were used as the cut-off values (Ribas and Penuelas, 2003, Pihl Karlsson *et al.*, 2004); and (2) using species-specific time intervals that better captured the time of damaging ozone episodes as tested here for the AOT40 (clover) model.

In contrast to the AOT40 maps, the risk maps based on the flux parameter, $POD3_{gen}$, provided a better representation of the areas where ozone effects occurred for all four types of effect data. Flux-effect relationships were improved when a species-specific model ($POD3_{gen}$ (clover)) was used. These important results are supported by locally parameterised flux models for sites in, for example, Spain (Filella *et al.,* 2005) and Italy (Fagnano and Merola, 2007). They are also supported by analyses of data from early ICP Vegetation experiments that identified the importance of locally measured conductance modifying factors such as VPD and rainfall as contributory factors for the response to ozone (Benton *et al*., 2000; Ball *et al*., 2000). Further support for the improved performance of $POD3_{gen}$ maps compared to AOT40 maps comes from the many locations in Germany, The Netherlands and Sweden where ozone effects were noted in grid squares predicted to have relatively low AOT40 (crop) (< 3 ppm h) but mid-range $POD3_{gen}$ (crop) (18 - 30 mmol m^{-2}). This paper presents some evidence to support critical levels for $POD3_{gen}$ (clover) of ca. 40 - 50 mmol m^{-2} for biomass effects and ca. 3 mmol m^{-2} for appearance of visible injury, but these would need to be tested further with site-specific measured data. Since data from ACE, CCE and MED are contributing to the response function shown in Figure (9d), it can be concluded that it is appropriate to combine data sets from the different regions of Europe for the derivation of a flux-based critical level for Europe-wide application.

Conclusion

Our study has provided evidence that ambient concentrations of the pollutant ozone have repeatedly induced damage to vegetation across 17 European countries during the period 1990 to 2006. Species exhibiting visible injury in the field match those identified in exposure experiments to be sufficiently sensitive to ozone to have reduced biomass or yield at concentrations within the European range of ozone concentrations. Biomass reductions have been found in ambient air for the sentinel bioindicator species, *Trifolium repens*. Unfortunately, data records compiled for this study were too inconsistent for identification of long-term trends in effects in response to the changing ozone profile. Overall, flux-based risk maps were better predictors of the areas where ozone damage occurred than AOT40–based risk maps, with predictive ability improving for time-period accumulation and effect data matched maps. In many areas of Europe (e.g. Belgium, northern Germany, southern Sweden) there is evidence of effects where AOT40 values (crop and clover) are predicted to be low, whilst ozone flux (crop and clover) is moderate to high due to the climatic conditions being conducive to high stomatal uptake. Thus, this study provides important validation data to provide support to the use of the biologically more meaningful flux-based approach for risk assessment.

The most disconcerting outcome of this study is that the current ambient ozone climate of Europe is already having extensive impacts on vegetation across Europe. Since ozone damage has also been detected in other parts of the world including the USA (e.g. Bennett *et al*., 2006, Davis & Orendovici, 2006, Booker *et al*., 2009) and south-east Asia (Emberson *et al*., 2009), the problem appears to be global. Even with implementation of current legislation, ozone concentrations are predicted to continue to rise across most of the world over the coming decades (Royal Society, 2008) and thus it is likely that ozone impacts on vegetation will worsen. Indeed, the Royal Society report (2008) predicted that by 2030, tropospheric ozone pollution could pose as big a threat to global food security as climate change. Thus, there is an urgent global need for coordinated effort to reduce the emissions of the precursors of ozone pollution to benefit security of food supplies, improve human health and help reduce global warming.

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Annex. The estimation of ozone flux to the ICP clover biomonitors.

The procedures specified in the Modelling and Mapping Manual (LRTAP Convention, 2004) for calculating PODY (described as AFstY in LRTAP Convention, 2004) or indeed AOTX, and implemented in the EMEP chemical transport model (Simpson *et al.*, 2003), have been designed on the assumption that the vegetation in question provides a homogeneous canopy (`big-leaf'), so that vertical O_3 gradients above the canopy can be derived with standard turbulence profiles (Tuovinen et al., 2009).

The ICP Vegetation biomonitoring programme consists of potted clover plants, sitting above a short vegetation (or bare-soil) surface, surrounded usually by grassland or other low vegetation. Such a situation is theoretically difficult to model, and does not conform to the assumptions of the Modelling and Mapping Manual methods. The O_3 concentration impinging on the upper leaves of these clover plants will be affected more by the characteristics of the surrounding vegetation than of the clover plants themselves. To generate a flux-estimate for potted clover (F_{st}(clover)) which accounts for this situation, we calculate

$$F_{st}(\text{clover}) = O_3(\text{grass}) \,.\, g_{sto}(\text{clover})$$

where g_{sto}(clover) is the effective stomatal conductance of clover found by applying the DO_3SE model, using the NC-S/NC-R parameterisation derived as described below and presented in Table 5. O_3(grass) is the O_3 concentration found at the top of a grass canopy, estimated using the grassland parameterisation from the standard EMEP model (Simpson *et al.*, 2003). The effective stomatal conductance represents that fraction of the O_3 flux entering via the stomata, as opposed to that lost through deposition to the external leaf surface. The relation between these terms is discussed in Tuovinen et al., (2009).

The g_{sto}(clover) model uses the following formulation based on the stomatal conductance algorithm described in the Modelling and Mapping Manual, see LRTAP Convention (2004) for further details.

$$g_{sto}(\text{clover}) = g_{max} * f_{phen} * f_{light} * \max\{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$$

The g_{sto}(clover) model is parameterized to represent a hybrid NC-S/NC-R clover plant based on a dataset described in Mills et al. (2003) that includes over 5000 stomatal conductance measurements made at nine sites in Europe from 1998 – 2000 (Austria-Seibersdorf, Belgium-Tervuren, Germany-Essen, Germany-Trier, Italy-Milan, Italy-Rome, Spain-Ebro Delta, Sweden-Gothenburg, UK-Bangor). Data was supplied by G Mills, P Büker, F Hayes, W Werner, B Gimeno, I Fumagalli, B Köllner, F Manes, G Pihl Karlsson, G. Soja and K Vandermeiren.

g_{max} and f_{min}

g_{max} is derived from the 90th percentile and g_{min} from the 10th percentile values of the entire NC-S and NC-R stomatal conductance dataset. g_{max} was converted from a conductance for water vapour (H_2O) to a conductance for O_3 using a ratio of 0.662 based on the diffusivities of H_2O to O_3 in air after Massman (1998).This gave a value of 532 mmol O_3 m^{-2} s^{-1} for the mean of the two biotypes. The f_{min} is expressed as a fraction of g_{max}. All values are shown in Table A1.

Boundary line derivation for f_{light}, f_{temp} and f_{VPD}

For the derivation of f_{light}, f_{temp} and f_{VPD} relationships a boundary line approach was used. This method defined the 90th percentile values of stomatal conductance within different incremental classes of environmental data which were: 100 µmol m^{-2} s^{-1} for

f_{light}; 2°C for f_{temp} and 0.1 kPa for f_{VPD}. Figure A1 shows the resulting boundary lines, fitted by eye, for each biotype of clover using the generic functions described in the Mapping Manual (LRTAP Convention, 2004). To derive the NC-S/NC-R parameterization, mean values (i.e. the mean of the co-efficient for f_{light} and means of the threshold values for f_{temp} and f_{VPD}) were calculated from the individual biotypes; all values are presented in Table A1. Note: for f_{VPD}, data classes with fewer than 10 data points were used to guide the derivation for high VPDs; these are denoted by grey shading in the figures.

