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AN OVERVIEW OF THE IMPACT OF AMBIENT OZONE ON WHITE CLOVER AT ICP VEGETATION SITES (1996–2003)

A technical report prepared by the Coordination Centre of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation)

Introduction

1. Experiments have been conducted each year since 1996 using ozone-sensitive (NC-S) and ozone-resistant (NC-R) biotypes of white clover (*Trifolium repens* cv Regal) originally selected in North Carolina (NC) as described by Heagle et al. (1995). This biomonitoring system was chosen because the forage biomass for both biotypes was similar at low ozone concentrations, but lower for the NC-S biotype at high ozone concentrations (12-hour mean > 40–50 part per billion (ppb); Heagle et al. 1995). By exposing plants to ambient air, the reaction to ozone episodes could be considered without any confounding influences of an exposure chamber on the flux of ozone to the plant. This report reviews the results from the clover biomonitoring system during the period from 1996 to 2003.

2. The main objective was to quantify the effects of ambient ozone in the ECE region using an inexpensive biomonitoring system.

3. The experiment has been conducted at 20–35 sites across Europe and the United States of America each year since 1996 according to a standard protocol (e.g. ICP Vegetation 2003). Impacts of ozone were monitored by weekly assessments of the health of the leaves, including the presence of ozone injury (small pale yellow spots on the leaf). The dry weight of foliage and stems was measured at 28-day intervals. Harvest intervals 1–2 (28–56 days), 2–3 (56–84 days) and 3–4 (84–112 days) were used in the data analysis presented here. From 1998 to 2001, participants from nine sites collected stomatal conductance measurements according to a standard protocol (e.g. ICP Vegetation 2001).

I. OZONE POLLUTION TRENDS

4. AOT40 (accumulated over a threshold of 40 parts per billion (ppb)) denotes 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. The AOT40s were calculated for the period from harvest 1 to harvest 4 (termed “three months”) for selected sites over the period from 1997 to 2003 (table 1). A geographical trend is indicated, with the highest AOT40s occurring in southern Europe, medium AOT40s in central Europe and the lowest AOT40s occurring in northern Europe. No specific trends in time were observed over the seven years but there was considerable variation from year to year. For example, Italy-Naples had a range in AOT40 in the selected three-month period from 9.4 parts per million times hours (ppm h) in 2002 to 32.4 ppm h in 1998. The three-month AOT40 exceeded the critical level for crops of an AOT40 of 3 ppm h every year at five of the eight sites shown in table 1 (when ozone concentrations were analyzed).

Table 1. AOT40 (in ppm h) over 3 months at selected rural ICP Vegetation biomonitoring sites during the period 1997–2003; n.a. = not analysed

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

II. FREQUENCY OF OCCURRENCE OF LEAF OZONE INJURY

5. Long-term trends were difficult to determine due to both the year to year variation in ozone concentrations at individual sites and due to moving of sites or to gaps in the funding of participants. With the exception of Belgium-Tervuren and UK-Bangor, ozone injury was detected on at least one 28-day harvest at all sites with a relatively long record of participation in all years (table 2). Injury was detected on at least 75% of harvests in all years in Germany-Trier, Italy-Isola Serafini and Slovenia-Ljubljana, and on 60% or more of the harvests every year at Switzerland-Cadenazzo. In northern Europe, ozone injury was frequently detected at Finland-Jokioinen and Sweden-Östad, but infrequently detected at United Kingdom-Bangor.

Table 2. Frequency of occurrence of leaf ozone injury on white clover (*Trifolium repens*) at selected ICP Vegetation biomonitoring sites (1996–2003). Data are presented as percentage of 28-day harvests per site per year when injury was detected; n.a. = not analysed

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

III. AOT40-BASED DOSE-RESPONSE FUNCTIONS FOR EFFECTS ON BIOMASS

6. Atmospheric ozone concentrations were measured at different heights across biomonitoring sites. Therefore, data were standardized for a canopy height of 1 metre (m) according to a simple gradient estimated from the ozone deposition module (Emberson et al. 2000a) for an artificial crop of 1 m height (for further details see ICP Vegetation 2004a). Using the gradient-corrected values for ozone had little effect on the fit of the data in the dose-response relationship, with linear regression providing an r^2 of 0.58 for the three month gradient-corrected AOT40 and 0.56 for the uncorrected AOT40 (fig. I). However, the gradient correction reduced the AOT40 values and thus the slope of the regression was steeper for fig. I.b than for fig. I.a. The importance of this difference became apparent when the regression was used to calculate the

critical level for a 5% reduction in the biomass of the NC-S biotype relative to that of the NC-R biotype. The critical level without height correction was 2.8 ppm h whilst that with correction was 2.2 ppm h.

