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THE OZONE UMBRELLA PROJECT

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EXECUTIVE SUMMARY

CEH Edinburgh

- Field measurements of ozone deposition fluxes have been measured to a range of terrestrial surfaces, including grassland, sugar beet, wheat and oats over 4 growing seasons in Nottinghamshire and central Scotland.

- The field measurements obtained, provide fluxes covering the expected range encountered in the UK (10 to 1000ng O_3 m^{-2}s^{-1}) for vegetation and a wide range of O_3 concentrations and surface and atmospheric conditions.

- Data capture for the micrometeorological flux measurements during the intensive measurements at Easter Bush during 2001, were typically 90%, with fetch requirement satisfied about 65% of the time. The O_3 fluxes were made by gradient (aerodynamic methods and by eddy covariance) n parallel, using two different O_3 sensors. Fluxes by the two methods agreed well on average, but from hour to hour differences of a factor of two were common.

- For two of the crops (grassland and wheat), simultaneous measurements of water vapour fluxes enable the stomatal conductance and the stomatal fluxes to be quantified and by difference the cuticular fluxes to be determined. These measurements show that non-stomatal ozone fluxes represent a substantial fraction of the total and are being used to understand the underlying process of O_3 destruction at leaf surfaces.

- The full energy balance at the crop/ atmosphere interface has been measured to identify the underlying data quality for the micrometeorological fluxes. These measurements will be used in estimating the uncertainty in the O_3 flux measurements.

- AOT 40 maps for the UK have been calculated and are included in the NEGTAP report. The maps show exceedances of the critical levels for crops and semi-natural vegetation throughout eastern and southern Britain.

- An alternative method of calculating AOT40 maps for short vegetation, taking into account the reduction in concentration close to crop canopies, greatly reduces the magnitude of the exceedances, effectively limiting the problem to southern Britain.

University of Lancaster

Critical levels of tropospheric ozone pollution are defined by the AOT40 (accumulated exposure over a threshold concentration of 40ppb ozone), calculated using total ozone from 1st April to 30th September. This project aimed to determine whether the response of beech trees to ozone varies with the timing of ozone pollution and, hence, whether AOT40 might be refined by taking account of any seasonal variation in ozone sensitivity.

- Ozone pollution early in the growing season (mid-May to mid July) significantly reduces the ability of beech to fix carbon, but comparable pollution later in the season (mid July- mid September) had no significant effect. The response to ozone in spring occurs at AOT40s less than approximately 2.0 ppmh., i.e. well below the threshold of 10 ppmh generally accepted for effects on plant growth.

- Experiments in which plants were "forced" in order to allow plants at different developmental stages to be fumigated at the same time confirmed that increased sensitivity to ozone early in the season was a function of plant developmental stage, not differences in environmental conditions between spring and summer.

- Although the mechanism of early season ozone damage remains unclear, it appears to lie in direct ozone effects on stomata rather than with photosynthetic metabolism.

- Early season ozone damage coincided with the period of maximum rates of photosynthesis, and reduced biomass increase between May and July by approximately 20%. However, in the absence of continuing ozone exposure beech was able to recovery to some extend from initial damage.

- Plants exposed to ozone throughout the growing season (mid May-mid September) were less able than unfumigated plants to fix carbon late in the season. However, carbon fixation late in the season was low compared with that in June and July, and these late-season effects of ozone have little effect on plant growth, although they may contribute to a reduction in root reserves during autumn.

- On the basis of data obtained to date, we suggest that calculation of AOT40 for ozone might be "weighted" to take account for the greater effects of pollution in late spring and early summer.

University of Bradford

- Ozone effects are more closely related to flux into the leaves than to ambient concentrations. Wetland plants, which typically have a high stomatal conductance and suffer less water stress in ozone episodes, maybe thus be particularly sensitive to this pollutant.

- The objectives of this research were to determine the sensitivity of wetland plant species to ozone and to identify physiological or ecological characteristics which are associated with high sensitivity to ozone.

- The effects of ozone on wetland plant species were investigated in season-long fumigation in open-top chambers and in shorter-term fumigation in closed chambers within a controlled environment.

- The effects of ozone on a group of damp woodland species and a group of rare species were also investigated in the shorter-term fumigation experiments.

- The eight wetland species in the longer-term experiment received AOT40 exposures in the range 9-11,000 ppb.h over about two months. The results from this study were combined with those from a study in the previous season, using AOT40 values in the range 11-15,000 ppb.h.

- Eight of the twelve species studied in the two seasons showed visible leaf injury in ozone, but only three species had a significant reduction in above-ground biomass.

- Root/shoot ratios were generally lower in ozone, with three of the twelve species showing a significant reduction.