The g_{sto}(clover) model assumes that f_{phen} and f_{SWP} are both equal to 1. This maintains consistency with the crop generic flux model (LRTAP Convention, 2004).

Table A1 Parameterisation for the NC-S and NC-R clover clones and the hybrid NC-S/NC-R clover clone used to parameterise the g_{sto}(clover) model.

	NC-S	NC-R	Combined NC-S/NC-R values used
g_{max} (mmol O_3 m^{-2} PLA s^{-1})	534	527	530
g_{min}	0.12	0.09	0.105
f_{light}	-0.02	-0.018	-0.019
T_{min}	-5	8	1
T_{opt}	27	30	28
T_{max}	59	52	55
VPD_{max}	3.7	4.3	4
VPD_{min}	7.54	7.05	7.30

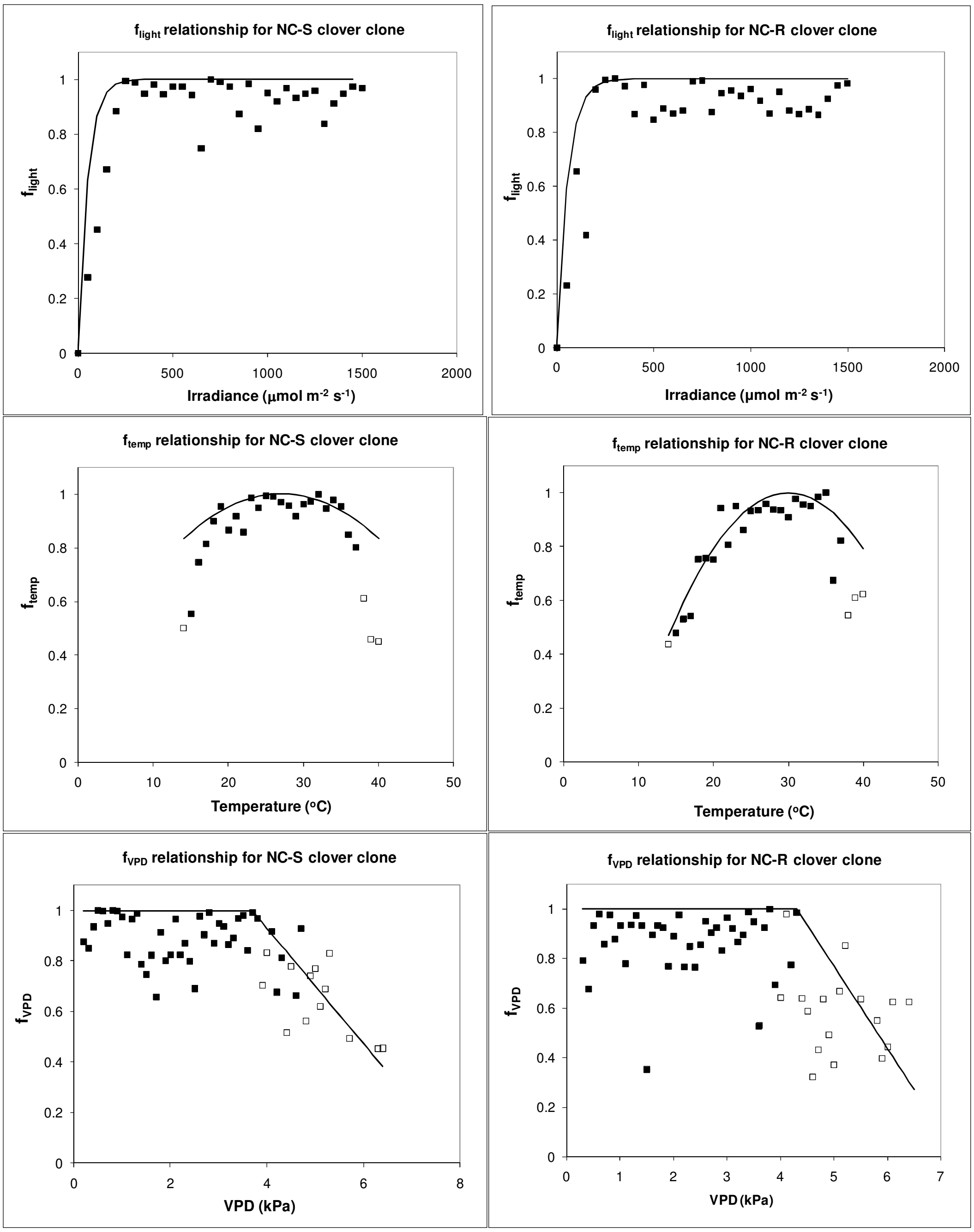


Figure A1. Boundary line derivation of f_{light}, f_{temp} and f_{VPD} for the NC-S and NC-R clover bio-types. Solid line represents the boundary line; filled squares represent data points with equal or greater than 10 data points within each environmental class; open squares represent data points with less than 10 data points within each environmental class.

Figure Legends

Figure 1: Locations of records of visible injury attributed to ozone on crops, (semi-)natural vegetation and shrub species for the period 1991 – 2006 (includes published data and previously unpublished observations).

Figure 2: Number of published and unpublished records of visible leaf injury symptoms attributed to ambient ozone for each year (1991 – 1996). Note: the fluctuation in number of records per year may be more strongly associated with recording effort than with severity of ozone effect.

Figure 3: Locations where ozone injury has been detected in the field and published in scientific papers for the period 1995 – 2004 superimposed on EMEP modelled (a) AOT40 (crop), ppm h (b) and $POD3_{gen}$ (crop), mmol m^{-2} using methods described in LRTAP Convention (2004), and averaged over the same time period. Data sources: Benton *et al*. 2000; Benton *et al*. 1996; Bermejo *et al*. 2003; Bermejo, *et al*. 2002; Bungener *et al*. 1999; Bussotti *et al.* 2003; Carrasco-Rodriguez *et al*. 2001; Faoro and Iriti, 2003; Gimeno *et al*. 1996; Innes *et al*. 2001; Manning *et al*. 2002; Manning and Godzik, 2004; Novak *et al*. 2003; Persson *et al*. 2003; Pihl Karlsson, *et al*. 1995; Piikki *et al*. 2004; Pleijel *et al*. 1994; Pleijel *et al*. 1999; Pleijel *et al*. 1997; Ribas and Penuelas, 2000; Saitanis *et al*. 2004; Saitanis, 2003; Skelly *et al*. 1999; VanderHeyden *et al.* 2001; Velissariou, 1999; Velissariou and Kyriazi, 1996; Velissariou *et al.* 1996; Velissariou *et al*. 1992 and Velissariou, 1999. Note: some dots for injury location overlap.

Figure 4: Cumulative frequency of number of records for (a) AOT40(clover), (b) $AOT30_{VPD}$ (clover) and (c) $POD3_{gen}$ (clover) accumulated from either day 0 of the experiment (■) or for the 8 days prior (□), to the first occurrence of visible ozone injury on NC-S white clover.

Figure 5: Five-year mean ozone injury score (June to August) on ozone-sensitive white clover (NC-S) at ICP Vegetation sites for the period 2000 – 2004 superimposed on the EMEP five-year mean for (a) AOT40 (crop), ppm h, and (b) AOT40 (clover), ppm h (c) $POD3_{gen}$ (crop), mmol m^{-2} and (d) $POD3_{gen}$ (clover), mmol m^{-2} for the same years. The injury score data by country was (n, mean, SE mean): Austria (4, 1.3, 0.37); Belgium (3, 1.4, 0.35); Germany (15, 1.7, 0.79); Italy (7, 3.5, 0.4); The Netherlands (1, 0.7, -); Slovenia (5, 2.3, 0.3); Spain (3, 4.07, 0.04); Sweden (5, 1.7, 0.3); Switzerland (4, 4.0, 0.3); UK (5, 0.7, 0.3).

