Article (refereed) - postprint

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1   **Evidence of widespread effects of ozone on crops and (semi-)natural vegetation**

2   **in Europe (1990 - 2006) in relation to AOT40 - and flux-based risk maps**

3

4

5   **Running title**

6   Ozone effects on vegetation in Europe

7

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23   **Key words**

24   Ozone, crops, (semi-)natural vegetation, clover, ozone injury, biomass reduction,

25   ambient air, AOT40, flux, risk assessment

1   **Abstract**

2   Records of effects of ambient ozone pollution on vegetation have been compiled for

3   Europe for the years 1990 – 2006.   Sources include scientific papers, conference

4   proceedings, reports to research funders, records of confirmed ozone injury symptoms

5   and an international biomonitoring experiment coordinated by the ICP Vegetation.

6   The latter involved ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of

7   white clover (*Trifolium repens* L.) grown according to a common protocol and

8   monitored for ozone injury and biomass differences in 17 European countries, from

9   1998 to 2006. Effects were separated into visible injury or growth/yield reduction.

10   Of the 644 records of visible injury, 39% were for crops (27 species), 38.1 % were for

11   (semi-)natural vegetation (95 species) and 22.9% were for shrubs (49 species). Due

12   to inconsistencies in reporting effort from year to year it was not possible to determine

13   geographical or temporal trends in the data. Nevertheless, this study has shown

14   effects in ambient air in 18 European countries from Sweden in the north to Greece in

15   the south. These effects data were superimposed on AOT40 (accumulated ozone

16   concentrations over 40 ppb) and $POD3_{gen}$ (modelled accumulated stomatal flux over a

17   threshold of 3 nmol m^{-2} s^{-1}) maps generated by the EMEP Eulerian model (50 km x 50

18   km grid) that were parameterised for a generic crop based on wheat and NC-S/NC-R

19   white clover. Many effects were found in areas where the AOT40 (crops) was below

20   the critical level of 3 ppm h. In contrast, the majority of effects were detected in grid

21   squares where $POD3_{gen}$ (crops) were in the mid-high range (> 12 mmol m^{-2}). Overall,

22   maps based on $POD3_{gen}$ provided better fit to the effects data than those based on

23   AOT40, with the $POD3_{gen}$ model for clover fitting the clover effects data better than

24   that for a generic crop.

25

1   **Introduction**

2

3   Concentrations of ozone in the troposphere have increased over recent decades

4   (Vingarzan, 2004, Derwent *et al*., 2007), with health-based guidelines regularly

5   exceeded across much of Europe (Meleux *et al*., 2007, EEA, 2009). In addition to

6   health impacts (described in WHO, 2008), ozone is considered to be more damaging

7   to vegetation than any other air pollutant (Ashmore, 2005). Experimental exposures

8   at concentrations that can be experienced in Europe, especially in hot summers like

9   2003, have shown that crops and (semi-)natural vegetation could be damaged by

10   reduced growth and seed production (e.g. Mills *et al*., 2007a, Hayes *et al*., 2007a,

11   Booker *et al*., 2009), premature senescence (e.g.Tonneijck *et al*., 2004), reduced

12   ability to over-winter (e.g. Hayes *et al*., 2006) and withstand stresses such as drought,

13   (e.g. Wilkinson and Davies, 2009), and by producing visible injury symptoms such as

14   chlorotic and bronze stippling of leaves (e.g. Manning *et al*., 2002). Earlier European

15   surveys and biomonitoring experiments conducted during the 1980s and 1990s

16   indicated that ambient ozone concentrations were sufficiently high to induce visible

17   injury on over 20 crops growing in countries extending from Sweden in the north to

18   Italy and Spain in southern Europe (Benton *et al*., 2000, Fumagalli *et al*., 2001). With

19   northern hemispheric ozone concentrations predicted to continue to rise, at least for

20   the next few decades (Royal Society, 2008), we undertook a study to review the

21   evidence for effects of current ambient ozone on crops and (semi-)natural vegetation

22   in Europe over the period 1990 – 2006, and related this evidence to maps being used

23   by the LRTAP Convention[1] to indicate the areas of greatest risk of effects over these

24   years. For one biomonitoring system (ozone-sensitive and –resistant white clover,

[1] Convention on Long-range Transboundary Air Pollution

4

1   described later) we also related biomass effects and visible injury to site- and year-

2   specific modelled ozone parameters that describe both ozone concentration and ozone

3   flux (uptake *via* the stomatal pores on the leaf surface). Thus, we provide here field-

4   based biological validation of mapped European ozone risk assessment indices,

5   information identified as urgently required by both Manning (2003) and Simpson *et*

6   *al*. (2007).

7   The general increase in ozone concentrations in the Northern hemisphere in recent

8   decades has been associated with a change in the profile of maximum seasonal and

9   diurnal ozone concentrations. Owing to implementation of precursor emission

10   controls, peak ozone concentrations in the USA and Western Europe have declined

11   from 1980 to present, with the decline being less steep in recent years (Derwent *et al.*,

12   2007, Vingarzan, 2004, Jenkin, 2008, Lefohn *et al.*, 2008, Solberg *et al.*, 2005). At

13   the same time, background ozone concentrations have increased due to hemispheric

14   transport of precursors from the developing areas of the world (NEGTAP, 2001,

15   Dentener *et al.*, 2006). Looking to the future, significant declines in background

16   ozone in Europe will only be achievable if strict global controls on ozone precursor

17   emissions are implemented (Royal Society, 2008, Dentener *et al.*, 2006). A

18   confounding factor in predicting future ozone climates is that higher temperatures and

19   reduced cloudiness and precipitation as a result of climate change may increase

20   summer peak and average ozone concentrations (Meleux *et al.*, 2007). Current

21   predictions are that by 2030, effects of ground level ozone pollution will have major

22   implications for global food security with global yield losses due to ozone rising to 9

23   – 18% for wheat and 4 – 8% for rice under scenarios that allow for implementation of

24   current legislation to control pollutant emissions (Van Dingenen *et al.*, 2009). It has

25   also recently emerged that ozone may reduce the ability of vegetation to absorb

1   carbon dioxide resulting in even greater carbon dioxide concentrations in the

2   atmosphere in the future, further increasing radiative forcing (Felzer *et al.*, 2005, Sitch

3   *et al.*, 2007). Predictions for the future are complicated by enhanced temperature

4   increasing ozone production over and above that associated with global increases in

5   precursor emissions, and the ameliorating effect of increasing carbon dioxide

6   concentration on ozone effects (by reducing stomatal uptake), see reviews by Booker

7   *et al.*, 2009, Feng and Kobayashi, 2009. Within the context of evaluating potential

8   effects of ozone in the future, it is timely to synthesise data on the current evidence of

9   effects of ozone in Europe on crops and (semi-)natural vegetation, and to draw

10   attention to the urgent need to further reduce the emissions of ozone precursors on a

11   global scale.

12

13   A recent synthesis of published dose-response function data from field-based chamber

14   experiments revealed that the most ozone-sensitive crops include wheat, soybean,

15   pulses and tomato, with potato, sugar beet, rape and maize being moderately sensitive

16   (Mills *et al.*, 2007a). In a similar study for (semi-)natural vegetation, Mills *et al.*

17   (2007b), compiled published dose-response functions for individual species and

18   proposed that grasslands (especially uplands, dry grasslands and woodland fringes),

19   heaths and wetlands are amongst the most ozone-sensitive habitats in Europe.

20   Although these compilations of exposure-response data provide an indication of the

21   relative sensitivity of different species, they do not provide actual evidence that

22   current ozone climates are damaging vegetation in the open field without any

23   confounding influence of an exposure chamber (as described in Heagle, 1989 and

24   Sanders *et al.*, 1991). Field-release studies with ozone are relatively few, and have

25   provided mixed results. For example, Morgan *et al.* (2006) found that yield

6

1   reductions for soybean were even larger than those predicted from open-top chamber

2   experiments whilst Bassin *et al*. (2009) found that alpine vegetation was relatively

3   resistant to ozone despite the sensitivity of the component species.

4

5   Several previously un-collated sources of evidence of visible ozone injury in ambient

6   air in Europe exist in the literature including surveys of crops and (semi-)natural

7   vegetation together with *ad hoc* observations. Examples include a survey of ozone

8   injury symptoms on vegetation in an alpine valley (Bussotti *et al*., 2003b); surveys of

9   symptoms on crops in Belgium, France, Spain and Switzerland (Benton *et al*., 2000)

10 and surveys conducted in countries in the Carpathian mountain range area of central

11 Europe (Manning *et al*., 2002). Researchers have also reported the presence of visible

12 injury symptoms on crops grown in ambient air plots included as part of open-top

13 chamber based ozone exposure experiments (e.g. De Temmerman *et al*., 2002).

14 These types of data are useful sources of evidence of ozone effects in the field

15 providing (as in the examples quoted), the assessors of ozone injury are well trained,

16 follow a clearly defined protocol and preferably, that the ozone symptoms have been

17 confirmed using ozone exposure experiments (Bussotti *et al*., 2006, Lorenz et al.,

18 2008, Manning and Godzik, 2004).

19

20 Further evidence of ozone effects in ambient air derives from surveys conducted by

21 placing a sentinel bioindicator species of known sensitivity to ozone at a series of sites

22 in a local area and assessing the plants for injury symptoms at intervals thereafter.

23 Ozone-sensitive tobacco (*Nicotiana tabacum* L. cv BELW3) has been used in several

24 such surveys, particularly in Southern Europe (e.g. Spain, Ribas and Penuelas, 2003

25 and Italy, Nali *et al*., 2006). Over the last fifteen years, the participants of the ICP

1 Vegetation[2] have compiled evidence of ozone effects in ambient air by conducting a

2 series of coordinated experiments across Europe with ozone-sensitive- (NC-S) and

3 ozone-resistant (NC-R) biotypes of a commercial cultivar of white clover (*Trifolium*

4 *repens* cv Regal) originally selected in Raleigh, North Carolina (Heagle *et al.*, 1994).

5 The NC-S strain develops ozone injury symptoms following an ambient ozone

6 episode involving ozone concentrations of 50 – 60 ppb and higher, and after

7 prolonged exposure to ozone has reduced above-ground growth whilst the NC-R

8 biotype only responds to ozone at substantially higher concentrations. In addition to

9 ozone injury data, this biomonitoring system also provides evidence of ambient ozone

10 effects of ambient concentrations on biomass – an effect of potentially greater

11 importance than visible injury.