- The shorter-term experiments, which lasted three weeks, provided an AOT40 exposure of about 3500 ppb.h, close to the current provisional critical level for semi-natural vegetation.

- Only two of the sixteen wetland and damp woodland species showed characteristic visible leaf injury in ozone. However, seven species showed a significant adverse effect of ozone on their relative growth rate. Two species showed a significant reduction in root-shoot ratio

- Nine of the more sensitive species were selected for a further exposure-response experiment. Estimates of the AOT40 value required to produce a 10% reduction in relative growth rate (RGR) were made for eight species.

- Two of these eight species showed a threshold AOT40 of about 3000 ppb.h for above-ground relative growth rate, with three further species having values of 5-6000 ppb.h.

- When threshold AOT40 values were calculated for root relative growth rates, lower values were found for most species. For two species, the threshold values were in the range 1000-1500 ppb.h.

- There was a significant positive correlation between the size of the reduction in RGR caused by ozone and the stomatal conductance of the species in the short-term experiments. Similarly, there was a significant positive correlation between stomatal conductance and the reduction in biomass below-ground, but not above-ground, in the longer-term experiments.

- The most sensitive species in the short-term experiments were characterised by high relative growth rates, a high stomatal conductance and a high specific leaf area. However, the correlation coefficients between reduction in RGR in ozone and both growth rate and specific leaf area were not significant.

- Rare species as a group showed little evidence of adverse effects of ozone. This group had a lower mean stomatal conductance and relative growth rate than wetland plants, a finding which is consistent with the trends in the data for wetland species.

- There was no significant association between Ellenberg ecological indicator values for moisture, light, pH and fertility and ozone sensitivity in the short-term experiments. However, this may reflect the low range of values between the species.

- Overall, the experimental results clearly demonstrate the sensitivity of wetland species to ozone and the significance of a high stomatal conductance in enhancing sensitivity. They provide strong support for the current critical level for semi-natural vegetation of 3000 ppb.h, and indeed suggest that lower critical levels may be needed to prevent adverse effects on the most sensitive UK wetland species.

- Analysis was made of a database of previous experimental studies of ozone effects on semi-natural species, primarily of grasslands. As for the experimental data, no significant association was found with Ellenberg indicator values. However the analysis suggested that there was a high proportion of sensitive species in groups associated with woodlands and woodland edge habitats.

- New data on changes in frequency of individual species in the Countryside Vegetation Survey over the period 1978-2000 was analysed. There was no evidence that species declining in frequency are more sensitive to ozone. However, this analysis is crude and needs to be further developed to provide a more rigorous test of the hypothesis.

- An attempt was made to compare the possible impacts of ozone on the range of grassland communities identified in the National Vegetation Classification. The approach used the ozone exposure, levels of soil moisture deficit and the proportion of dominant or frequent species which are ozone-sensitive. The results showed that mesotrophic grasslands were overall at higher risk than calcareous grasslands. Acid grasslands proved impossible to quantify because of the low proportion of dominant species which had been studied.

University of Newcastle

- There is significant variation in the ozone response between populations of the common wetland species, *Epilobium hirsutum*.

- Ozone response is not associated with any particular regions of the UK so exceedance of the critical level would be expected to affect some populations in which ever part of the UK that it occurs.

- Early-season fumigation of a sensitive population leads to significant effects but they are transient.

- The effects of early-season exposure are modified by water stress. Ozone <u>reduces water use and the effects of water stress.</u>

- Intra-specific competition among dense patches of seedlings alters leaf morphology and makes them more susceptible to acute injury. Therefore <u>competition reduces the critical level for acute injury</u>.

- Late-season exposure has a detectable effect on the shoots but it has a much larger effect on stolon mass. Stolons are the over-wintering organs so ozone reduces the potential for vegetative growth early in the following season. The long-term effects of this are unknown. The critical level for this effect is around 5000 ppbh.

- Because of the seasonality of the work, an experiment is still underway on the effects of ozone and water stress on fecundity. It will be finished in October and a supplementary report will be issued soon after.

- The need to express critical levels in terms of flux rather than exposure is reinforced by the results of the ozone-water stress experiment. To do this measurements of stomatal conductance have been made over two years of OTC experiments. It is hoped that Ms Haley will be able to develop an ANN model to enable us to express the critical level in terms of flux.

- Finally, the work has shown that further work is needed on the interaction between ozone and water stress, and on the carry-over effects of ozone from one season to another.