Figure 6: Mean normalised percentage biomass reduction in ozone-sensitive white clover (NC-S relative to NC-R) at ICP Vegetation sites in 2000 – 2004 superimposed on the EMEP five-year mean for (a) AOT40 (crop), ppm h, and (b) AOT40 (clover), ppm h, (c) $POD3_{gen}$ (crop), mmol m^{-2} and (d) $POD3_{gen}$ (clover), mmol m^{-2}. The biomass data is described in Table 4.

Figure 7 Response functions for clover biomass reduction and site- and year-specific grid square values for AOT40 (crop), (a) and (c), and AOT40 (clover), (b) and (d). Figures (a) and (b) show the complete data set and Figures (c) and (d) show only the values for effects at AOT40 > 3 ppm h.

Figure 8 Response functions for clover biomass reduction and site- and year-specific grid square values for $POD3_{gen}$ (crop), (a) and (c), and $POD3_{gen}$ (clover), (b) and (d). Figures (a) and (b) show the complete data set and Figures (c) and (d) show only the values for effects at (c) $POD3_{gen}$ (crop) > 15 mmol m^{-2} and (d) $POD3_{gen}$ (clover) > 40 mmol m^{-2}.

Figure 9: Relationship between clover site and year specific values for AOT40 (clover) and $POD3_{gen}$ (clover) accumulated over 3 months from experimental start date. (a) whole dataset, (b) data set separated into regions, with the key: NE (Northern Europe); ACE (Atlantic Central Europe); CCE (Continental Central Europe) and Med (Mediterranean Europe). Note: linear function not fitted for NE as there were only 3 data points.

Table 1: Agricultural and horticultural crops exhibiting visible leaf ozone injury in the field (ad hoc observations, surveys). Note: Records of injury on clover species (*Trifolium* spp.) where they occur as components of managed pasture are included within this table.

Region	Country	Species	Reference
Northern Europe	Sweden	*Raphanus sativus*	Pleijel *et al.* 1999
		Solanum tuberosum	Persson *et al.* 2003, Piikki et al. 2004
		Trifolium repens	Karlsson *et al.* (1995)
		Trifolium subterraneum	Pleijel *et al.* 1994, Pihl Karlsson *et al.* 1995, Benton *et al.* 2000
		Trifolium pratense	Pleijel *et al.* 1994, Karlsson *et al.* 1995
		Triticum aestivum	Pleijel *et al.* 1996
Atlantic Central Europe	Belgium	*Phaseolus lunatus, Phaseolus vulgaris, Solanum tuberosum, Trifolium subterraneum, Triticum aestivum, Zea mays*	Benton *et al.* 2000
	Netherlands	*Phaseolus vulgaris, Trifolium subterraneum*	Benton *et al.* 2000
	UK	*Trifolium repens*	Benton *et al.* 2000
Continental Central Europe	Austria	*Phaseolus vulgaris, Trifolium subterraneum*	Benton *et al.* 2000
	France	*Glycine max, Phaseolus vulgaris, Trifolium repens*	Benton *et al.* 2000
	Germany	*Phaseolus vulgaris*	Bender (unpublished))
		Trifolium repens	Benton *et al.* 2000
	Hungary	*Phaseolus vulgaris*	Benton *et al.* 2000
	Poland	*Phaseolus vulgaris, Trifolium repens*	Benton *et al.* 2000
	Russian Federation	*Phaseolus vulgaris, Trifolium subterraneum*	Benton *et al.* 2000
	Switzerland	*Vitis vinifera*	Innes *et al.* 2001
		Solanum tuberosum, Trifolium subterraneum	Benton *et al.* 2000
		Vitis spp.	Skelley *et al.* 1998
Eastern Mediterranean	Greece	*Chicorium endive, Cucurbita pepo, Trifolium alexandrinum*	Velissariou *et al.* 1996
		Allium cepa, Petroselinum crispum, Phaseolus vulgaris, Beta vulgaris, Nicotiana tabacum, Beta vulgaris, Vitis vinifera, Citrullus lanatus, Zea mays	Velissariou *et al.* 1996, Velissariou 1999

		Solanum tuberosum	Velissariou *et al*. 1996
		Vitis vinifera	Saitanis *et al.* 2004, 2003, Saitanis (un-published)
	Slovenia	*Phaseolus vulgaris, Trifolium repens*	Benton *et al.* 2000
Western Mediterranean	Italy	*Glycine max, Lycopersicon esculentum, Phaseolus vulgaris*	Gerosa (unpublished))
		Triticum durum, Cucurbita pepo	Schenone (unpublished))
		Phaseolus vulgaris	Schenone *et al.* 1995, Postiglione and Fagnano 1995, Manes and Vitale (unpublished)
		Trifolium repens, Phaseolus vulgaris	Benton *et al*. 2000
		Allium cepa, Glycine max, Triticum aestivum, Triticum durum	(Quaroni *et al*. 2003), Faoro and Iriti (unpublished)
		Beta vulgaris, Vitis vinifera	Bussotti *et al.* 2003a
		Prunus persica	Paolacci *et al.* 1995
		Trifolium subterraneum, Phaseolus vulgaris	Postiglione and Fagnano 1995
	Spain	*Citrullus lanatus*	Benton *et al.* 2000
		Phaseolus vulgaris	Gimeno and Bermejo (unpublished)), Gimeno *et al.* 1996, Ribas and Penuelas 2000
		Lycopersicon esculentum	Bermejo *et al.* 2002, Gimeno *et al* 1995
		Avena sativa	Carrasco-Rodriguez and del Valle-Tascon, 2001
		Citrus clementina	Iglesias *et al*. 2006
		Arachis hypogaea, Glycine max, Nicotiana tabacum, Vitis vinifera	Gimeno *et al.* 1995
		Solanum tuberosum	Calvo and Sanz (unpublished))
		Citrullus lanatus	Gimeno *et al.* 1992

Table 2: Species of (semi-)natural vegetation (grasses and forbs) exhibiting visible leaf ozone injury in the field (ad hoc observations, surveys). Note: Records of injury on clover species (*Trifolium* spp.) where they occur as components of meadows and unmanaged grasslands are included within this table.

Region	**Country**	**Species**	**Reference**
Northern Europe	Sweden	*Trifolium subterraneum*	Benton *et al.* 200)
Atlantic Central Europe	Belgium	*Malva sylvestris*	Benton *et al.* 1996
		Trifolium subterraneum	Benton *et al.* 2000
	Netherlands	*Trifolium subterraneum*	Benton *et al.* 2000
	United Kingdom	*Centaurea nigra, Eupatorium cannabinum*	Hayes (unpublished)
		Trifolium repens, Trifolium subterraneum	Benton, *et al.* 2000
Continental Central Europe	Austria	*Trifolium subterraneum*	Benton, *et al.* 2000
	France	*Rubus idaeus*	Schelfaut *et al* (unpublished)
		Trifolium repens	Benton, *et al.* 2000
		Epilobium angustifolium, Calamagrostis villosa, Cephalaria brevipalea, Dryas octopetala, Fragaria viridis, Geum montanum, Rubus articus, Rubís saxatilis, Centaurea scabiosa	Bussotti *et al.* 2003a
		Rubus idaeus	Bussotti *et al.* 2003), Gillot (unpublished)
	Germany	*Achillea millefolium*	Bender (unpublished)
		Mentha aquatica	Biostress 2002
	Poland	*Trifolium repens*	Benton, *et al.* 2000
		Alchemilla spp., Angelica sylvestris, Astrantia major, Centaurea jacea, Centaurea mollis, Chaerophyllum aromaticum, Geranium palustre, Impatiens parviflora, Lapsana communis, Thymus alpestris	Manning *et al.* 2002, Manning and Godzik 2004
	Russian Federation	*Trifolium subterraneum*	Benton, *et al.* 2000
	Switzerland	*Centaurea jacea, Knautia arvensis, Leucanthemum vulgare, Plantago lanceolata, Rumex obtusifolius, Salvia pratensis*	Bungener *et al.* 1999