12

13 During the last fifteen years, the ozone-effects research community in Europe has

14 been establishing methods for determining the critical levels for ozone, above which

15 effects on sensitive species can be expected. The overall aim has been to develop

16 methods for mapping the areas of Europe where vegetation is at highest risk of ozone

17 damage. The resulting maps are used within the LRTAP Convention as part of the

18 negotiations for reductions in emissions of the precursors of ozone from the 51

19 signatories to the Convention (for details, see

20 http://www.unece.org/env/lrtap/lrtap_h1.htm). Contributing countries agree to

21 reductions by signing Convention Protocols, the most recent being the Gothenburg

22 Protocol (1999) to abate acidification, eutrophication and ground-level ozone which is

23 currently under review. The negotiations concerning ozone for the Gothenburg

24 Protocol were based on exceedance of a concentration-based long-term critical level

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

1   of ozone for crops and (semi-)natural vegetation. This value, an AOT40[3] of 3 ppm h

2   accumulated over three months was set at the Kuopio Workshop in 1996 (Kärenlampi

3   and Skärby, 1996) and is still considered to be the lowest AOT40 at which significant

4   yield or biomass loss due to ozone can be detected for agricultural crops and (semi-

5   )natural vegetation dominated by annuals, according to current knowledge (LRTAP

6   Convention, 2004).   A critical level for visible injury has also been derived based on

7   analysis of many ozone parameters in the days preceding ozone injury development

8   on *Trifolium subterraneum* (Pihl-Karlsson *et al.,* 2004). The "short-term" critical level

9   is a VPD-modified AOT30 ($AOT30_{VPD}$[4]) of 0.16 ppm.h accumulated over the eight

10   days prior to injury development (LRTAP Convention, 2004).

11

12   Recent research for the LRTAP Convention has led to a new index being developed

13   that has a stronger biological basis than AOT40. It models the flux of ozone from the

14   exterior of the leaf through the stomatal pores to the sites of damage ("stomatal flux"

15   or "flux") using algorithms describing the species-specific effects of temperature,

16   Photosynthetic Photon Flux Density (PPFD), soil water potential, vapour pressure

17   deficit (VPD) and plant growth stage on stomatal functioning (Emberson *et al*., 2000,

18   Pleijel *et al*., 2007). To date, flux-based critical levels have been derived for wheat,

19   potato and provisionally for beech and birch, and flux-based risk assessment methods

20   have been developed for a generic crop and generic tree species for use in large-scale

21   integrated assessment modelling (LRTAP Convention, 2004). The latter are

22   simplified full flux models that do not take into account genetic variability in

23   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.

1   conductance with the notation, $PODY_{gen}$[5] and units mmol m^{-2}, where Y = 3 for the

2   generic crop flux model used in this study. The threshold Y is similar in concept to

3   "40" in AOT40 and represents an amount of ozone flux that can be tolerated (or

4   detoxified) by the plant before negative effects begin to occur. The $POD3_{gen}$ (crop)

5   model is based on the parameterisation for wheat described in Pleijel *et al*. (2007).

6   Unfortunately, there is no generally-accepted $POD3_{gen}$ for clover yet, and in any case

7   the environmental and pollution-exposure conditions of potted plants within the ICP

8   programme differ from those that would apply to homogeneous canopies of clover,

9   but we use here available knowledge to derive a flux estimate for this network. This

10   new index, $POD3_{gen}$(Clover) is used alongside the more standard AOT40 and

11    $POD3_{gen}$ (Crop) functions in this paper.

12

13   Risk maps produced using AOT40 and generic flux (to crops or trees) provide very

14   different spatial patterns of ozone impacts (Simpson *et al*., 2007, Karlsson *et al.,*

15   2009). For both metrics, ozone impacts are predicted for southern Europe where

16   ozone concentrations are highest. However, risk maps based on generic flux to crops

17   indicate effects in central and north-west Europe where lower ozone concentrations

18   and AOT40 values below the critical level occur in climatic conditions (moderate

19   temperatures, moist climates) that are conducive to relatively high ozone flux. In the

20   current study, we superimpose maps of ozone effects detected in the field onto

21   AOT40- and $POD3_{gen}$ flux-based risk maps (for crops and NCS/NCR white clover) to

22   determine whether the more biologically meaningful flux approach is a better

23   predictor of vegetation damage than AOT40. Ideally ozone risk maps should be

24   generated from measurements of AOT40 and flux, however, measured ozone

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[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 $AF_{st}Y$.

1   concentrations are only available from ca. 130 sites in Europe (reported in

2   EMEP/CCC reports available at www.emep.int such as Fjæraa and Hjellbrekke,

3   2008), and spatial distribution of measurements is not uniform with many parts of

4   eastern and southern Europe poorly covered. For ozone fluxes, hardly any measured

5   data exist, with just a few research sites providing values (e.g. Tuovinen *et al*., 2004,

6   2007). Thus, assessment of risk of effects to vegetation is dependant on modelling

7   procedures that can provide a geographically broad-scale prediction of both the

8   AOT40 and the $POD3_{gen}$ metrics. Within Europe, the model of the European

9   Evaluation and Monitoring Programme (EMEP) plays a key role in developing air

10   pollution control strategies for both the LRTAP Convention and the European Union

11   (see Simpson *et al*., 2003 for details). The model results for AOT40 have been

12   compared with observations in, for example, Fowler *et al*. (1999) and Simpson *et al*.

13   (2003), and in general fair agreement (R>0.7) was obtained. Far fewer data are

14   available to evaluate the model's flux predictions, but studies by, for example,

15   Tuovinen *et al*. (2004) and Klingberg *et al*. (2008), provide support for the basic

16   formulation and deposition rates. The uncertainties associated with the EMEP model

17   are considered further in the Discussion.

18

19   In conducting this study, we set out to answer the following questions: How

20   widespread is the occurrence of ozone injury and ozone-induced biomass reduction in

21   Europe and which species of crops, (semi-)natural vegetation and shrubs are affected?

22   Is there any evidence of temporal or spatial trends in ozone effects as a result of

23   changing ozone profiles? How well do AOT40-based and flux-based maps predict the

24   areas where ozone injury and/or biomass reductions have been detected in ambient

25   air? This paper provides an overview of analysis of ozone effects data for the period

1   1990 – 2006; further details including the regional distributions of effects can be

2   found in a report by Hayes *et al*. (2007b).

3

4

5   **Materials and methods**

6   *Sources of ozone–effects data*

7   Data were sourced from scientific papers and conference proceedings published up to

8   2007 (see Tables 1 - 3), national-scale reports to funders of research (listed in Tables

9   1-3 as unpublished), the ICP Vegetation database for the clover experiment (described

10   below) and specialists that have published papers on ozone effects that had noted

11   confirmed ozone injury symptoms in the field (listed in Tables 1 - 3 as unpublished).

12   Each record of an effect was entered into a database together with the species name,

13   location of species (pot, experimental plot, commercial field, natural vegetation), date

14   of observation, nature and extent of effect (injury, biomass or yield), local ozone and

15   climate data if available, grid reference and data source. Only data with a known grid

16   reference were included in the database.

17

18   *Records of observations of visible-injury symptoms in ambient air*

19   Records of visible injury symptoms attributed to ozone included data observed on

20   grasses, forbs, shrubs and crop species growing in experimental pots or plots, natural

21   vegetation communities and commercial crops from surveys conducted during spring

22   and summer months over the period 1990 - 2006. The database includes records of

23   visible injury from biomonitoring experiments conducted for the ICP Vegetation

24   using nine species from 1994 - 1996 (see Benton *et al*., 2000 for details), and white

25   clover conducted from 1996 – 2006 (see below for details). There are also records

1   included from both unpublished data and *ad-hoc* observations supplied by ozone-

2   specialists that participate in the ICP Vegetation. Overall, this part of the database

3   contained 644 records, of which 70.6% were published in the scientific literature and

4   29.3% were unpublished data.

5

6   With such a diverse dataset, there was a need to apply quality assurance procedures to

7   ensure the data were comparable.   Records of the highest quality were supplied by

8   scientists that have collected seeds or cuttings from species showing injury in the

9   field, grown the species and exposed it to ozone experimentally to confirm the

10   symptoms.   Since such records only accounted for ca. 6% of the data available,

11   records were also included if collected by scientists who research and publish papers

12   on ozone pollution effects and therefore are familiar with 'typical' ozone injury

13   symptoms and could confirm that the symptoms seen matched those published

14   following ozone exposure of the same or related species. No symptoms described as

15   'possibly' due to ozone were included in the analysis presented here. Assessments of

16   the amount of injury per leaf are subject to variation between researchers with

17   underestimates of damage commonly occurring (e.g. Bussotti *et al*., 2006).   To avoid

18   such confounding factors when comparing across survey types, ozone leaf injury data

19   were only included if the presence, rather than the extent, of symptoms was recorded.

20

21   *Evidence of ozone effects from ICP Vegetation biomonitoring studies with white*

22   *clover*

23   The ICP Vegetation biomonitoring programme has involved exposure of an ozone

24   sensitive (NC-S) biotype of white clover (*Trifolium repens* L. cv. Regal) to ambient

25   air since 1996. Cuttings of clover were sent by the Programme Coordination Centre

1 at the Centre for Ecology and Hydrology, Bangor (UK) to participants across Europe,

2 who established the plants according to a standard protocol (see Mills *et al*., 2000 for

3 details). Twenty-eight days after establishing the cuttings, 10 to 25 plants of the NC-

4 S clover were placed in 15 l pots and exposed to ambient air for four to six months at

5 each site. The date for the start of the exposure varied between sites and between

6 years, according to local growing seasons and experimental needs. The majority of

7 sites began exposure of plants in May or June, and the last assessments were carried

8 out in September or October. Plants were cut back to a height of 7cm every 28 days

9 to allow new leaves to develop. At the time of these harvests, the plants were

10 assessed for ozone-specific leaf injury using a common protocol. Injury was apparent

11 in varying magnitudes ranging from pale cream stipples on the leaf surface to large

12 necrotic patches with leaves severely damaged. For comparison across sites, ozone

13 injury was scored as the percentage of leaves visibly damaged by ozone using the

14 following key: 1 = <1% of leaves affected, 2 = 1-5%, 3 = 5-25%, 4 = 25-50%, 5 = 50-

15 90% and 6 = 90-100%. At some sites, ozone-resistant plants (NC-R) were also grown

16 according to the same protocol; the ratio of the biomass of NC-S to NC-R

17 (accumulated over harvests 2, 3 and 4 representing three months of growth) provided

18 an indication of ambient ozone effects on growth at these sites. For those sites visited

19 daily, the date of first appearance of ozone injury symptoms, defined as the date when

20 20% of the plants have one or more injured leaves, was recorded. All data were

21 checked for quality assurance prior to inclusion in the dataset as described in Mills *et*

22 *al*. (2000).