Measurement of Ozone Dry Deposition to Crops and Grassland
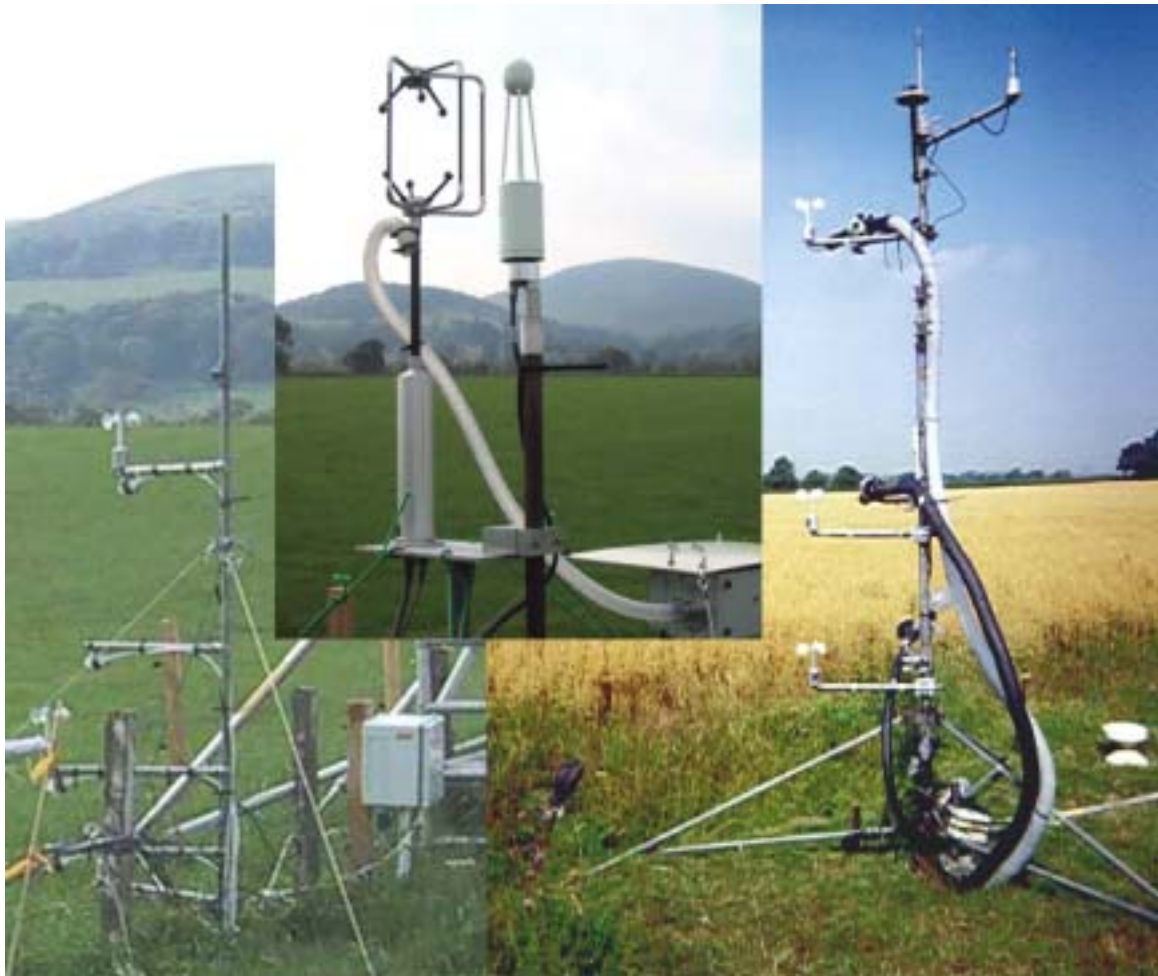
C01700 - 2001 Final Report
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Contents

Introduction

The field studies were designed primarily to obtain high quality flux measurements of O_3 flux averaged over a field scale ($\geq 10^4$ m^2) with sufficient additional measurements to quantify hourly flux density and it's partitioning between the different sinks present at the surface. This is entirely new and has not been achieved before. To simplify the interpretation of the field data, a single plant species which is representative of substantial areas of the UK, was selected (Perennial ryegrass - *Lolium perenne*). Measurements of the major components of the surface energy balance, net radiation, sensible (or corrective) heat flux, latent heat and soil heat fluxes were measured to provide clear estimates of the stomatal resistance of the crop canopy. The measurements reported include 4 years, semi-continuous flux measurements at Sutton Bonnington over a range of crops, and a single season of measurements over *lolium perenne* at Easter Bush, close to the CEH laboratories.

The measurements of ozone flux also provide the means of directly modifying AOT40 at a reference height above the crop to the canopy height, to show the magnitude of the correction that should be appied to quantify the exceedances of critical levels by crops throughout the UK.