		Epilobium hirsutum, Oenothera biennis, Rubus fruticosus, Tragopogon pratensis, Artemisia vulgaris, Plantago major, Impatiens parviflora, Calystegia sepium, Epilobium angustifolium, Geranium sylvaticum, Parthenocissus quinquifolia, Rumex obtusifolius	Innes *et al.* 2001
		Malva sylvestris, Trifolium subterraneum	Benton *et al.* 1996
		Alchemilla spp., Artemisa vulgaris, Calamentha grandifolia, Calystegia spp. Centaurea paniculata, Chenopodium spp., Convovulus arvensis, Epilobium angustifolium, Epilobium hirsutum, Impatiens parviflora, Lamium galeobdolon, Lapsana communis, Malva spp. Oenothera spp., Parthenocissus quinquifolia, Plantago major, Polygonum spp., Reynoutria japonica, Rubus fruticosus, Rudbeckia lacinata, Rumex obtusifolius, Solidago canadensis, Stachys officinalis, Succisa pratensis	Skelley *et al.* 1998
		Rumex obtusifolius	VanderHeyden *et al.* 2001
	Ukraine	*Betonica officinalis, Centaurea nigra, Centaurea scabiosa, Gentiana asclepiada, Vincetoxium officianalis*	Manning *et al.* 2002, Manning and Godzik 2004
Eastern Mediterranean	Greece	*Sonchus spp*	Velissariou 1999
	Slovenia	*Trifolium repens*	Benton *et al.* 2000
Western Mediterranean	Italy	*Astrantia major, Cyclamen spp., Euphorbia dulcis, Gentiana asclepiadea, Globularia nudicaulis, Pastinaca sativa, Polygonatum spp., Stachys spp., Centaurea spp., Helleborus níger, Rubia peregrina*	Bussotti *et al.* 2006
		Astrantia major, Centaurea nigra, Helleborus niger	ICP Forests 2003

		Astrantia major, Centaurea nigra, Geranium nodosum, Mycelis muralis, Veronica urticifolia, Lamium spp. Rubus ulmifolius	Bussotti *et al.* 2003b
		Centaurea jacea	Bungener *et al.* 1999
		Eupatorium cannabinum, Origanum vulgare	Bussotti *et al.* 2003a.)
	Spain	*Briza maxima, Bromas hordaceus, Cynosurus echinatus, Trifolium striatum*	Sanz and Bermejo (unpublished)
		Aegilops geniculata, Aegilops triuncialis, Avena barbata, Avena sterilis, Biserrula pelecinus, Briza maxima, Lolium rigidum, Trifolium cherleri, Trifolium glomeratum, Trifolium subterraneum	Bermejo *et al.* 2003
		Agrimonia eupatoria, Abutilon theophrasti, Anthyllis cytisoides, Calystegia sepium, Capanula spp., Chenopodium album, Colutea arborescens, Cytisus patens, Epilobium angustifolium, Epilobium collium, Inula viscosa, Ipomea sagitatta, Lagersteroemia indica, Oenothera rosea, Plantago lanceolata, Rubina peregrina, Rubus ulmifolius, Rumex pulcher, Verbascum sinuatum, Vinca difformis	Skelley *et al.* 1998

1

Table 3: Species of shrubs exhibiting visible leaf ozone injury in the field (*ad hoc* observations, surveys).

Region	Country	Species	Reference
Continental Central Europe	Czech Republic	*Corylus avellana*	Manning *et al.* 2002, Manning and Godzik 2004
	France	*Cornus sanguinea, Clematis vitalba*	Remy (unpublished)
		Ampelopsis tricuspidata, Cornus sanguinea, Symphoricarpos alba	Garrec (unpublished)
		Cornus sanguinea	Rainouard (unpublished)
		Viburnum lantana, Berberis vulgaris, Cornus sanguinea, Prunus brigantina	Remy (unpublished)
	Poland	*Cornus sanguinea*	Manning *et al.* 2002, Manning and Godzik 2004
	Switzerland	*Clematis alpina, Ribes rubrum, Robinia pseudoacacia, Rosa canina, Rubís fruticosus*	Innes *et al.* 2001
		Alnus viridis, Berberis spp., Clematis spp., Corylopsis pauciflora, Euonymous europeaus, Forsythia spp., Ligustrum ovalifolium, Lilac spp., Lonicera caprifolium, Prunus spinosa, Ribes alpinum, Rosa canina, Salix pentrandra, Salix purpurea, Salix viminalis, Sambucus racemosa, Spirea spp., Viburnum lantana, Viburnum opulus, Viburnum plicatum	Skelley *et al.* 1999
		Cornus alba, Ribes alpinum, Vibernum opulus	Novak *et al.* 2003
		Frangula agnus, Rhamnus catharticus, Salix viminalis, Sambucus racemosa, Viburnum lantana	VanderHeyden *et al.* 2001
Western Mediterranean	Italy	*Clematis vitalba, Vaccinium myrtillus, Virburnum lantana, Robinia pseudoacacia*	Bussotti *et al.* 2005
		Clematis vitalba, Lonicera caprifolium, Rosa canina, Rubus idaeus, Sambucus racemosa	Bussotti 2003a

		Robinia pseudoacacia	Innes *et al.* 2001
		Euonymus europeaus, Syringa vulgaris	Bussotti *et al.* 2003a,
	Spain	*Arbutus unedo, Cistus salvifolius, Lagerstroemia indica, Lonicera etrusca, Lonicera implexa, Myrtus communis, Pathenocissus quinquefolia, Pistacia lentiscus, Pistacia terebinthus, Prunus spinosa, Ricinus communis, Rosa canina, Sambucus nigrum, Viburnum tinus*	Skelley *et al.* 1998

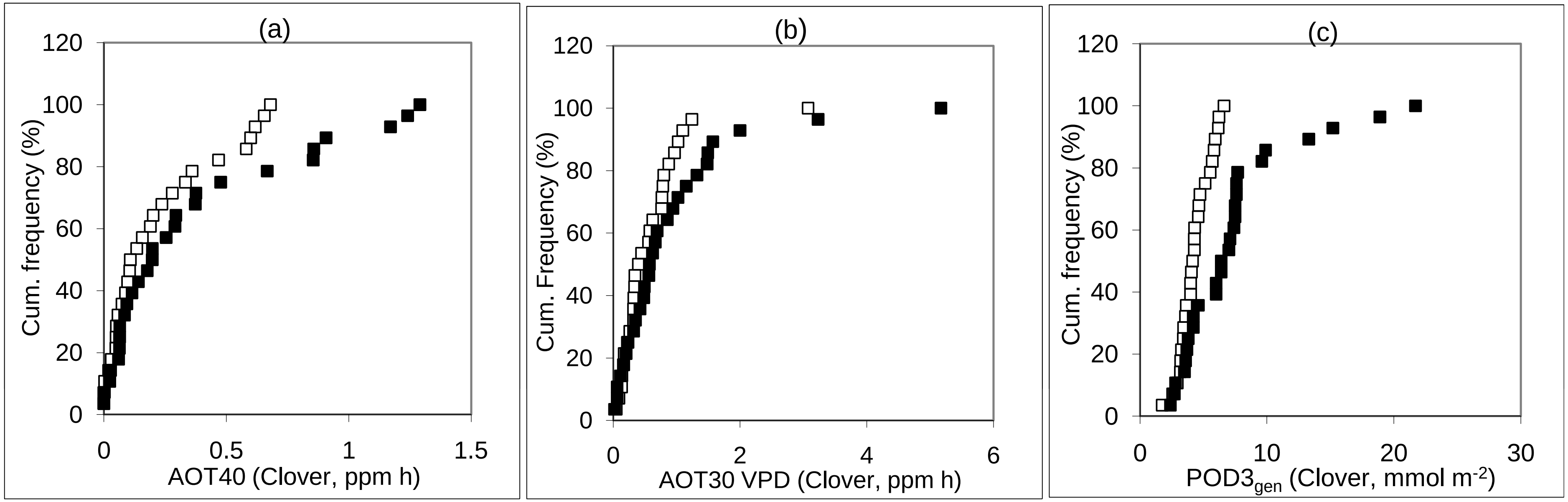
Table 4 Description of the ICP Vegetation biomonitoring experiment database (1996 – 2004) on ozone effects on the biomass of NC-white clover.