23

24 Scored injury data were available from a total of 45 sites, representing 16 countries

25 across Europe from 1998 to 2006 and biomass ratio data were available from 1996 to

1   2006 for a total of 41 sites from 15 countries (Table 4). However, each individual site

2   did not necessarily perform the investigation every year meaning there were very few

3   sites with a long time-run of data.

4

5   *Ozone risk maps*

6   The EMEP Eulerian model maps ozone concentrations and fluxes on an (approx.) 50

7   km x 50 km grid. Described in detail by Simpson *et al*. (2003), the EMEP model

8   simulates the emissions, transport, transformation and removal of pollutants, and

9   includes the calculation of ozone fluxes using the Deposition of Ozone and Stomatal

10   Exchange module (DO_3SE, described in Emberson *et al*., 2000, Simpson *et al*., 2003,

11   2007 and references therein). For use in this study, the EMEP model generated

12   AOT40 values for crops (termed here AOT40 (crop)) and generic crop flux ($POD3_{gen}$

13   (crop)) maps using the methods described in the Modelling and Mapping Manual of

14   the LRTAP Convention (LRTAP Convention, 2004) for the years 1995 through to

15   2004, inclusive. Parameterisation of the generic crop flux model is reproduced in

16   Table 5. The accumulation period for AOT40 (crop) and $POD3_{gen}$ (crop) was three

17   months, with the timing of the accumulation window reflecting the period of active

18   growth of wheat and centred on anthesis (LRTAP Convention, 2004). This approach

19   provided a moving time interval to reflect the early growing seasons in southern

20   Europe and later growing seasons in northern Europe.   The classification scales for

21   the EMEP risk maps presented here have been chosen to provide a fair comparison

22   between AOT40 and $POD3_{gen}$ maps. Each map has six categories for values

23   exceeding zero, plus a zero category, with the maximum whole number value

24   recorded in any of the years included being divided by six to provide the six evenly

25   spaced categories.

1

2   For this study we have also developed a clover flux model that is specific to NC-

3   S/NC-R clover ($POD3_{gen}$ (clover)) as used at the ICP biomonitoring network. The

4   basic formulation is similar to that of other vegetation but uncertainties are introduced

5   in calculating the ozone concentration at the canopy height because the clover plants

6   in the ICP Vegetation network were in pots surrounded by short vegetation (grass

7   species) – a complex situation for modelling. Thus, here we have assumed that the

8   dominant vegetation is grassland rather than clover itself, with the ozone

9   concentration gradients around the plants being driven more by the surroundings than

10   by the characteristics of clover. On the other hand, the stomatal conductance of the

11   clover itself still drives the uptake of O_3 into the plant. In an effort to account for this

12   complex situation, we calculate the stomatal conductance of clover with clover-

13   specific parameters, but make use of the O_3 gradients calculated for grassland to

14   calculate the O_3 at canopy top. The parameterisations for this model are provided in

15   Table 5, with the derivation and equations described in the Annex to this paper.

16   Clover $POD3_{gen}$ and clover AOT40 maps have been generated for values accumulated

17   over 84 days starting 15 June (the mean start date for ICP Vegetation clover

18   experiments) to represent the three 28d growth periods. Grid square values for these

19   maps are the average of five years data for 2000 – 2004.

20

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

22   Four sets of data were compared with EMEP modelled AOT40 and $POD3_{gen}$ maps

23   and/or grid square values. Firstly, the 50 x 50 km grid squares where visible injury

24   had been detected in published surveys and *ad hoc* observations and the ICP

25   Vegetation biomonitoring experiments described here during the period 1995 -2004

1 were mapped with the ten year mean values for crop AOT40 and crop POD3$_{gen}$. For

2 grid squares where an effect was detected in more than one year in the five-year

3 periods, the presence of effect was only counted once. The second dataset was the

4 mean injury score on white clover for the period June to August on NC-S white clover

5 over the five-year period 2000 – 2004. This data were mapped against AOT40

6 (clover), AOT40 (crop), POD3$_{gen}$ (clover) and POD3$_{gen}$ (crop) for illustration but

7 could not be used to produce dose-response functions due to the non-linearity in the

8 injury score used in the plant-level assessments. The third dataset was for a sub-set of

9 the clover biomonitoring sites where the first day of ozone injury was recorded.

10 Clover AOT40, AO30$_{VPD}$ and POD3$_{gen}$ were calculated for the days from the start of

11 the experiment and the eight days prior to ozone injury for use in an assessment of the

12 short-term critical level for visible injury. Lastly, the fourth dataset used was the

13 white clover biomass ratio (NC-S/NC-R) calculated from the total biomass for the

14 months May, June and July, normalised for the 1.05 ratio recorded at zero AOT40

15 (Hayes *et al.*, 2007b) and converted to percentage biomass reduction. The five-year

16 mean values per site were mapped against the five-year mean crop and clover AOT40

17 and POD3$_{gen}$ (2000 – 2004). In addition, the site- and year-specific values for clover

18 AOT40 (clover) and POD3$_{gen}$ (clover) using the site starting date were accumulated

19 over 84 days and plotted against biomass ratio.

20

21 *Statistical analysis*

22 The datasets described were deemed too inconsistent for analysis of temporal and

23 spatial trends as the number of sites surveyed for injury or included in the clover

24 biomonitoring experiment varied from year to year and were not systematically

25 selected for geographical representation.   Response functions were fitted to the

1   biomass data by non-linear regression within Minitab V.15. Where applicable, data

2   are presented visually for four regions of Europe Northern Europe (NE), Atlantic

3   Central Europe (ACE), Continental Central Europe (CCE), Mediterranean (Med),

4   with countries included in each region as listed in LRTAP Convention (2004).   In

5   Tables 1-3, the Mediterranean region is split into eastern and western Mediterranean.

6

7   **Results**

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

9   Over the years 1990 – 2006, ozone injury was detected in 16 countries of Europe

10 representing each of the five geographical regions studied (Tables 1 - 3 and Figures 1-

11 3). Records of injury were particularly common from Italy, Spain, and Switzerland,

12 but were also common for more northern countries such as Belgium and Sweden.

13 Overall, ozone injury was most commonly reported in central and Mediterranean

14 Europe with more than 200 published records available for each of these regions, with

15 fewer records reported in ACE and NE. There were large year-to-year differences in

16 the number of published and unpublished records reported across Europe (Figure 2).

17 Since this inconsistency may well have reflected the sporadic nature of surveys rather

18 than fluctuations in ozone climate, it was not possible to statistically analyse this

19 dataset for any geographical or temporal trends. Instead, the focus of the analysis of

20 the visible injury dataset has been the overall geographical spread of sites where

21 injury occurred together with the range of species injured in each region, based on the

22 combined records for the period 1990 - 2006.

23

24 Of the 644 records of visible injury over the period 1990 – 2006, 39% were for crops,

25 38.1 % were for forbs and grasses, and 22.9% were for shrubs.   Overall, 27 crop

1 species exhibited visible injury including agricultural crops such as maize, potato,

2 wheat, durum wheat and soybean, and horticultural crops such as lettuce, chicory,

3 radish, courgette and onion (Table 1). These effects were detected in 14 countries

4 representing each of the five geographical regions. Ten or more crops were injured in

5 Greece, Italy and Spain and it is also of note that ozone injury was detected on six

6 crop species in Sweden (NE).

7

8 Ninety-five species of grasses and forbs exhibited typical ozone injury in ambient air

9 at sites across Europe (Table 2). Overall, the vast majority of the species injured were

10 forbs, with injury being more difficult to identify on grasses and only reported for 8

11 species. Species from the same genus were injured at sites in several countries. For

12 example, *Centaurea jacea* was injured in Poland, Switzerland and Italy, *Centaurea*

13 *nigra* was injured in Italy, UK and Ukraine, *Centaurea paniculata* was injured in

14 Switzerland and *Centaurea scabiosa* was injured in France and the Ukraine. In

15 addition to the *Trifolium* spp. used in the ICP Vegetation biomonitoring programme,

16 other examples of genus' that were well represented within the database were *Rubus*

17 spp, with injury recorded on five species growing in France, Switzerland, Italy and

18 Spain and *Epilobium* spp. with three species injured in France, Spain and Italy.

19

20 Ozone injury was reported for 49 species of shrubs growing in France, Italy, Poland,

21 Spain and Switzerland (Table 3). Records for *Viburnum* spp. were the most

22 widespread with injury occurring on four species at sites in France, Italy, Spain and

23 Switzerland. *Rosa canina* was injured in three countries (Italy, Spain and

24 Switzerland) whilst many other species were injured in two countries such as

25 *Lonicera caprifolium*, *Robinia pseudoacacia* and *Sambucus racemosa*.

1

2   The geographical distribution of locations in Europe where visible injury was detected

3   and published in the scientific literature over the period 1995 – 2004 is shown in

4   Figure 3 superimposed on the 10 year average for AOT40 (crop) and $POD3_{gen}$ (crop).

5   There was a clear north-south increase for AOT40 (crop), with the highest modelled

6   values being found in Italy, whilst $POD3_{gen}$ (crop) was > 18 mmol m^{-2} across a large

7   region of Europe covering central and southern areas and spreading northwards into

8   southern UK and Scandinavia. Overall, 62% of injury locations were in grid squares

9   with AOT40 (crop) values below the critical level of 3 ppm h to protect crops against

10   effects on biomass and yield suggesting that some effects (even if not on biomass and

11   yield) can occur well below this value (Figure 3). No obvious threshold value was

12   apparent but most grid squares (29%) were in the category 2 - 3 ppm h for AOT40

13   (crop).   In contrast, only 9% of the injury locations were found within grid squares

14   with an $POD3_{gen}$ (crop) of < 12 mmol m^{-2}, with 7%, 27% and 47.3% of locations

15   falling in grid square categories 12 - 18, 18 – 24 and 24 – 30 mmol m^{-2} respectively.

16   The proportion of grid squares with injury falling within the three highest categories

17   was 22% for AOT40 (crop) and 56% for $POD3_{gen}$ (crop).   From Figure 3b, a

18    $POD3_{gen}$ (crop) of 12-18 mmol m^{-2} can be tentatively interpreted as a threshold for

19   likely occurrence of ozone injury. Unfortunately, published records of the absence of

20   ozone symptoms are extremely rare making it impossible from this evidence to

21   confirm this threshold.