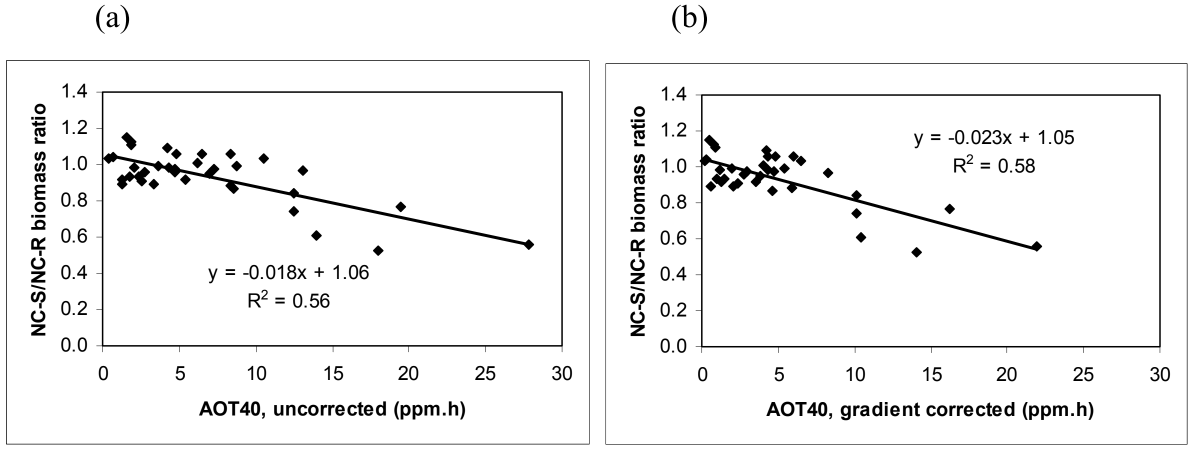

Figure I. Dose-response functions for effects on biomass using the three month (a) uncorrected AOT40 and (b) ozone gradient corrected AOT40 (data from 1996–2002)

IV. STOMATAL CONDUCTANCE MODELS TO CALCULATE OZONE FLUXES

7. Chapter 3 of the recently revised Mapping Manual of the Convention contained stomatal flux-based critical levels for crops that take into account the effect of varying climatic conditions and plant growth stage on the uptake of ozone into the plant (ICP Vegetation 2004b). This approach was considered more biologically realistic since it links plant responses with ozone uptake rather than ambient ozone concentration as with AOT40. The clover experiments of ICP Vegetation provided an ideal opportunity to model ozone flux using plants grown in ambient air at a wide range of sites in Europe. Three different modelling methods were applied to stomatal conductance measurements made by the participants at nine sites across Europe: multiple linear regression, artificial neural network (ANN) modelling and multiplicative stomatal conductance modelling using the approach developed by Emberson et al. (2000a,b). Ozone-flux-effect relationships were developed for each method, with the most successful ones described here.

8. At nine sites in Europe, stomatal conductance (g_s) measurements were made for the NC-S and NC-R plants exposed to ambient ozone as part of the main biomonitoring experiment. Measurements were always made on fully developed leaves positioned in full sun and normally between 10 a.m. and 4 p.m. on several days during the season at a range of climatic conditions; the fourth leaf from the tip of a stolon was selected for consistency. The dataset was collated and quality checked at the ICP Vegetation Coordination Centre (see Mills et al. 2000) and contained over 5000 g_s measurements covering the period 1998 to 2001. All g_s measurements were expressed as total conductance of the upper and lower surface of the leaf, based on the projected

leaf area (PLA). Each g_s measurement was accompanied by measurements of instantaneous photosynthetically active radiation (PAR), air temperature (T_{air}), vapour pressure deficit (VPD) and ozone concentration, together with the AOT40 since previous harvest, days since first harvest (DSFH), days since last harvest (DSLH), time (hour), date and harvest interval during which measurements were made. Mean g_s values were in the range 178 (Austria-Seibersdorf) to 585 mmol H_2O m^{-2} s^{-1} (Italy-Milan).