The objectives of the measurements were:

- To measure full-scale O_3 deposition fluxes with a time resolution of 1 hour over a range of crops at Sutton Bonnington.
- To measure field scale O_3 fluxes with hourly time resolution over grass at Easter Bush, and to provide sufficient additional measurements of Ra, C, λE and G to quantify the canopy resistance for H_2O (and hence) O_3 uptake by stomata.
- To separate stomatal (Rc_1) and non-stomatal (Rc_2) canopy resistance components and quantify the effects on Rc_2 of radiation and surface temperature.
- To quantify the change in AOT40 between a reference height above the crop to the canopy height.

Field Measurements at Sutton Bonnington

Measurements at Sutton Bonnington provide long term, field scale fluxes of O_3 at the same location as the SO_2 flux monitoring station. The measurements rely on the aerodynamic, flux-gradient technique in which the vertical flux is inferred from the vertical profiles in concentration, wind velocity and temperature, which develop over extensive uniform terrain. The vertical profile in ozone concentration is monitored using a UV absorption gas analyser, which samples sequentially from a profile mast, which extends to a height of 3 m above the surface. The wind velocity profile and temperature profiles are monitored from the same mast and are logged continuously. At this rural location, 15km south of Nottingham, the air is typical of the English Midlands with large concentrations of the major pollutants, including SO_2, NO, NO_2 and a range of VOC. It is necessary therefore to monitor the NO and NO_2 concentrations to provide corrections to the profiles of O_3 for the effects of NO/O_3 reaction in the surface layer according to Duyzer *et al* (1995). A more detailed description of the theory of gaseous pollutant flux measurement can be found in Monteith & Unsworth (1990) or Flechard (1998).

The measurements have continued at Sutton Bonnington during the growing season in 2001, a summary of the results, up to 31st September 2001, is given here. The location of the monitoring site is shown in Figure 1a and Figure 1b shows the position of the instrument mast on the boundary between two fields. The data are split into two fetch sectors, one for the west field and one for east as illustrated on Figure 2, which also shows the frequency of wind directions at the site. Table 1 gives the total data capture for all the measurements and Figure 3 shows how the data are split between each sector. Table 2 lists the various crops that have been planted each year. Typically, the data capture with site and atmospheric properties which satisfy the boundary conditions for flux gradient analysis amount to about half the time, with the majority in the west field and the minor fraction in the east field.
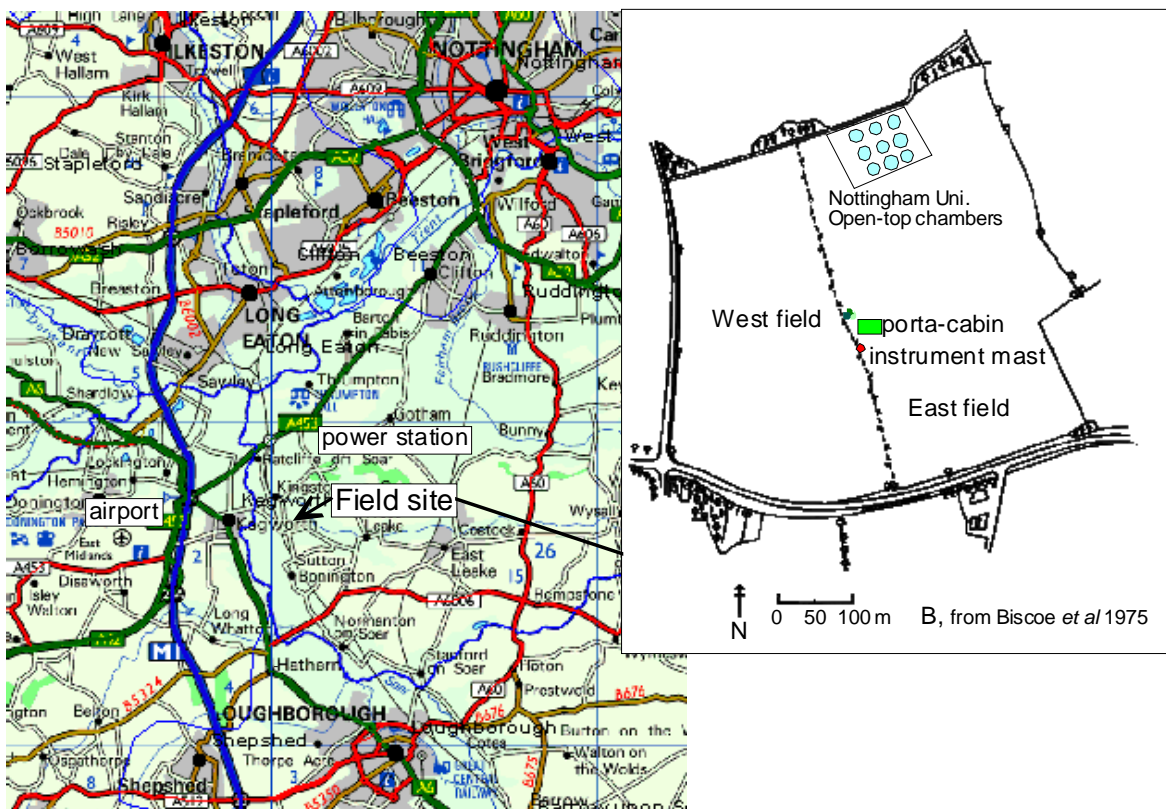
A, Bartholemews UK Road Map CD v 3 (50 km x 50 km grid)