22

23   *Visible injury occurrence in the ICP Vegetation white clover biomonitoring*

24   *experiments.*

1   Ozone injury occurred at almost all sites in the years included in this study (1998 –

2   2004), with only 5 of the 52 site/year combinations recording an absence of injury.   A

3   sub-set of sites (28 data points) recorded the first date of ozone injury on NC-S white

4   clover. For the 8 days prior to injury appearance or for all days since day 0 of the

5   experiment, the EMEP modelled AOT40 (clover) provided little evidence for a

6   threshold AOT40 value (Figure 4a). For both time periods, the cumulative frequency

7   of percentage of data points increased rapidly with increasing AOT40 (clover), with

8   42.9% and 35.7% of data being for AOT40s below 0.1 ppm h (for 8 days and day 0 to

9   first day of injury, respectively). A similar pattern existed for $AOT30_{VPD}$ (clover)

10   (Figure 4b) with 4 sites recording ozone injury at grid square $AOT30_{VPD}$ of below the

11   critical level of 0.16 ppm h (Italy-Isola Serafini 1999, Italy-Rome 2004, Belgium-

12   Tervuren 2004, UK-Ascot 2004). In contrast, there was stronger evidence of a

13   threshold for $POD3_{gen}$ (clover) of ca. 3-4 mmol m^{-2} with 3.5% of data points falling

14   below 2 mmol m^{-2}, 7.2% falling between 2 and 3 mmol m^{-2}, and 30.2% falling

15   between 3 and 4 mmol m^{-2} for $POD3_{gen}$ (clover) values accumulated for 8 days prior

16   to injury (Figure 4c).

17

18   The five-year mean injury score values were superimposed on crop and clover

19   AOT40 and $POD3_{gen}$ maps to illustrate the geographical distribution (Figure 5). The

20   AOT40 maps for the two time periods showed similar patterns but AOT40 (clover)

21   values were larger than AOT40 (crop) values. Site-specific AOT40 (clover) values,

22   determined for 84 days from 15 June were higher than AOT40 (crop) values (clover

23   AOT40 (clover) = 1.72 * AOT40 (crop), r^2 = 0.75, figure not presented) reflecting the

24   higher ozone concentrations in the later months of the clover experiment (mean start

25   dates were 33, 35 and 54 days later for ACE, CCE and Med respectively). $POD3_{gen}$

1 (clover) values were also higher than POD3$_{gen}$ (crop) values with the region of

2 medium - high fluxes (> 24 mmol m^{-2}) covering larger areas of Europe stretching as

3 far north as southern UK and southern Scandinavia. Overall, the highest injury scores

4 on white clover were detected in central and southern Europe, but not all of these sites

5 coincided with the highest mean crop or clover AOT40 values (Figure 5). In contrast,

6 the injury score data showed a closer correlation with POD3$_{gen}$ with the highest injury

7 scores being associated with medium to high fluxes. For example, all sites with a

8 mean score >1 had an POD3$_{gen}$ (clover) of > 36 mmol m^{-2}. The corresponding value

9 for AOT40 was an AOT40 (clover) of 1 ppm h.

10   .

11

12 *Effects on biomass in the ICP Vegetation white clover biomonitoring experiment*

13 Data from 10 countries contributed to this analysis, with the mean normalised %

14 biomass reduction being greatest in Italy (33.3 %, n=10) and Greece (30%, n= 2),

15 with no effects being consistently detected in the UK (Table 4). For other countries

16 there was a wide range in % biomass reduction reflecting year to year variation in

17 AOT40 (clover) and POD3$_{gen}$ (clover), with maximum reductions being as high as

18 25.2 %, 20.8%, and 24.8 % for Austria, Germany and Spain respectively. The mean

19 biomass reduction per site is shown in Figure 6 for crop and clover AOT40 and

20 POD3$_{gen}$ averaged over the period 2000 – 04. For both AOT40 (crop) and AOT40

21 (clover), mean biomass reductions of > 10% were detected in areas where the

22 modelled AOT40 was below the critical level of 3 ppm h (Figure 6). By comparison,

23 such higher mean effects were found in the grid squares with medium – high fluxes.

24

1   Site- and year-specific grid values for AOT40 (clover) and AOT40 (crop) were

2   calculated from the EMEP model outputs and plotted against percentage biomass

3   reduction (Figure 7). Not surprisingly there was a lot of scatter in this data (discussed

4   later). Separate plots of the data for sites with modelled AOT40 values that exceeded

5   the critical level of 3 ppm h revealed linear relationships that were significant for

6   AOT40(clover) ($r^2 = 0.58$, p <0.001) where data from CCE and MED contributed, but

7   not for AOT40 (crop) ($r^2 = 0.28$, p=0.179) where only data from MED had an AOT40

8   of > 3 ppm h. However, the relationship for AOT40 (clover) seems to be being

9   driven by the large number of points with an AOT40 of 3 - 4 ppm h, with relatively

10   few points for the higher AOT40 values. A similar approach was used for $POD3_{gen}$.

11   For both Figure 8a and Figure 8b there is some indication of a threshold value, above

12   which ozone effects start to occur consistently. For $POD3_{gen}$ (clover), this threshold is

13   ca. 40 mmol m^{-2}; a separate plot of the sub-set of data for $POD3_{gen}$ > 40 shows a

14   strong linear relationship ($r^2 = 0.58$, p <0.001), with data points from ACE and CCE

15   well spread along the regression line. The relationship between $POD3_{gen}$ (crops) and

16   clover biomass reduction was less strong for data points where $POD3_{gen}$ (crops) > 15

17   mmol m^{-2} especially for the higher fluxes, but was significant ($r^2 = 0.21$, p=0.002).

18

19   For the 57 data points within the clover biomass dataset, the grid square values were

20   compared for AOT40 (clover) and $POD3_{gen}$ (clover) (Figure 9). There was a strong

21   polynomial relationship between the two parameters (p = 0.93) which could be broken

22   down into region-specific linear relationships. At low AOT40s, $POD3_{gen}$ was higher

23   for CCE than for ACE presumably indicating climatic conditions that were more

24   conducive to ozone uptake. Between AOT40s of 0 and 3 ppm h, $POD3_{gen}$ for ACE

25   increased more slowly for CCE and MED than for ACE; this slower rate of

1 accumulation of $POD3_{gen}$ per unit AOT40 in CCE and MED continued at the higher

2 AOT40s not found in ACE.

3

4 **Discussion**

5 This study has clearly indicated that many crops and (semi-)natural vegetation

6 communities are responding to current ambient ozone in Europe. The most easily

7 recognisable and most commonly reported effect has been the development of

8 characteristic ozone injury on leaves.   Such effects have been noted every year over

9 the period 1990 – 2006, with over 170 species being reported as having developed

10 ozone injury. Injury was reported in each of the five geographical regions of Europe,

11 including in northern Europe where maps indicate relatively low AOT40. There is

12 also evidence from the ICP Vegetation clover experiment that ambient ozone

13 concentrations are sufficiently high at several locations in Europe to reduce the

14 growth of an ozone-sensitive species.   Injury and biomass effects were most

15 prevalent in southern European countries, but were also found in central and northern

16 Europe. The results presented here provide significant progress towards identifying

17 field evidence of the improved performance of flux-based compared to concentration-

18 based risk maps and provide some justification for the biologists' preference for flux-

19 based approaches (as outlined by Simpson *et al.,* 2007). They also highlight the

20 potential threat to vegetation from future increases in ozone pollution predicted for the

21 near decades.

22

23 The ozone effects data presented here have been subjected to quality assurance

24 procedures in order to reduce uncertainty. The highest potential source of uncertainty

25 was in the field observations whereby scientists may have wrongly assigned visible

1   injury caused by other stresses to ozone pollution. This was minimised by inclusion

2   of observations that were either verified by ozone exposure experiments or recorded

3   by experienced ozone-specialist scientists, the latter being the most consistent of 25

4   observers in a quality assurance trial described by Bussotti *et al*. (2006). Injury data

5   from the ICP Vegetation clover experiments were more robust as a common protocol

6   was followed using plant material originating from the same source. Photographs

7   were provided to guide assessments and the use of a broad range of injury scores

8   rather than % injury helped to prevent the problems of under-estimation of damage

9   described by Bussotti *et al*. (2003a). The NC-S and NC-R biotypes were originally

10   selected in the southern-Europe-like climate of North Carolina, USA (Heagle *et al.*,

11   1994), but have been extensively employed in many parts of the USA as

12   bioindicators. In the current study, rigorous quality assurance checks (described in

13   Mills *et al*., 2000) resulted in exclusion of about one-quarter of the data, including

14   some from northern Europe where the NC-R biotype was sensitive to downy mildew

15   in wetter summers.

16

17   Much of the evidence presented here is based on the occurrence of ozone injury on

18   the leaves of sensitive species.   Several authors have argued that visible injury is

19   sometimes of little biological significance to the plant in that growth or seed

20   production are not always reduced by ozone when injury symptoms are present (e.g.

21   review by Bassin *et al.*, 2007). We tested this argument by comparing lists of species

22   injured by ozone in ambient air with response functions we derived in earlier studies

23   for yield and biomass effects that were based on ozone-exposure experiments (Hayes

24   *et al.*, 2007a, Mills *et al.*, 2007a). Of the nine crop species with an AOT40 critical

25   level of $<= 5$ ppm h (Mills *et al*., 2007a), all except cotton and turnip exhibited ozone

1　injury in the surveys reported here. Similarly, 5 of the 8 species classified by Mills *et*

2　*al*. (2007a) as moderately sensitive (maize, sugar beet, potato, tobacco and grapevine)

3　were identified as showing ozone injury in the field whilst no visible effects were

4　reported for the crops classified by Mills *et al*. (2007a) as resistant to ozone. Of the

5　species of (semi-)natural vegetation exhibiting injury, 9 were reported by Hayes *et al.*

6　(2007a) as having a relative sensitivity based on biomass effects of <0.9, and 6 had a

7　relative sensitivity of 0.9 – 1. At the time of the Hayes *et al*. (2007a) study, there was

8　no/insufficient biomass response data available with which to classify the other

9　species that are reported here as developing ozone injury in the field. Although data

10　from injury surveys cannot be directly compared with yield or biomass response data,

11　there is thus clear evidence that those species known to respond negatively to ozone in

12　experiments by either reduced growth or reduced seed production often develop

13　ozone injury in the field in response to ambient ozone, whilst those known to be

14　ozone insensitive have not been reported as showing such symptoms.