9. Initial analysis of the dataset revealed, not surprisingly, that VPD and temperature were linearly correlated ($r^2 = 0.85$) and that the hourly mean ozone concentration was correlated with temperature ($r^2 = 0.51$) and AOT40 since previous harvest ($r^2 = 0.42$). There was a significant influence of site on g_s ($p < 0.001$ by analysis of variance (ANOVA)). As there was also a significant influence of site on VPD ($p < 0.001$), one of the main drivers of g_s, it was considered reasonable to combine the data from the nine sites when needed for subsequent analysis.Three methods were used to develop stomatal conductance models using these data (see below).

A. Multiple linear regression

10. ANOVA of the whole data set revealed that g_s was significantly ($p < 0.001$) lower for the NC-R biotype (number of accepted observation (n) = 2418, mean = 339 ± 199 mmol H_2O m^{-2} s^{-1}) than for the NC-S biotype (n = 2639, mean = 393 ± 209 mmol H_2O m^{-2} s^{-1}). Thus all subsequent analyses were conducted separately for the two biotypes. After splitting the data by biotype, best subsets multiple linear regression provided relationships between five input factors and g_s that accounted for 22.4% and 25.7% of the variation for NC-R and NC-S respectively. Best subsets analysis showed that air temperature and vapour pressure deficit were the most important influencing factors, that phenology was important (indicated by days since last harvest) and that ozone concentration and AOT40 since last harvest had a somewhat lower influence. The best fit using multiple linear regression was established using the following two equations:

NC-R ($r^2 = 0.47$, $p < 0.001$):

$$g_s = 27.8 – (1.84 * DSFH) + (23.8 * T_{air}) – (111 * VPD) + (0.0318 * AOT40)$$

NC-S ($r^2 = 0.44$, $p < 0.001$):

$$g_s = –136 – (1.04 * DSFH) + (37.4 * T_{air}) – (158 * VPD) + (0.0087 * AOT40)$$

11. However, the plots of measured versus calculated g_s from these equations were skewed towards the measured values (data not presented). Thus, the regression equations were underestimating g_s for conditions which led to high g_s values. This statistical approach seemed

suitable for development of parsimonious models of g_s, with reasonable fit to the data, but was limited by the non-linear nature of the effects of the parameters on g_s. A major benefit of this approach was the identification of those factors that were the prime drivers of g_s in each biotype of clover. This information was used to guide the multiplicative flux modelling described later.

B. Artificial Neural Network (ANN) modelling

12. Following comprehensive training and optimization procedures, the ANN model with the best performance for each biotype included all eight input parameters. The models had r^2 values for the test data of 0.78 for the NC-S biotype and 0.75 for the NC-R clover biotype. DSLH, VPD and T_{air} had the highest percentage contribution for both biotypes (13.4–15.9%). Time of day, PAR and ozone concentration had the lowest contribution for NC-S (10.1–11.6%) whilst time, DSFH and AOT40 were lowest for the NC-R model (9.1–10.9%). However, it was notable that no one factor dominated, and that the percentage contribution per input factor only varied from 10.1 to 14.7% for the NC-S model and 9.11–15.9% for the NC-R model. This confirmed the message from multivariate statistical analysis that stomatal conductance in clover was influenced by several factors in a complexity of interactions.

C. Multiplicative stomatal conductance modelling

13. The multiplicative algorithm (MA) that was used to model stomatal flux was described previously in Emberson et al. (2000a,b):

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

where g_s was the actual stomatal conductance in mmol H_2O m^{-2} s^{-1} and g_{max} was defined as the average maximum stomatal conductance expressed in mmol H_2O m^{-2} s^{-1} on a total leaf area basis.

14. The parameters g_{pot}, g_{light}, g_{temp} and g_{VPD} were all expressed in relative terms as a proportion of g_{max} where:

g_{pot} represented the modification to g_{max} due to phenological changes;
g_{light} represented the modification of g_{max} by irradiance described by PAR (mmol m^{-2} s^{-1});
g_{temp} represented the modification of g_{max} by temperature (^oC);
g_{VPD} represented the modification of g_{max} by vapour pressure deficit (VPD) (kPa);
g_{min} represented the minimum g_s that occurs during the daylight period.

15. Parameterization was achieved using a boundary line analysis technique whereby all g_s data points were plotted against each model variable (e.g. irradiance, temperature and VPD) individually. A boundary line was then fitted according to generic functions that were predefined for each model variable (for further details see Emberson et al. (2000a,b)).