Figure 1. Location of the Sutton Bonnington field site.

Figure 4 shows plots of the latest monthly mean meteorological parameters measured at Sutton Bonnington. The patterns are typical of a UK site with a seasonal cycle in temperature, solar radiation (peak in the summer months) and wind speed (peak in the winter months). The extensive micrometeorological data for this site provide the variables to simulate flux measurements for the gaps in the measured fluxes due to wind direction or instrument problems. The rainfall does not show an obvious seasonal cycle, with all months receiving sufficient rainfall for crop growth and development, thus the crops would have been well watered over most of this period of measurement.

Figure 5 summarises the monthly mean ozone fluxes measured during this period, the top plots show the average ozone flux and the middle plots the average deposition velocity and canopy resistance. These mean values conceal the hour to hour variability but provide a snapshot of the data record. The bottom pair of plots show the measured and modelled canopy heights, the log-normal relationship described below was fitted to periodic measurements of the canopy height to model the canopy growth.

canopy growth model		
$h = h0 + a*exp(-0.5*(ln(x/x0)/b)^2)$		
x = time code in the Excel system, a, b, h0 and x0 are contants		
	E	W
a	0.32	0.46
b	0.0014	0.0013
x0	37158	37084
h0	0.084	0.077

Table 1. Monthly percentage data capture at Sutton Bonnington.