15

16　Before considering the link between measured effects and modelled ozone exposure it

17　is important to consider the uncertainty associated with mapping AOT40 and $POD3_{gen}$

18　within the EMEP model. Both of these indices are sensitive to the characteristics of

19　the frequency distribution of ozone concentrations (Tuovinen *et al*., 2007, 2009) with

20　both showing increased sensitivity with increasing threshold. However, as lower

21　ozone concentrations contribute more to $POD3_{gen}$ than to AOT40 (ca. 7 ppb for the

22　$POD3_{gen}$ (clover) model included here), this parameter is less sensitive to threshold

23　effects than AOT40 (LRTAP Convention, 2004, Tuovinen *et al*., 2007). Additional

24　sources of uncertainty associated with the simulation of the emissions, transport and

25　deposition of ozone and its precursors are described in Simpson *et al.* (2003a, 2003b,

1   2007). Because of the disparity between both the AOT40 (crop) and $POD3_{gen}$ (crop)

2   accumulation periods in relation to the timing of the ICP Vegetation experiments

3   (which usually start in June, 30 – 55 days after the latitude-derived $POD3_{gen}$ (crop)

4   accumulation period starts, for further details, see Hayes *et al.*, (2007b)), and the

5   physical nature of the ICP network (potted plants surrounded by short vegetation), we

6   devised for this study a generic clover flux model for the pot-based clover

7   biomonitoring system (Annex 1). The higher g_{max}, lower f_{min} and higher T_{max} for

8    $POD3_{gen}$ (clover) compared to $POD3_{gen}$ (crop), together with the higher ozone

9   concentrations and warmer climate for the mid-June to mid-August period result in

10   higher values for $POD3_{gen}$ (clover) than $POD3_{gen}$ (clover) for the same geographical

11   region. A major uncertainty in the clover model is the calculation of canopy-height

12   ozone concentration. This uncertainty is caused by a number of factors including the

13   dense canopy and high stomatal conductance of the potted clover in relation to the

14   surrounding grassland, and the experimental design with pots usually placed on grass

15   and being well-spaced out, resulting in an heterogeneous surface roughness that is

16   difficult to model. The method for calculating ozone concentration provided here for

17   the clover model was thus a first attempt to account for such factors. It provides a

18   consistent methodology across all sites, but it is impossible to assess any biases

19   associated with the difficulty of modelling ozone concentration. In addition, the

20   clover stomatal conductance parameterisation was generalized to provide one

21   parameterisation for the 8 countries contributing data and for the two biotypes (NC-S

22   and NC-R). This introduces some uncertainty into the analysis as within the gs

23   datasets there was some evidence that plants showed variable acclimatisation to local

24   conditions (e.g. in cooler climates the stomates show a tendency for closure at lower

25   VPDs than in warmer climates, Mills *et al.*, 2003). Unfortunately, the limited range of

1   key environmental variables measured at individual sites did not allow for climate

2   specific parameterisations to be developed; a generic "pan-European" flux modelling

3   approach was selected enabling direct comparison with the crop maps shown.

4

5   At the farm scale, injury-causing ozone episodes can have a catastrophic effect, for

6   example, one farmer in Greece lost a chicory crop worth Euro 15000 as a result of one

7   ozone episode (Velissariou, *pers. comm.* and described in more detail in Hayes *et al.*,

8   2007b). Thus, there is a need to develop a method for assessing the risk of such

9   catastrophic effects on a pan-European scale. Although the number of incidences of

10   ozone injury per area strongly reflects the reporting effort, this study has nevertheless

11   shown that ambient ozone over the period 1990 – 2006 induced visible injury on

12   ozone sensitive species in many parts of Europe, including in northern Europe where

13   ozone concentrations are generally lower than in central and southern Europe.   Injury

14   was detected in areas where there is a long history of relatively high ozone

15   concentrations and some resistance to ozone has been detected in the field (e.g.

16   *Centaurea jacea*, Bassin et al., 2004, *Plantago major*, Reiling and Davison, 1992). To

17   fully understand the thresholds above which ozone injury occurs, more data is needed

18   on the conditions that do not lead to ozone injury. In the absence of such data, we

19   have analysed the data from the ICP Vegetation clover biomonitoring experiment by,

20   for the first time, applying the EMEP model to short-time periods to determine

21   whether exceedance of the modelled $AOT30_{VPD}$ critical level was associated with

22   occurrence of visible injury. For 24 of the 28 data points this was found to be the

23   case. However, a threshold was more evident for $POD3_{gen}$ (clover), with a rapid

24   increase in incidences of ozone injury for values between 2 and 3 mmol m^{-2} for the 8

25   days prior to injury and from day 0 to injury. Using either index, the EMEP model

1   could be used to predict for current and future ozone conditions the frequency of

2   injury-causing episodes. Such maps and data could be used to assess the risk of

3   damage to leafy vegetable crops, the economic value of which depends on the

4   appearance of the leaf.

5

6   We tested here the efficacy of the AOT40-based critical level for crops using the

7   clover biomass data and the EMEP modelled data for AOT40. Whether AOT40 was

8   calculated for the crop growth period or the clover growth period, ca. 40% of sites had

9   a > 10% biomass reduction in NC-S at modelled AOT40 values below the critical

10   level of 3 ppm h. For both accumulation periods, a relatively small number of points

11   for higher effects at high AOT40 values led to a significant linear relationship

12   between AOT40 and % biomass reduction above the current critical level.

13   Furthermore, there was widespread occurrence of visible injury in grid squares that

14   were below the critical level for yield reduction. Although, as already discussed, the

15   latter cannot necessarily be equated with an effect of biological significance to the

16   plant, when taken with the biomass reduction data such widespread occurrence does

17   tend to suggest that the AOT40-based critical level is insufficiently robust for

18   predicting the damaging effects of ozone on vegetation in Europe. This approach to

19   mapping risk could possibly be improved by (1) using a different cut-off value instead

20   of 40 ppb as correlations between ozone injury and locally measured ozone

21   concentrations were improved in both Spain and Sweden when 20 and 30 ppb were

22   used as the cut-off values (Ribas and Penuelas, 2003, Pihl Karlsson *et al*., 2004); and

23   (2) using species-specific time intervals that better captured the time of damaging

24   ozone episodes as tested here for the AOT40 (clover) model.

25

1   In contrast to the AOT40 maps, the risk maps based on the flux parameter, $POD3_{gen}$,

2   provided a better representation of the areas where ozone effects occurred for all four

3   types of effect data. Flux-effect relationships were improved when a species-specific

4   model ($POD3_{gen}$ (clover)) was used. These important results are supported by locally

5   parameterised flux models for sites in, for example, Spain (Filella *et al.,* 2005) and

6   Italy (Fagnano and Merola, 2007). They are also supported by analyses of data from

7   early ICP Vegetation experiments that identified the importance of locally measured

8   conductance modifying factors such as VPD and rainfall as contributory factors for

9   the response to ozone (Benton *et al*., 2000; Ball *et al*., 2000).   Further support for the

10   improved performance of $POD3_{gen}$ maps compared to AOT40 maps comes from the

11   many locations in Germany, The Netherlands and Sweden where ozone effects were

12   noted in grid squares predicted to have relatively low AOT40 (crop) (< 3 ppm h) but

13   mid-range $POD3_{gen}$ (crop) (18 - 30 mmol m^{-2}). This paper presents some evidence to

14   support critical levels for $POD3_{gen}$ (clover) of ca. 40 - 50 mmol m^{-2} for biomass

15   effects and ca. 3 mmol m^{-2} for appearance of visible injury, but these would need to

16   be tested further with site-specific measured data. Since data from ACE, CCE and

17   MED are contributing to the response function shown in Figure (9d), it can be

18   concluded that it is appropriate to combine data sets from the different regions of

19   Europe for the derivation of a flux-based critical level for Europe-wide application.

20

21   **Conclusion**

22   Our study has provided evidence that ambient concentrations of the pollutant ozone

23   have repeatedly induced damage to vegetation across 17 European countries during

24   the period 1990 to 2006. Species exhibiting visible injury in the field match those

25   identified in exposure experiments to be sufficiently sensitive to ozone to have

1   reduced biomass or yield at concentrations within the European range of ozone

2   concentrations.   Biomass reductions have been found in ambient air for the sentinel

3   bioindicator species, *Trifolium repens*. Unfortunately, data records compiled for this

4   study were too inconsistent for identification of long-term trends in effects in response

5   to the changing ozone profile. Overall, flux-based risk maps were better predictors of

6   the areas where ozone damage occurred than AOT40–based risk maps, with

7   predictive ability improving for time-period accumulation and effect data matched

8   maps. In many areas of Europe (e.g. Belgium, northern Germany, southern Sweden)

9   there is evidence of effects where AOT40 values (crop and clover) are predicted to be

10   low, whilst ozone flux (crop and clover) is moderate to high due to the climatic

11   conditions being conducive to high stomatal uptake. Thus, this study provides

12   important validation data to provide support to the use of the biologically more

13   meaningful flux-based approach for risk assessment.

14

15   The most disconcerting outcome of this study is that the current ambient ozone

16   climate of Europe is already having extensive impacts on vegetation across Europe.

17   Since ozone damage has also been detected in other parts of the world including the

18   USA (e.g. Bennett *et al*., 2006, Davis & Orendovici, 2006, Booker *et al*., 2009) and

19   south-east Asia (Emberson *et al*., 2009), the problem appears to be global.   Even with

20   implementation of current legislation, ozone concentrations are predicted to continue

21   to rise across most of the world over the coming decades (Royal Society, 2008) and

22   thus it is likely that ozone impacts on vegetation will worsen. Indeed, the Royal

23   Society report (2008) predicted that by 2030, tropospheric ozone pollution could pose

24   as big a threat to global food security as climate change.   Thus, there is an urgent

25   global need for coordinated effort to reduce the emissions of the precursors of ozone

1 pollution to benefit security of food supplies, improve human health and help reduce

2 global warming.

3

15

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

2

3   The procedures specified in the Modelling and Mapping Manual (LRTAP

4   Convention, 2004) for calculating PODY (described as AFstY in LRTAP Convention,

5   2004) or indeed AOTX, and implemented in the EMEP chemical transport model

6   (Simpson *et al.*, 2003), have been designed on the assumption that the vegetation in

7   question provides a homogeneous canopy (`big-leaf'), so that vertical O_3 gradients

8   above the canopy can be derived with standard turbulence profiles (Tuovinen et al.,

9   2009).