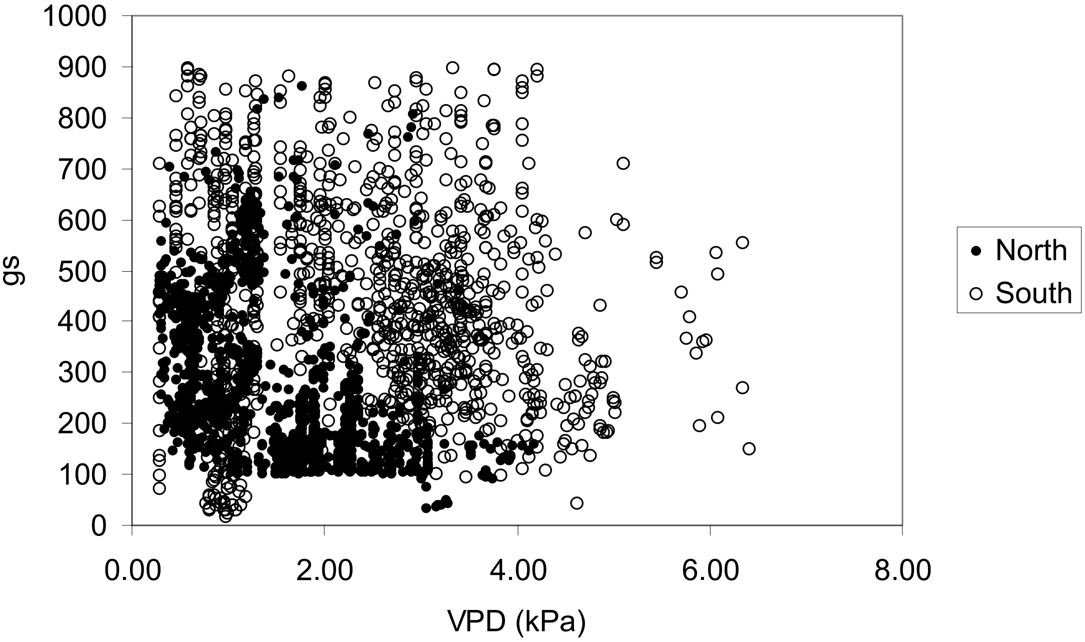

Figure II. The ICP Vegetation data on stomatal conductance g_s (mmol H_2O m^{-2} s^{-1}) versus VPD, and separated into data from northern sites (Austria, Belgium, Germany-Essen, Germany-Trier, United Kingdom, Sweden) and southern sites (Italy-Milan, Italy-Rome, Spain)

16. The initial aim of this MA modelling was to use the clover g_s dataset to produce one model of g_s per biotype that was applicable to all of Europe. However, it soon became apparent that this approach might not be appropriate since boundary line analysis would be dominated by data from southern Europe (fig. II). For this reason, the data were separated into a North dataset (Austria, Belgium, Germany-Essen, Germany-Trier, United Kingdom, Sweden) and a South dataset (Italy-Milan (only these data were used for g_{max} and g_{min} derivation), Italy-Rome, Spain) for all subsequent model development. It allowed separate parameterizations to be established for each biotype for northern and southern Europe.

V. STOMATAL FLUX-BIOMASS EFFECT MODELLING

The NC-S and NC-R flux algorithms described in the previous section were used to develop flux-effect relationships for the 28-day and three-month data for the nine sites contributing g_s data. The stomatal ozone flux, F_{st} (mol O_3 m^{-2} s^{-1}), was first calculated as g_s (mol H_2O m^{-2} s^{-1}) * O_3 concentration (mol mol^{-1}) * 0.613, where 0.613 is the ratio of diffusivities for water vapour and ozone, and is used to convert g_s from mol H_2O m^{-2} s^{-1} to mol O_3 m^{-2} s^{-1}. The highest correlations with NC-S/NC-R biomass ratio were found for $AF_{st}Y$ (accumulated flux above a flux threshold of Y nmol m^{-2} s^{-1}, accumulated over a stated time period during daylight hours) calculated using the MA modelled for the NC-R biotype in sourthern Europe, since this provided the best fit to the data with either 40 ppb ($r^2 = 0.46$) or 5 nmol m^{-2} s^{-1} ($r^2 = 0.50$) as a threshold. The preceding analysis used ozone concentrations that were uncorrected for measurement height. To provide a flux-response relationship for the whole dataset, ozone concentration was first corrected for the concentration gradient as described above. The $AF_{st}5$ was calculated for three months for each site using the MA modelled for the NC-R biotype in sourthern Europe. The r^2 for the resulting linear regression was 0.55 (fig. III). Thus, the critical level for a 5% reduction in the biomass ratio, relative to the biomass at an $AF_{st}5$ of 0 mmol m^{-2} PLA, was an $AF_{st}5$ of 1.7 mmol m^{-2} PLA.