Country	Sites	No of data points	AOT40 (clover), ppm h		POD3gen (clover), mmol m^{-2}		Biomass Reduction (NC-S dry weight/NC-R dry weight), %		
			Mean	SE Mean	Mean	SE Mean	Mean	SE Mean	Range
Austria	Seibersdorf	6	3.38	0.21	54.9	0.5	12.7	3.2	1.8 – 25.2
Belgium	Tervuren	5	1.67	0.34	39.7	2.6	4.9	3.0	-4.1 – 12.9
Germany	Braunschweig, Cologne, Deuselbach, Essen, Giessen, Trier	17	2.84	0.21	49.5	0.9	9.1	1.6	-0.9 – 20.8
Greece	Kalamata, Thessalonika	2	2.13	1.8	46.5	10.1	30.0	13.0	17 - 43
Ireland	Carlow	1	0.43	-	32.4	-	-1.6	-	-
Italy	Isola Serafini, La Casella, Milan, Naples, Pisa, Rome	10	6.53	0.48	66.0	1.3	33.3	3.32	19.8 – 49.7
The Netherlands	Waageningen	1	1.23	-	39	-	7.7	-	-
Slovenia	Iskbra, Llubjiana, Rakican	5	2.93	0.44	51.6	1.9	3.5	4.0	-7.8 – 10.5
Spain	Ebro delta, Navarra, Valencia	4	1.73	0.68	43.6	5.1	11.1	5.7	-2.7 – 24.8
UK	Bangor	3	0.11	0.03	21.9	0.7	2.8	0.7	1.5 – 3.5

Table 5 : Parameterisation for the generic crop and the NC-S/NC-R clover flux models. For the clover flux model (see Annex for further details), the start of the growing season was defined as the 15th June (the mean start date for the experiments across all sites), each clover experiment lasted for 84 days with the end of the experiment defining the end of the growing season. Full details of model formulations are provided in the Mapping Manual (LRTAP, 2004).

Functions and constants	**Generic crop**	**NC-S/NC-R Clover**
g_{max}	450 mmol O_3 m^{-2} PLA s^{-1}	530 mmol O_3 m^{-2} PLA s^{-1}
f_{min}	0.01	0.11
f_{phen}	1	1
f_{light}	$Light_a$ = 0.0105	$Light_a$ = 0.019
f_{temp}	T_{min} = 12 ºC T_{opt} = 26 ºC T_{max} = 40 ºC	T_{min} = 1.5 ˚C T_{opt} = 28.5 ˚C T_{max}[1] = 50 ˚C
f_{vpd}	VPD_{max} = 1.2 kPa VPD_{min} = 3.2 kPa	VPD_{max} = 4 kPa VPD_{min} = 7.3 kPa
ΣVPD routine[2]	ΣVPD_{crit} = 8 kPa	ΣVPD_{crit}[2] = 1000 kPa
f_{SWP}	1	1
f_{O3}	1	1
Y	3 nmol m^{-2} PLA s^{-1}	3 nmol m^{-2} PLA s^{-1} [3]
SAI[4]	5 m^2 PLA m^{-2}	-[4]
Green LAI[4]	3.5 m^2 PLA m^{-2}	5 m^2 PLA m^{-2} [4]
h	1 m	0.3 m[4]
L	0.02 m	0.03 m

[1] estimate from curves
[2] This routine prevents stomatal re-opening within the model in the late afternoon under declining VPD conditions (see LRTAP Convention, 2004, for further details). We have assumed that this function is not operating in NC-S/NC-R clover as included in the clover model already in DO_3SE.
[3] assumed to be same as for generic crop
[4] Green LAI (Leaf Area Index), SAI (surface area index = green leaf area index + senescent leaf area index) and h are used for the calculation of O_3 concentrations. Here, grassland parameters from Simpson & Emberson (2006) have been used for clover (except grassland height, h, set to 30 cm for this study), for further details see Annex.


(a)
Cum. frequency (%)
AOT40 (Clover, ppm h)
(b)
Cum. Frequency (%)
AOT30 VPD (Clover, ppm h)
(c)
Cum. frequency (%)
POD3gen (Clover, mmol m-2)

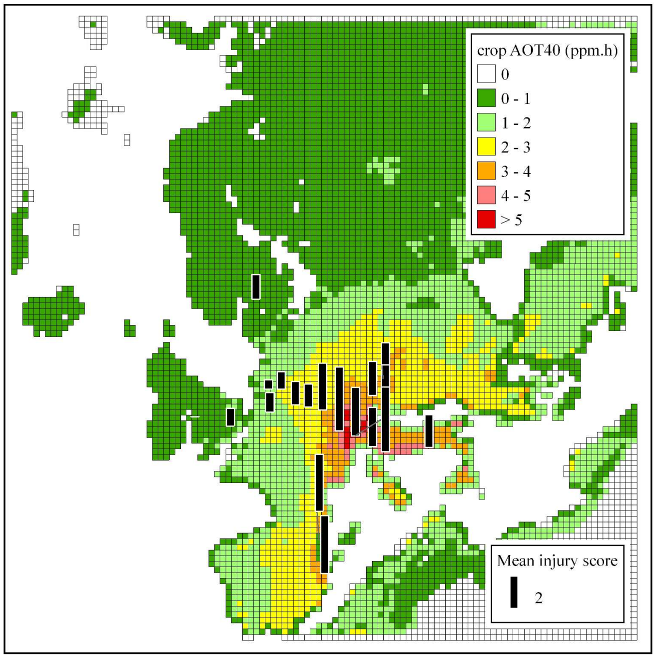


Figure 5a (see ms for legend)
297x209mm (400 x 400 DPI)

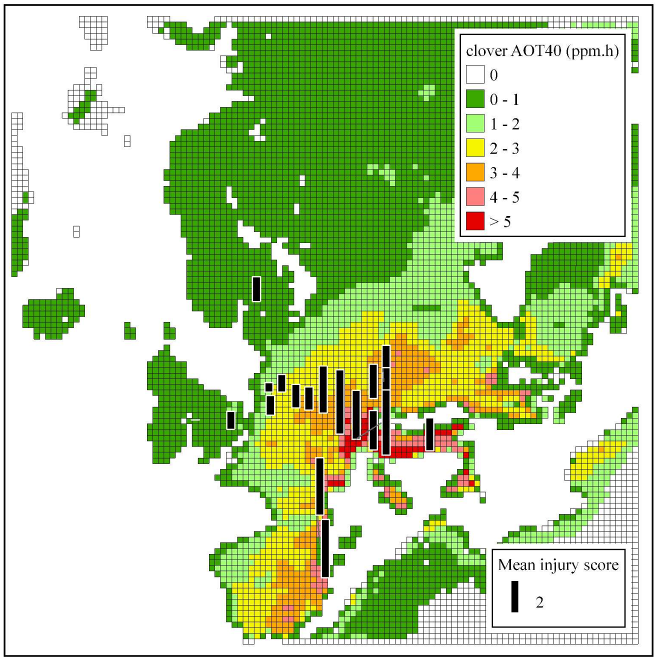


Figure 5b (see ms for legend)
297x209mm (400 x 400 DPI)