10

11   The ICP Vegetation biomonitoring programme consists of potted clover plants, sitting

12   above a short vegetation (or bare-soil) surface, surrounded usually by grassland or

13   other low vegetation. Such a situation is theoretically difficult to model, and does not

14   conform to the assumptions of the Modelling and Mapping Manual methods. The O_3

15   concentration impinging on the upper leaves of these clover plants will be affected

16   more by the characteristics of the surrounding vegetation than of the clover plants

17   themselves. To generate a flux-estimate for potted clover (F_{st}(clover)) which accounts

18   for this situation, we calculate

19
20   F_{st}(clover) = O_3(grass) . g_{sto}(clover)
21
22   where g_{sto}(clover) is the effective stomatal conductance of clover found by applying

23   the DO_3SE model, using the NC-S/NC-R parameterisation derived as described below

24   and presented in Table 5. O_3(grass) is the O_3 concentration found at the top of a grass

25   canopy, estimated using the grassland parameterisation from the standard EMEP

26   model (Simpson *et al.*, 2003). The effective stomatal conductance represents that

27   fraction of the O_3 flux entering via the stomata, as opposed to that lost through

1　　deposition to the external leaf surface. The relation between these terms is discussed

2　　in Tuovinen et al., (2009).

3

4　　The g_{sto}(clover) model uses the following formulation based on the stomatal

5　　conductance algorithm described in the Modelling and Mapping Manual, see LRTAP

6　　Convention (2004) for further details.

7
8　　$g_{sto}(clover) = g_{max} * f_{phen} * f_{light} * max \{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\}$
9
10　　The g_{sto}(clover) model is parameterized to represent a hybrid NC-S/NC-R clover plant

11　　based on a dataset described in Mills et al. (2003) that includes over 5000 stomatal

12　　conductance measurements made at nine sites in Europe from 1998 – 2000 (Austria-

13　　Seibersdorf, Belgium-Tervuren, Germany-Essen, Germany-Trier, Italy-Milan, Italy-

14　　Rome, Spain-Ebro Delta, Sweden-Gothenburg, UK-Bangor). Data was supplied by G

15　　Mills, P Büker, F Hayes, W Werner, B Gimeno, I Fumagalli, B Köllner, F Manes, G Pihl

16　　Karlsson, G. Soja and K Vandermeiren.

17
18　　**g_{max} and f_{min}**
19　　g_{max} is derived from the 90th percentile and g_{min} from the 10th percentile values of the

20　　entire NC-S and NC-R stomatal conductance dataset. g_{max} was converted from a

21　　conductance for water vapour (H_2O) to a conductance for O_3 using a ratio of 0.662

22　　based on the diffusivities of H_2O to O_3 in air after Massman (1998).This gave a value

23　　of 532 mmol O_3 m^{-2} s^{-1} for the mean of the two biotypes. The f_{min} is expressed as a

24　　fraction of g_{max}. All values are shown in Table A1.

25
26　　**Boundary line derivation for f_{light}, f_{temp} and f_{VPD}**
27　　For the derivation of f_{light}, f_{temp} and f_{VPD} relationships a boundary line approach was

28　　used. This method defined the 90th percentile values of stomatal conductance within

29　　different incremental classes of environmental data which were: 100 μmol m^{-2} s^{-1} for

1   f_{light}; $2^{o}C$ for f_{temp} and 0.1 kPa for f_{VPD}. Figure A1 shows the resulting boundary lines,

2   fitted by eye, for each biotype of clover using the generic functions described in the

3   Mapping Manual (LRTAP Convention, 2004). To derive the NC-S/NC-R

4   parameterization, mean values (i.e. the mean of the co-efficient for f_{light} and means of

5   the threshold values for f_{temp} and f_{VPD}) were calculated from the individual biotypes;

6   all values are presented in Table A1. Note: for f_{VPD}, data classes with fewer than 10

7   data points were used to guide the derivation for high VPDs; these are denoted by

8   grey shading in the figures.

9

10 The g_{sto}(clover) model assumes that f_{phen} and f_{SWP} are both equal to 1. This maintains

11 consistency with the crop generic flux model (LRTAP Convention, 2004).

12
13 **Table A1** Parameterisation for the NC-S and NC-R clover clones and the hybrid NC-

14 S/NC-R clover clone used to parameterise the g_{sto}(clover) model.

15

	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

16
17

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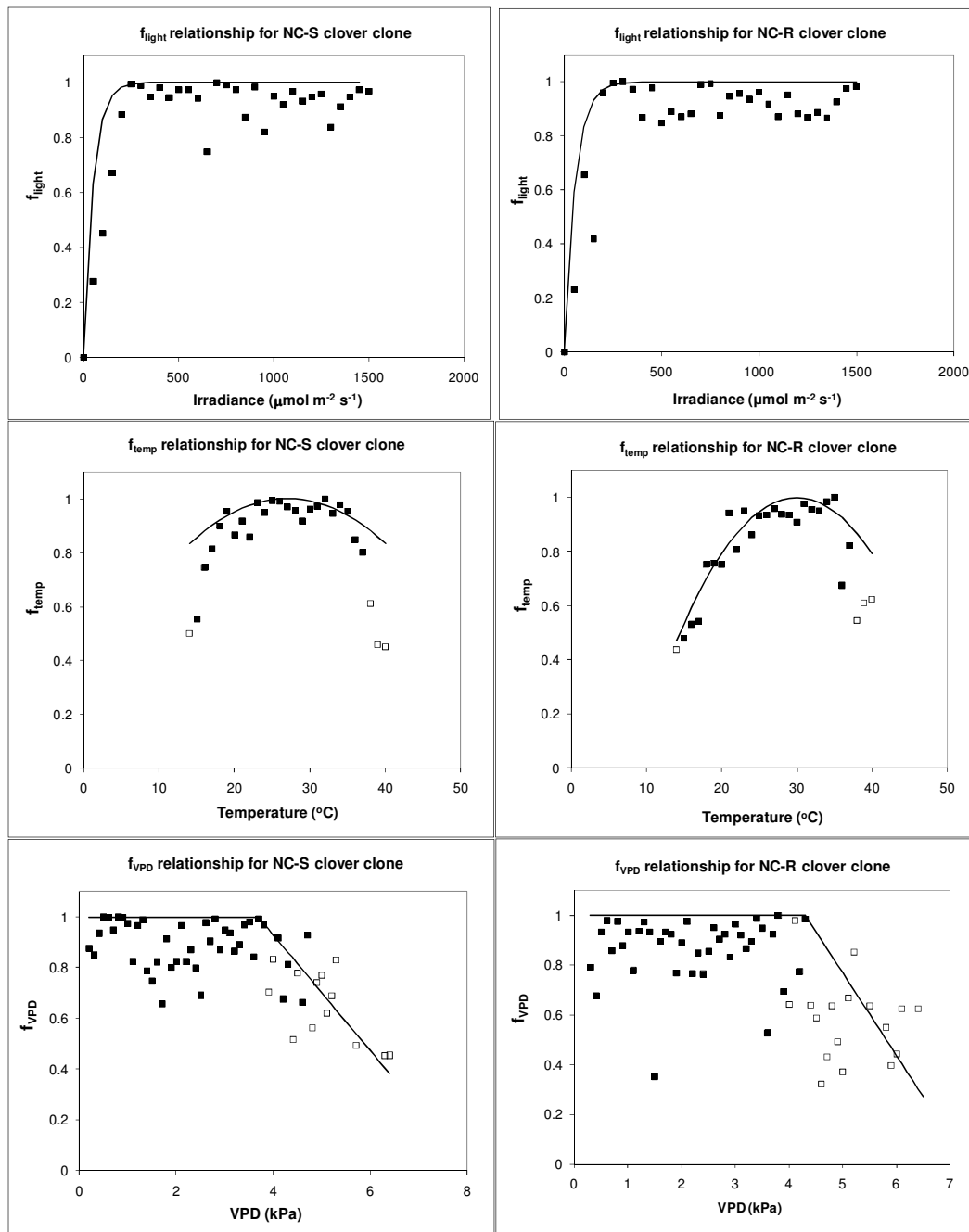
Figure A1. Boundary line derivation of f_{light}, f_{temp} and f_{VPD} for the NC-S and NC-R clover bio-types. Solid line represents the boundary line; filled squares represent data points with equal or greater than 10 data points within each environmental class; open squares represent data points with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

1   **Figure Legends**
2
3   **Figure 1:** Locations of records of visible injury attributed to ozone on crops, (semi-
4   )natural vegetation and shrub species for the period 1991 – 2006 (includes published
5   data and previously unpublished observations).
6
7   **Figure 2**: Number of published and unpublished records of visible leaf injury
8   symptoms attributed to ambient ozone for each year (1991 – 1996). Note: the
9   fluctuation in number of records per year may be more strongly associated with
10   recording effort than with severity of ozone effect.
11
12   **Figure 3:** Locations where ozone injury has been detected in the field and published
13   in scientific papers for the period 1995 – 2004 superimposed on EMEP modelled (a)
14   AOT40 (crop), ppm h (b) and POD3$_{gen}$ (crop), mmol m^{-2} using methods described in
15   LRTAP Convention (2004), and averaged over the same time period. Data sources:
16   Benton *et al*. 2000; Benton *et al*. 1996; Bermejo *et al*. 2003; Bermejo, *et al*. 2002;
17   Bungener *et al*. 1999; Bussotti *et al*. 2003; Carrasco-Rodriguez *et al*. 2001; Faoro and
18   Iriti, 2003; Gimeno *et al*. 1996; Innes *et al*. 2001; Manning *et al*. 2002; Manning and
19   Godzik, 2004; Novak *et al*. 2003; Persson *et al*. 2003; Pihl Karlsson, *et al*. 1995;
20   Piikki *et al*. 2004; Pleijel *et al*. 1994; Pleijel *et al*. 1999; Pleijel *et al*. 1997; Ribas and
21   Penuelas, 2000; Saitanis *et al*. 2004; Saitanis, 2003; Skelly *et al*. 1999; VanderHeyden
22   *et al.* 2001; Velissariou, 1999; Velissariou and Kyriazi, 1996; Velissariou *et al*. 1996;
23   Velissariou *et al*. 1992 and Velissariou, 1999. Note: some dots for injury location
24   overlap.
25
26   **Figure 4**: Cumulative frequency of number of records for (a) AOT40(clover), (b)
27   AOT30$_{VPD}$ (clover) and (c) POD3$_{gen}$ (clover) accumulated from either day 0 of the
28   experiment (■) or for the 8 days prior (□), to the first occurrence of visible ozone
29   injury on NC-S white clover.
30
31   **Figure 5:** Five-year mean ozone injury score (June to August) on ozone-sensitive
32   white clover (NC-S) at ICP Vegetation sites for the period 2000 – 2004 superimposed
33   on the EMEP five-year mean for (a) AOT40 (crop), ppm h, and (b) AOT40 (clover),
34   ppm h (c) POD3$_{gen}$ (crop), mmol m^{-2} and (d) POD3$_{gen}$ (clover), mmol m^{-2} for the same
35   years. The injury score data by country was (n, mean, SE mean): Austria (4, 1.3,
36   0.37); Belgium (3, 1.4, 0.35); Germany (15, 1.7, 0.79); Italy (7, 3.5, 0.4); The
37   Netherlands (1, 0.7, -); Slovenia (5, 2.3, 0.3); Spain (3, 4.07, 0.04); Sweden (5, 1.7,
38   0.3); Switzerland (4, 4.0, 0.3); UK (5, 0.7, 0.3).
39
40   **Figure 6**: Mean normalised percentage biomass reduction in ozone-sensitive white
41   clover (NC-S relative to NC-R) at ICP Vegetation sites in 2000 – 2004 superimposed
42   on the EMEP five-year mean for (a) AOT40 (crop), ppm h, and (b) AOT40 (clover),
43   ppm h, (c) POD3$_{gen}$ (crop), mmol m^{-2} and (d) POD3$_{gen}$ (clover), mmol m^{-2}. The
44   biomass data is described in Table 4.
45
46   **Figure** 7 Response functions for clover biomass reduction and site- and year-specific
47   grid square values for AOT40 (crop), (a) and (c), and AOT40 (clover), (b) and (d).
48   Figures (a) and (b) show the complete data set and Figures (c) and (d) show only the
49   values for effects at AOT40 > 3 ppm h.
50