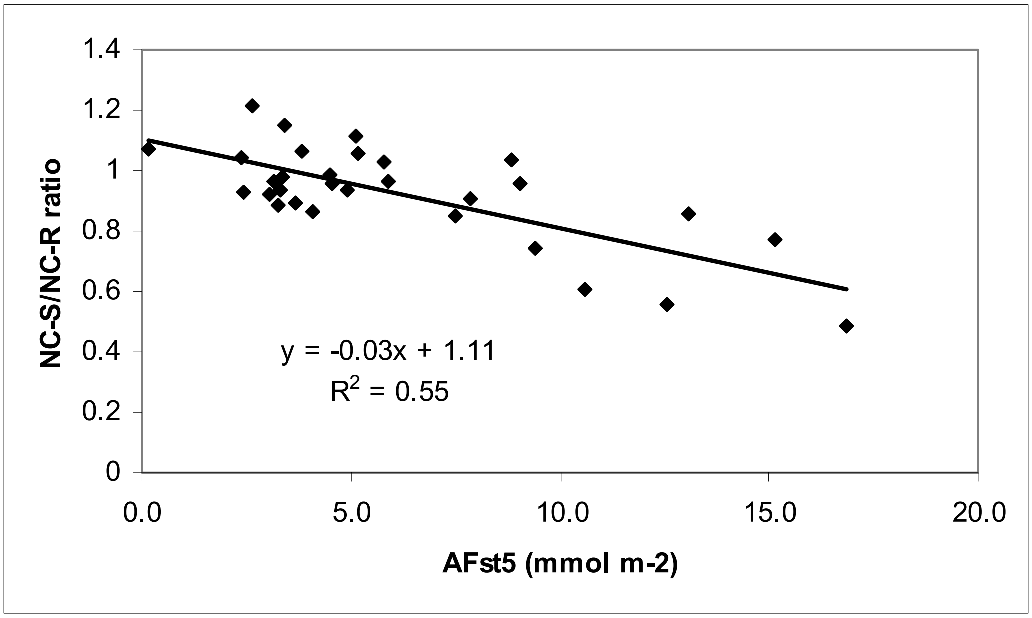

Figure III. Flux-effect relationship developed using the MA modelled for the NC-R biotype in sourthern Europe to accumulate $AF_{st}5$ over three months. Data are for all sites in the network (1996–2002) and hourly mean concentrations of ozone have been corrected to canopy height using the concentration gradient method

VI. DEVELOPING A RISK ASSESSMENT FOR CLOVER IN EUROPE

17. The biomonitoring experiments have indicated the extent of damage at sites across Europe and the resulting response functions could be used to establish critical levels for clover. Methods for applying the response functions to mapping ozone impacts across Europe are being investigated. Prelimary results based on the AOT40 approach show that the greatest risk for clover biomass reduction due to ozone exposure occurs across France and parts of central

Europe with additional "risk areas" located in the Mediterranean region of northern and southern Italy. At most of the example sites investigated, $AF_{st}Y$ appeared to be better correlated with biomass reduction than AOT40 (ICP Vegetation 2004a). However, across the different sites and years there is considerable variability in the relative merits of both indices and further analysis of the biomonitoring data and development of mapping procedures is required.

VII. CONCLUSIONS AND CHALLENGES

18. The ozone biomonitoring experiment clearly showed that ambient ozone can cause both visible injury and biomass reductions in a sensitive crop species. These types of damage were both widespread across Europe and present in most years. Ozone critical levels for biomass reduction could be established as an AOT40 of 2.2 ppm h or an $AF_{st}5$ of 1.7 mmol m^{-2} PLA, both accumulated over three months. First efforts to apply the AOT40 response function to a risk assessment for Europe indicated that biomass reductions potentially as high as 20% could be experienced in central and southern Europe. At most of the example sites investigated, $AF_{st}Y$ appeared to be better correlated with biomass reduction than AOT40.

19. There are still many uncertainties associated with mapping white clover at risk from ozone damage across Europe and further development of methods for mapping is continuing. So far, the flux-based method was based on modelling ozone flux into single leaves, however, efforts are ongoing to develop a canopy flux model for white clover. The canopy flux model for white clover will take into account increasing total ozone flux as the canopy develops and will be used to set a whole canopy flux-based critical level.

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<ins>Note</ins>: The references have been reproduced as received by the secretariat.