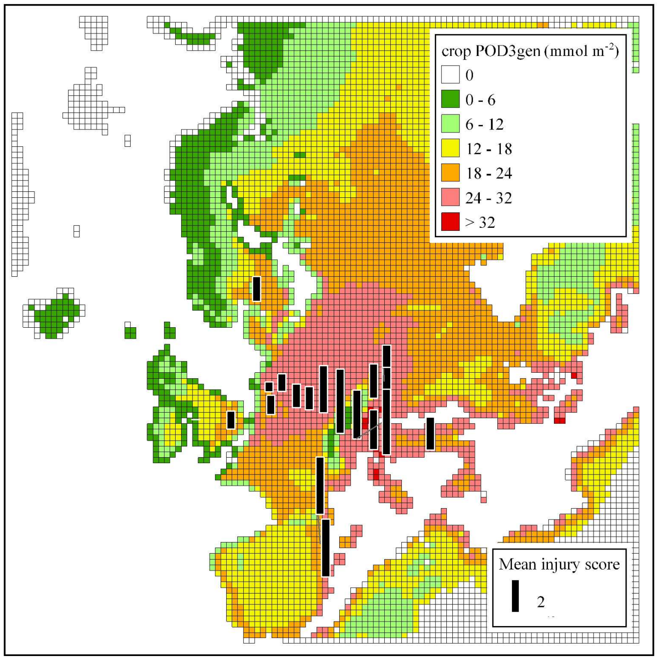


Figure 5c (see ms for legend)
296x210mm (400 x 400 DPI)

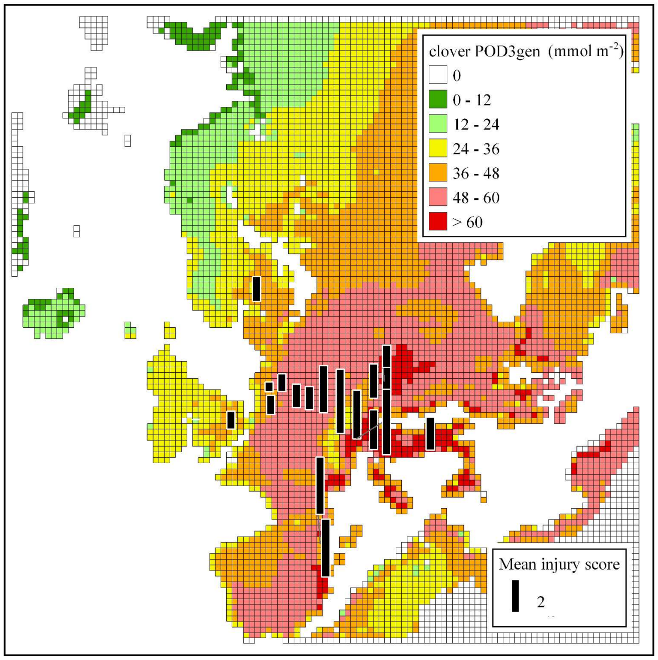


Figure 5d (see ms for legend)
296x210mm (400 x 400 DPI)

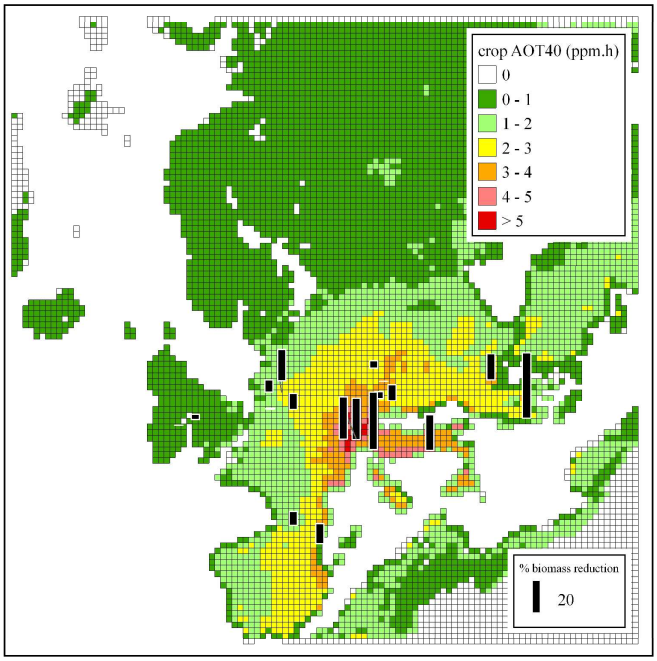


Figure 6a (see ms for legend)
297x209mm (400 x 400 DPI)

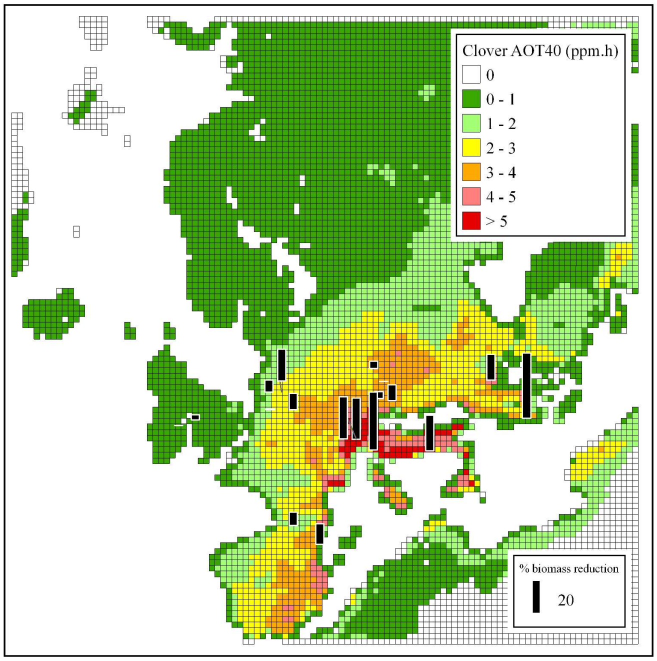


Figure 6b (see ms for legend)
297x209mm (400 x 400 DPI)

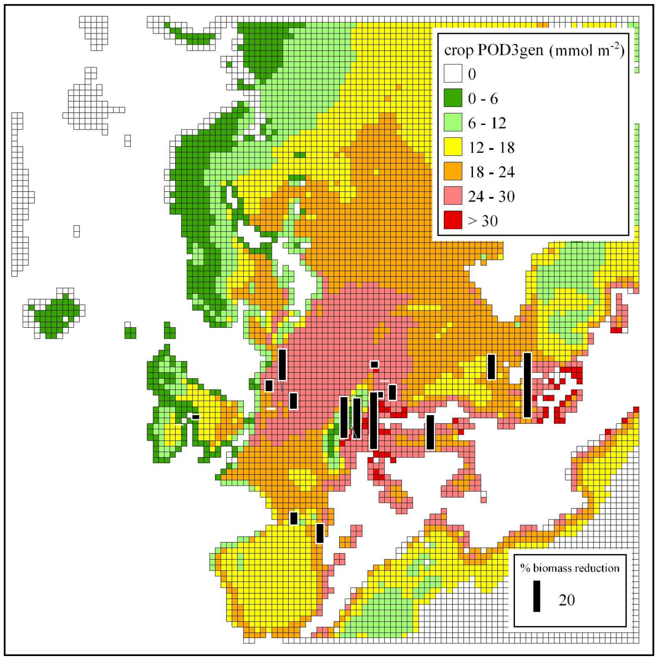


Figure 6c (see ms for legend)
296x210mm (400 x 400 DPI)