1   **Figure 8** Response functions for clover biomass reduction and site- and year-specific
2   grid square values for $POD3_{gen}$ (crop), (a) and (c), and $POD3_{gen}$ (clover), (b) and (d).
3   Figures (a) and (b) show the complete data set and Figures (c) and (d) show only the
4   values for effects at (c) $POD3_{gen}$ (crop) > 15 mmol m^{-2} and (d) $POD3_{gen}$ (clover) > 40
5   mmol m^{-2}.
6
7   **Figure 9:** Relationship between clover site and year specific values for AOT40
8   (clover) and $POD3_{gen}$ (clover) accumulated over 3 months from experimental start
9   date. (a) whole dataset, (b) data set separated into regions, with the key: NE
10 (Northern Europe); ACE (Atlantic Central Europe); CCE (Continental Central
11 Europe) and Med (Mediterranean Europe). Note: linear function not fitted for NE as
12 there were only 3 data points.
13
14

1   **Table 1**: Agricultural and horticultural crops exhibiting visible leaf ozone injury in the field
2   (ad hoc observations, surveys). Note: Records of injury on clover species (*Trifolium* spp.)
3   where they occur as components of managed pasture are included within this table.
4

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

1
2 **Table 2:** Species of (semi-)natural vegetation (grasses and forbs) exhibiting visible leaf
3 ozone injury in the field (ad hoc observations, surveys). Note: Records of injury on clover
4 species (*Trifolium* spp.) where they occur as components of meadows and unmanaged
5 grasslands are included within this table.
6
7

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

1

2 **Table 3:** Species of shrubs exhibiting visible leaf ozone injury in the field (*ad hoc*

3   observations, surveys).

4

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

1   **Table 4** Description of the ICP Vegetation biomonitoring experiment database (1996 – 2004)
2   on ozone effects on the biomass of NC-white clover.
3

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

1   **Table 5 :** Parameterisation for the generic crop and the NC-S/NC-R clover flux models. For
2   the clover flux model (see Annex for further details), the start of the growing season was
3   defined as the 15th June (the mean start date for the experiments across all sites), each clover
4   experiment lasted for 84 days with the end of the experiment defining the end of the growing
5   season. Full details of model formulations are provided in the Mapping Manual (LRTAP,
6   2004).
7

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

8
9   [1] estimate from curves
10   [2] This routine prevents stomatal re-opening within the model in the late afternoon under
11   declining VPD conditions (see LRTAP Convention, 2004, for further details). We have
12   assumed that this function is not operating in NC-S/NC-R clover as included in the clover
13   model already in DO_3SE.
14   [3] assumed to be same as for generic crop
15   [4] Green LAI (Leaf Area Index), SAI (surface area index = green leaf area index + senescent
16   leaf area index) and h are used for the calculation of O_3 concentrations. Here, grassland
17   parameters from Simpson & Emberson (2006) have been used for clover (except grassland
18   height, h, set to 30 cm for this study), for further details see Annex.
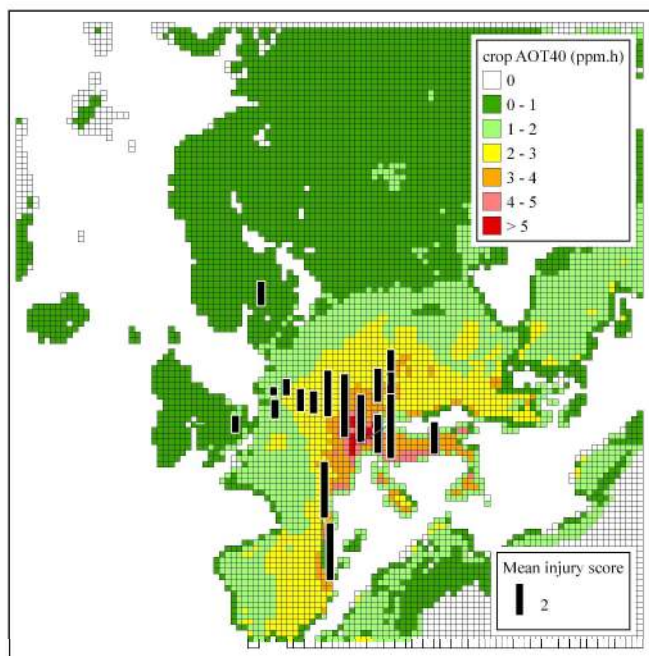19

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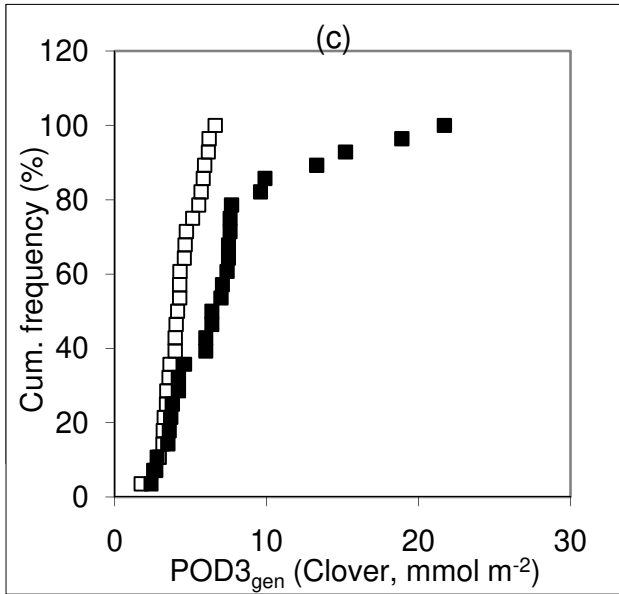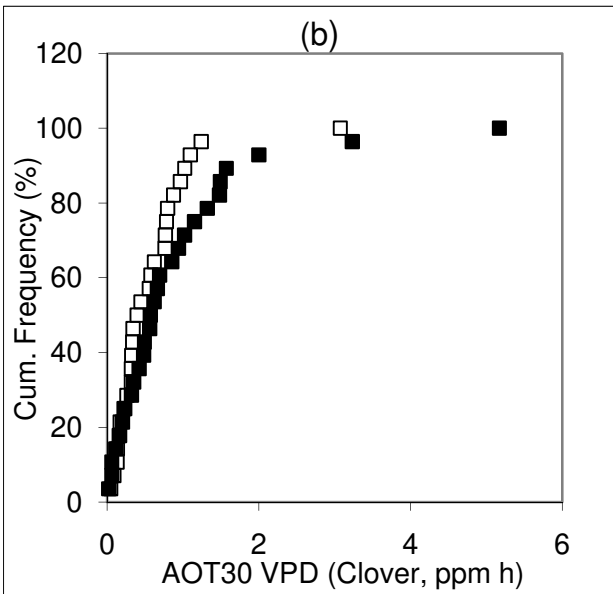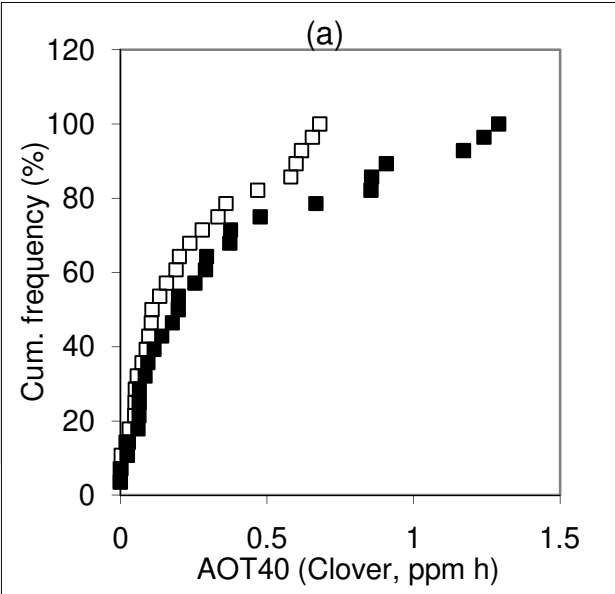
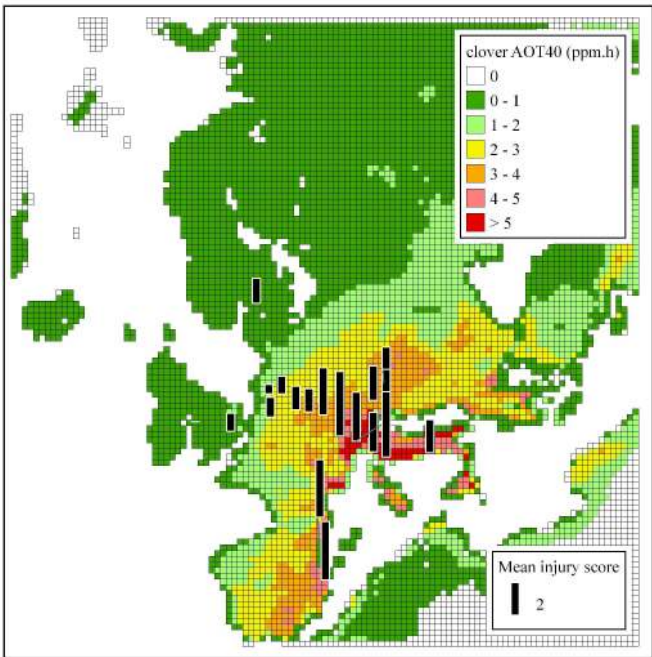Figure 5a (see ms for legend)
297x209mm (400 x 400 DPI)