| Month	O_3	NOx	Solar Rad., St	Wind speed	Rainfall	Temp.	Wetness	Wdir	Dew point	Net Rad.	Soil heat flux	Soil Temp
May-98	98.88	96.91	96.57	99.19	78.36	91.29	99.19	99.19	-	-	-	-
Jun-98	95.69	95.69	99.93	100	100	100	100	100	-	-	-	-
Jul-98	97.54	80.51	99.46	100	100	99.73	100	100	-	-	-	-
Aug-98	97.72	-	100	100	100	100	100	100	-	-	-	-
Sep-98	100	-	100	100	100	100	100	100	-	-	-	-
Oct-98	14.11	-	100	100	100	99.84	100	100	-	-	-	-
Nov-98	-	-	100	100	100	100	100	95	-	-	-	-
Dec-98	94.35	-	100	100	100	100	100	100	-	-	-	-
Jan-99	88.55	-	100	100	100	100	100	100	-	-	-	-
Feb-99	97.02	30.58	100	98.26	100	100	100	100	-	-	-	-
Mar-99	93.95	62.90	100	100	100	100	100	100	-	-	-	-
Apr-99	98.22	50.72	98.89	98.89	98.89	98.89	98.89	98.89	36.83	-	-	-
May-99	98.84	98.84	99.13	98.90	99.13	98.36	99.13	99.13	94.31	59.14	59.14	59.14
Jun-99	86.46	80.81	87.22	87.22	87.22	87.18	87.22	87.22	62.34	86.67	87.22	87.22
Jul-99	98.32	98.32	100	100	100	100	100	100	93.21	100	100	100
Aug-99	98.59	41.80	100	100	100	100	100	100	91.55	100	52.42	52.42
Sep-99	100	-	100	100	100	98.77	100	100	94.49	7.78	-	-
Oct-99	92.54	77.35	81.72	81.72	81.72	77.89	81.72	81.72	70.32	-	-	-
Nov-99	23.33	23.33	100	100	100	100	100	100	22.13	-	-	-
Dec-99	35.35	-	98.59	98.59	98.52	98.52	98.52	98.52	-	-	-	-
Jan-00	100	-	100	88.58	100	100	100	100	-	-	-	-
Feb-00	100	-	100	94.85	100	100	100	100	-	-	-	-
Mar-00	100	-	100	87.25	100	100	100	100	-	-	-	-
Apr-00	96.46	12.01	100	81.81	100	99.95	100	100	-	-	-	-
May-00	99.53	99.53	100	86.11	100	100	100	100	-	-	-	-
Jun-00	97.08	97.08	97.08	80.74	97.08	97.04	100	100	-	-	-	-
Jul-00	96.91	96.91	100	78.25	100	99.04	100	100	-	-	-	-
Aug-00	80.71	80.71	90.73	70.79	90.73	90.73	90.73	90.73	-	-	-	-
Sep-00	97.78	97.78	97.78	88.15	97.78	97.78	97.64	97.78	-	-	-	-
Oct-00	100	34.07	100	92.94	100	99.93	100	100	-	-	-	-
Nov-00	100	-	100	94.88	100	100	100	100	-	-	-	-
Dec-00	100	-	100	91.71	100	100	100	100	-	-	-	-
Jan-01	100	-	100	79.48	100	100	100	100	-	-	-	-
Feb-01	99.70	-	99.70	86.14	99.70	99.70	99.70	99.70	-	-	-	-
Mar-01	99.73	-	99.93	90.59	99.93	90.52	99.93	99.93	-	-	-	-
Apr-01	100	-	100	93.50	100	100	100	100	-	-	-	-
May-01	95.50	24.66	100	83.62	100	98.25	100	100	-	-	-	-
Jun-01	99.44	68.13	100	80.67	100	100	100	100	-	-	-	-
Jul-01	82.46	85.35	98.32	77.62	98.32	98.32	98.32	98.32	-	-	-	-
Aug-01	94.83	94.83	95.43	69.31	95.43	95.43	95.43	95.43	-	-	-	-
Sep-01	100	100	100	86.57	100	99.79	100	100	-	-	-	-
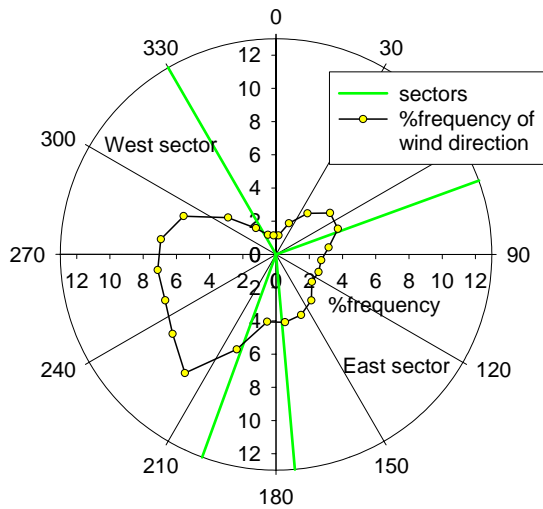
Figure 2. Wind rose showing the fetch sectors for each field and the % frequency of wind directions, (May 1998 to Sept. 2001)
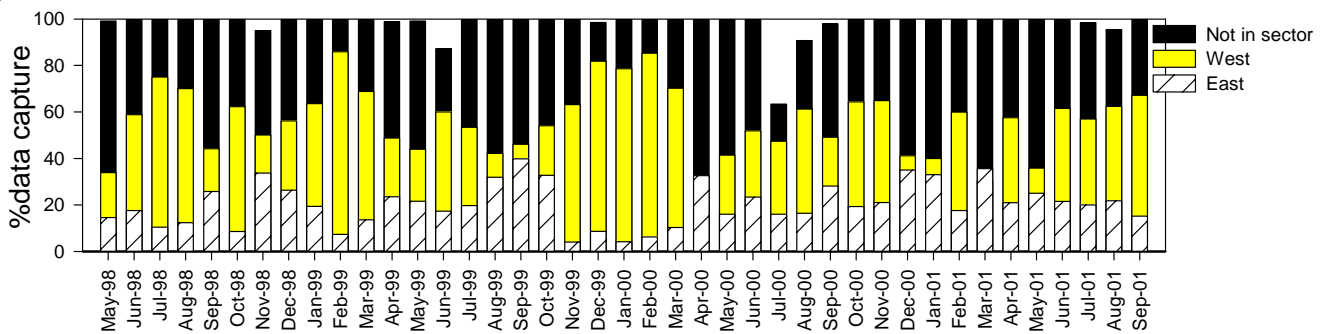

Figure 3. Wind direction % data capture for whole month (not in sector) and within the westerly and easterly sectors.

Table 2 Sutton Bonnington crops.