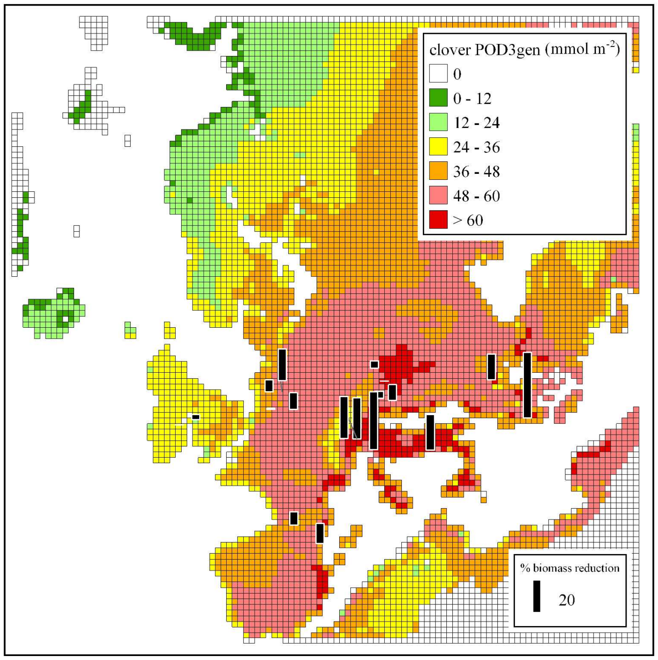
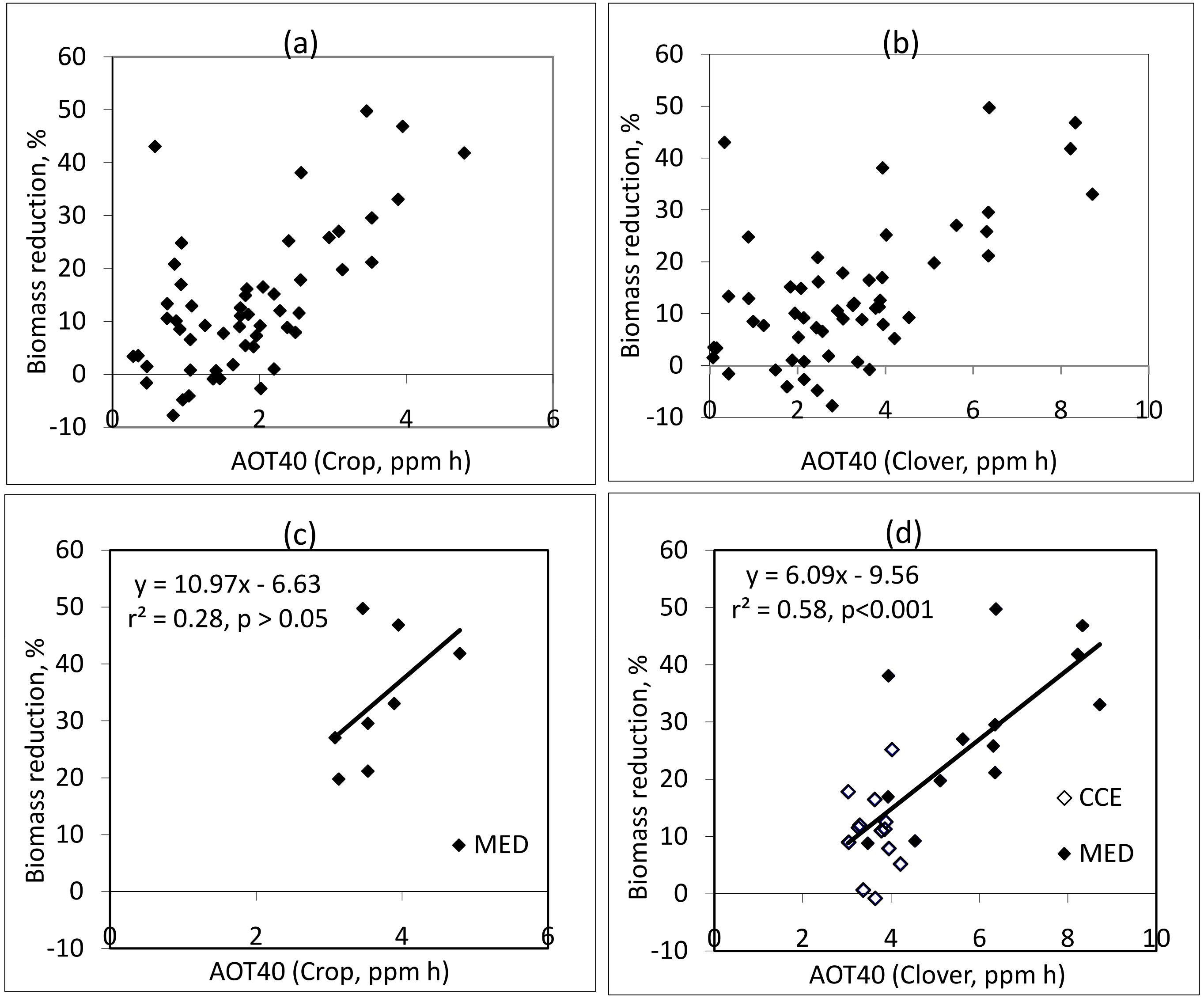
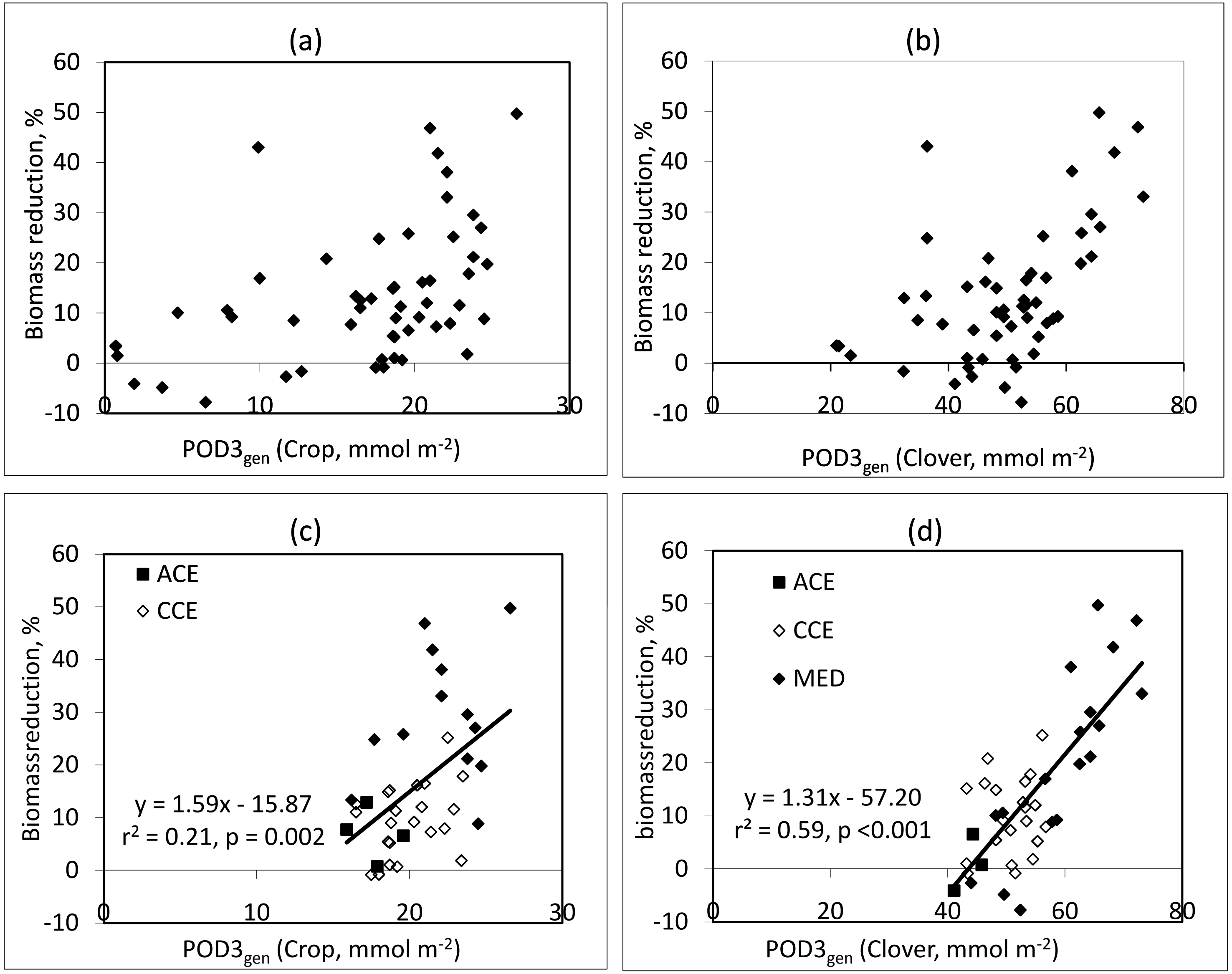
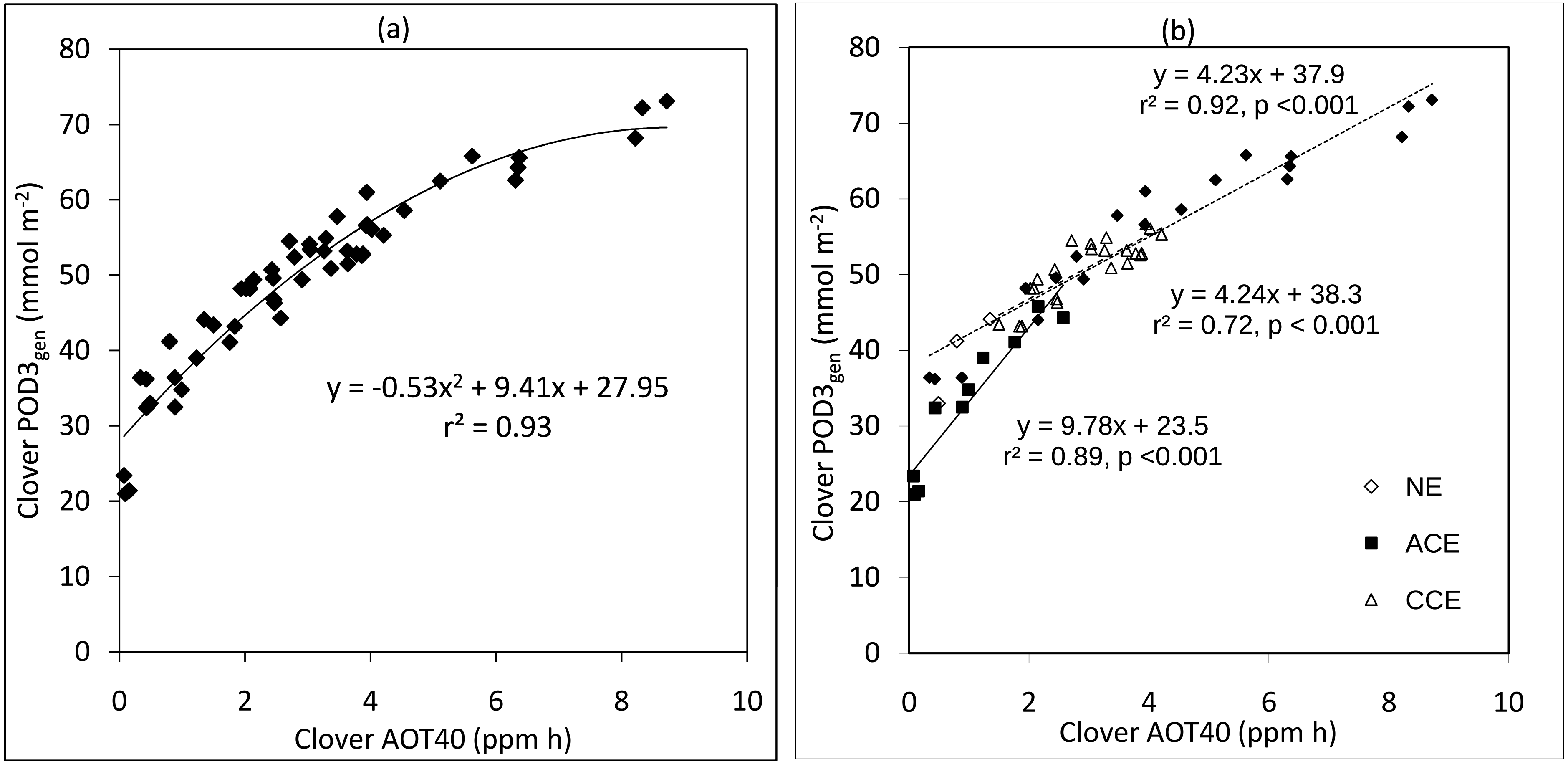