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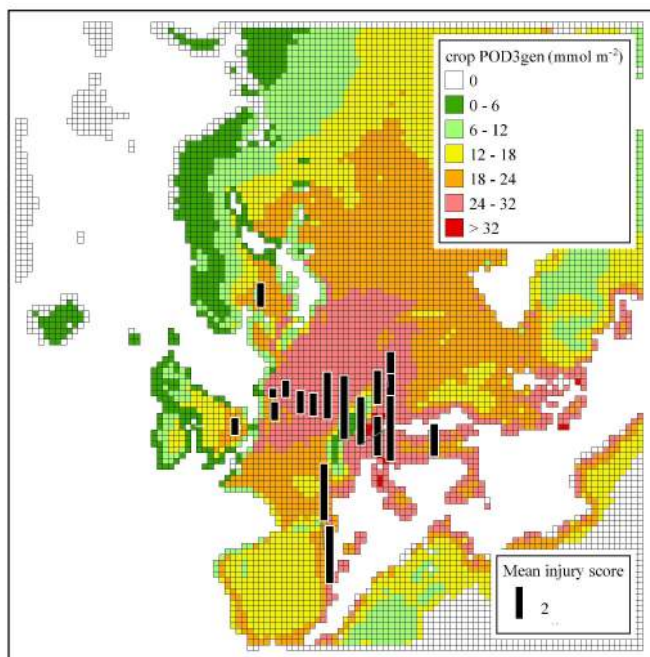297x209mm (400 x 400 DPI)

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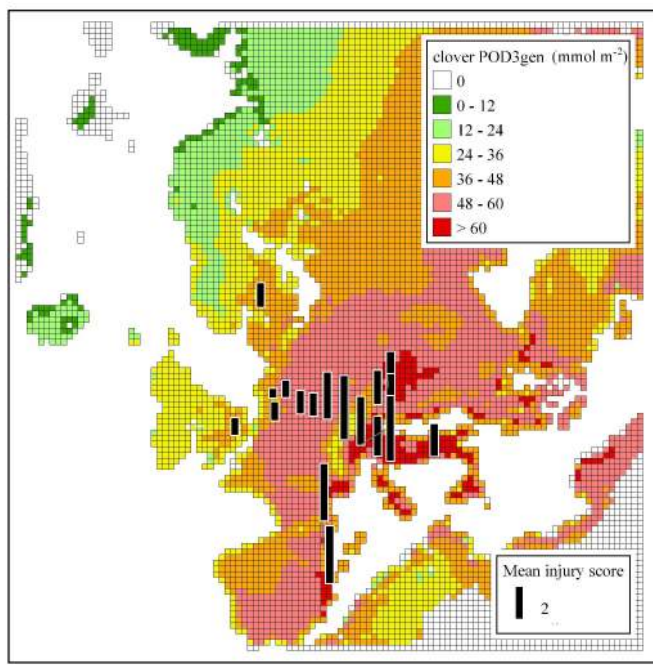296x210mm (400 x 400 DPI)

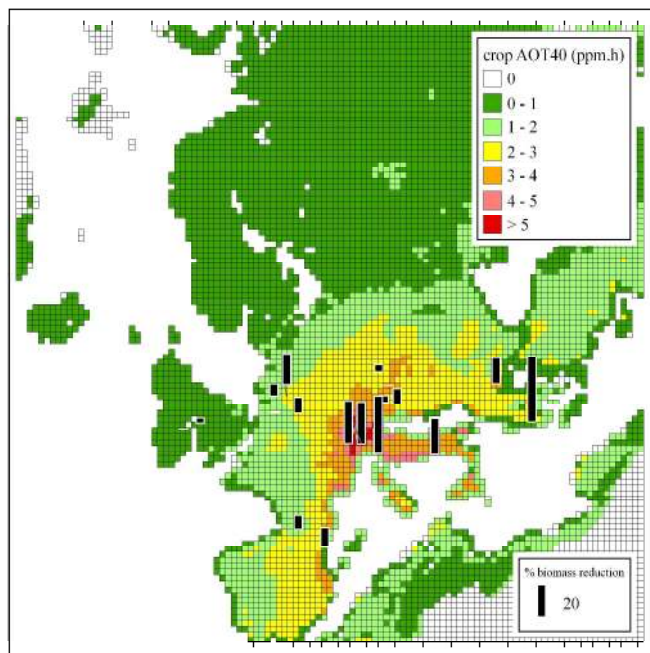Figure 5d (see ms for legend)
296x210mm (400 x 400 DPI)

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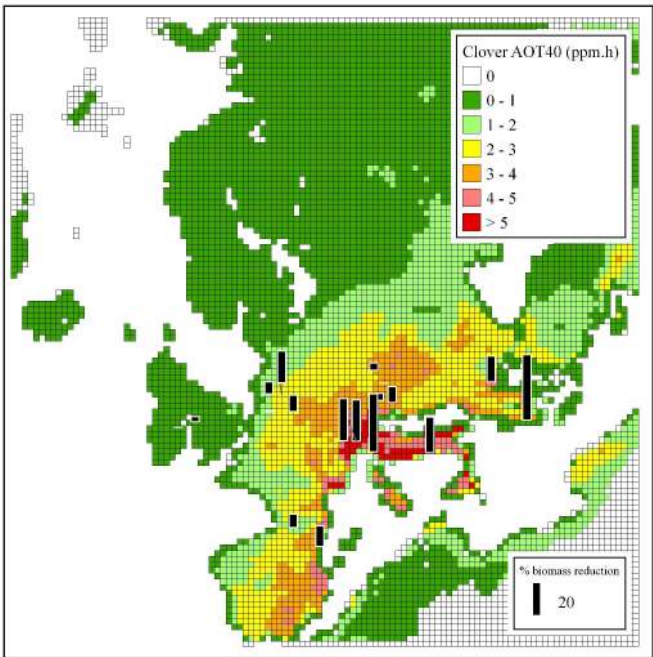297x209mm (400 x 400 DPI)

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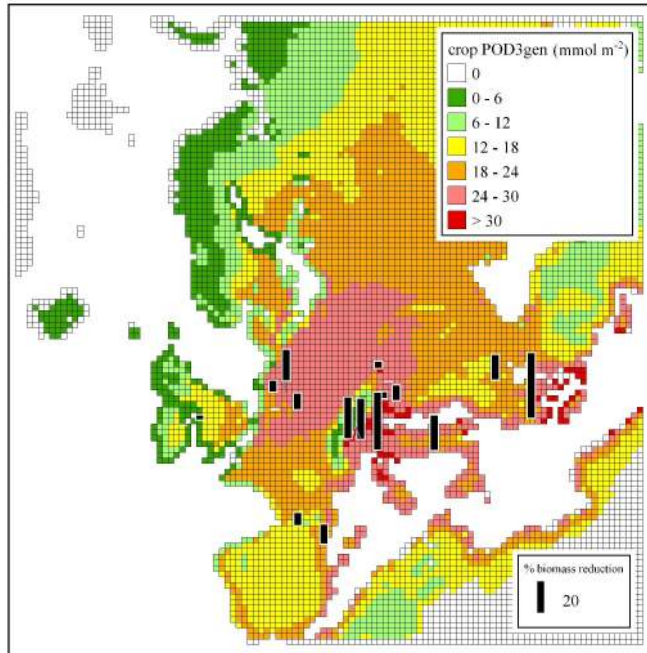297x209mm (400 x 400 DPI)

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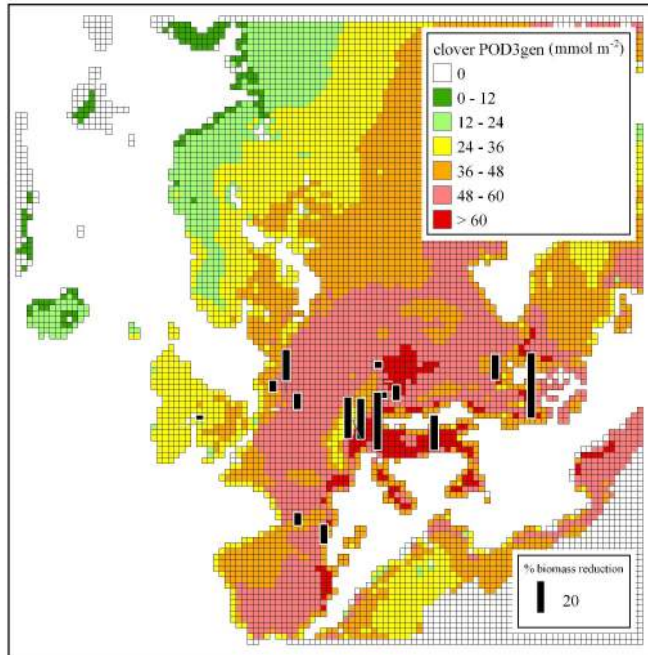
Figure 6d (see ms for legend)
296x210mm (400 x 400 DPI)

(a) AOT40 (Crop, ppm h)

(b) AOT40 (Clover, ppm h)

(c) AOT40 (Crop, ppm h)
y = 10.97x - 6.63
r² = 0.28, p > 0.05
♦ MED

(d) AOT40 (Clover, ppm h)
y = 6.09x - 9.56
r² = 0.58, p<0.001
◇ CCE
♦ MED

(a)

Biomass reduction, %

POD3$_{gen}$ (Crop, mmol m^{-2})

(b)

Biomass reduction, %

POD3$_{gen}$ (Clover, mmol m^{-2})

(c)

Biomassreduction, %

■ ACE
◇ CCE

y = 1.59x - 15.87
r^2 = 0.21, p = 0.002

POD3$_{gen}$ (Crop, mmol m^{-2})

(d)

biomassreduction, %

■ ACE
◇ CCE
◆ MED

y = 1.31x - 57.20
r^2 = 0.59, p <0.001

POD3$_{gen}$ (Clover, mmol m^{-2})

Figure (a): Clover POD3$_{gen}$ (mmol m^{-2}) vs Clover AOT40 (ppm h)

$y = -0.53x^2 + 9.41x + 27.95$
$r^2 = 0.93$

Figure (b): Clover POD3$_{gen}$ (mmol m^{-2}) vs Clover AOT40 (ppm h)

$y = 4.23x + 37.9$
$r^2 = 0.92, p < 0.001$

$y = 4.24x + 38.3$
$r^2 = 0.72, p < 0.001$

$y = 9.78x + 23.5$
$r^2 = 0.89, p < 0.001$

◇ NE
■ ACE
△ CCE