West	East
1998	
Saxon sugar beat drilled 30/4/98 harvested 12/11/98	Consort wheat drilled 2/11/97 harvested 30/8/98
1999	
Rialto wheat drilled 1/12/98 harvested 17/8/99	Gerald oats drilled 12/10/98 harvested 20/8/99
2000	
Jalna oats sown (*avena satvia*) variety 14/10/99 harvested 25/8/00	Rialto wheat sown (*triticum aestivum*) 17/9/99 harvested 29/08/00
2001	
wheat sown (incl. trial plots) 6/9/00 harvested 28/8/01	sugar beet drilled March 2001 harvest in November
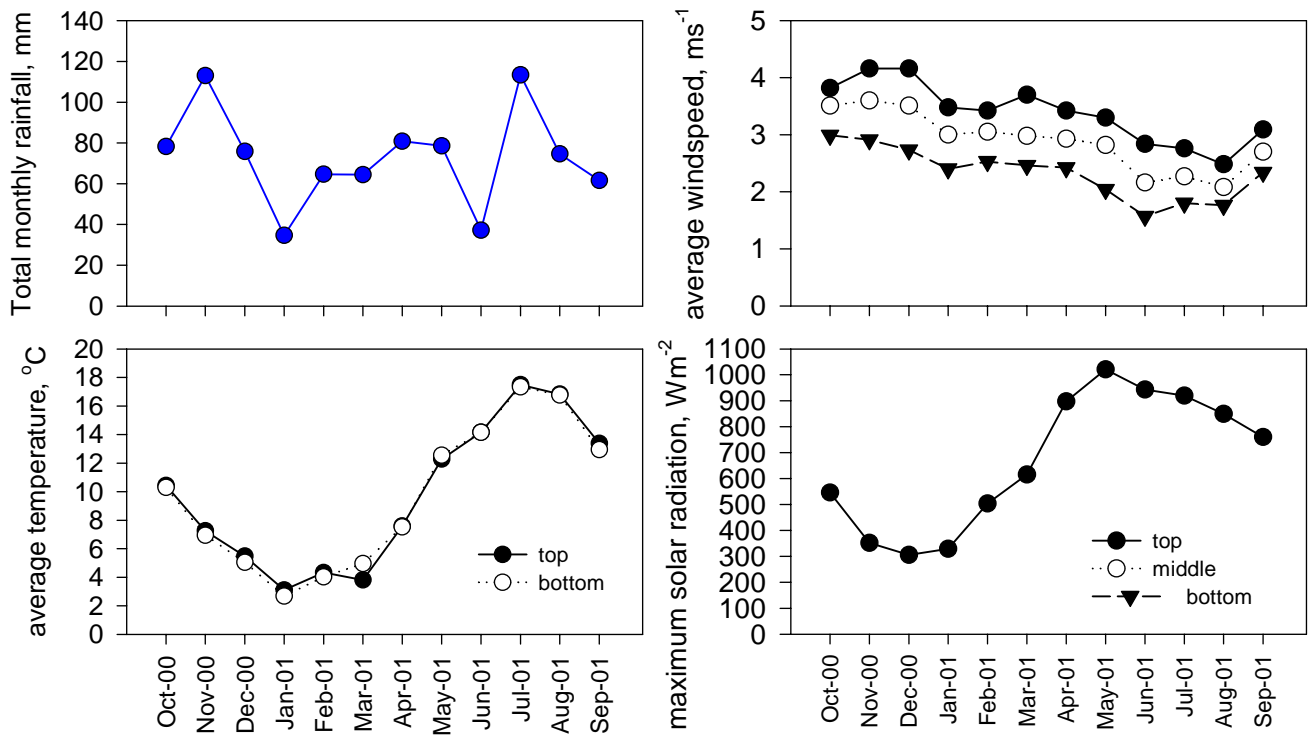
Figure 4. Summary plots of total monthly rainfall (mm), average temperature (^{o}C), average windspeed (ms^{-1}) and maximum solar radiation (Wm^{-2}) from October 2000 to September 2001.
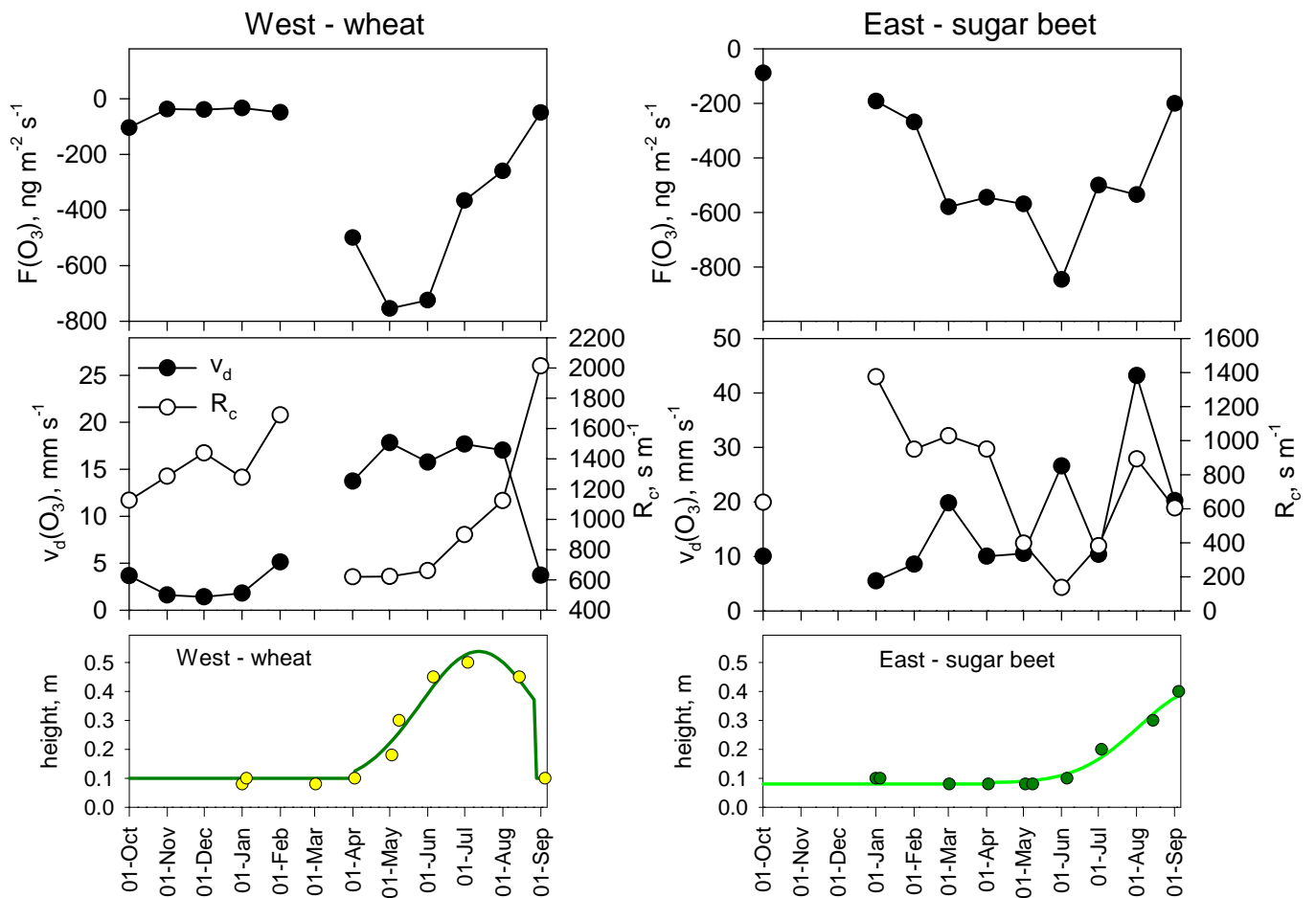
A   B