Figure 6d (see ms for legend)
296x210mm (400 x 400 DPI)


(a)
Biomass reduction, %
AOT40 (Crop, ppm h)
(b)
Biomass reduction, %
AOT40 (Clover, ppm h)
(c)
y = 10.97x - 6.63
r² = 0.28, p > 0.05
MED
Biomass reduction, %
AOT40 (Crop, ppm h)
(d)
y = 6.09x - 9.56
r² = 0.58, p<0.001
CCE
MED
Biomass reduction, %
AOT40 (Clover, ppm h)


(a)
Biomass reduction, %
POD3gen (Crop, mmol m-2)
(b)
Biomass reduction, %
POD3gen (Clover, mmol m-2)
(c)
ACE
CCE
Biomassreduction, %
y = 1.59x - 15.87
r2 = 0.21, p = 0.002
POD3gen (Crop, mmol m-2)
(d)
ACE
CCE
MED
biomassreduction, %
y = 1.31x - 57.20
r2 = 0.59, p <0.001
POD3gen (Clover, mmol m-2)


(a)
80
70
60
50
40
30
20
10
0
Clover POD3gen (mmol m-2)
y = -0.53x2 + 9.41x + 27.95
r2 = 0.93
0
2
4
6
8
10
Clover AOT40 (ppm h)
(b)
y = 4.23x + 37.9
r² = 0.92, p <0.001
y = 4.24x + 38.3
r² = 0.72, p < 0.001
y = 9.78x + 23.5
r² = 0.89, p <0.001
NE
ACE
CCE
Clover POD3gen (mmol m-2)
Clover AOT40 (ppm h)