Figure 5 Monthly mean ozone flux (F(O$_3$)), deposition velocity (v$_d$), canopy resistance (R$_c$), measured and modelled canopy height for (A) the westerly sector and (B) the easterly. Missing data occur where the data capture is low for that month.

Ozone deposition over both sectors gradually increases from March to peak in May for the westerly sector and then June for easterly sector. This cycle is partly controlled by the ozone concentration that follows a similar cycle (see Figure 6A). However the peaks in deposition do not correspond to the peak in concentration (April). For the westerly fetch, when the wheat begins to grow in April the canopy resistance to ozone deposition declines as the plants take up ozone through their stomata during the day, and the deposition increases. The deposition peaks in May and June during the main period of growth, consistent with the peak in the leaf area index and the minimum in the canopy resistance. In July the wheat had reached its maximum height and its ozone uptake declines as the crop matures, the green leaf area declines and the crop becomes senescent. During this period the canopy resistance steadily increases.

For the sugar beet the deposition peak occurs in June, just at the beginning of its main growth period and declines again in the following months. However these data are inherently less accurate due to the lower data capture for this (easterly) wind sector and so it is not possible to distinguish the effect of the vegetation from other factors, such as surface roughness and ozone concentration.

Figure 6A shows the monthly mean ozone concentration measured in all wind directions at each height. The effect of ozone deposition can clearly be seen in the reduction of ozone concentration from the top to the bottom height. Although the monthly averages are less than 30 ppb, several periods where hourly mean concentrations exceeded 40 ppb where recorded, as shown by the monthly total AOT40 values (for all wind directions) plotted on Figure 6B. The effect of deposition can be seen in the reduction of AOT40 values from the top height to the bottom.
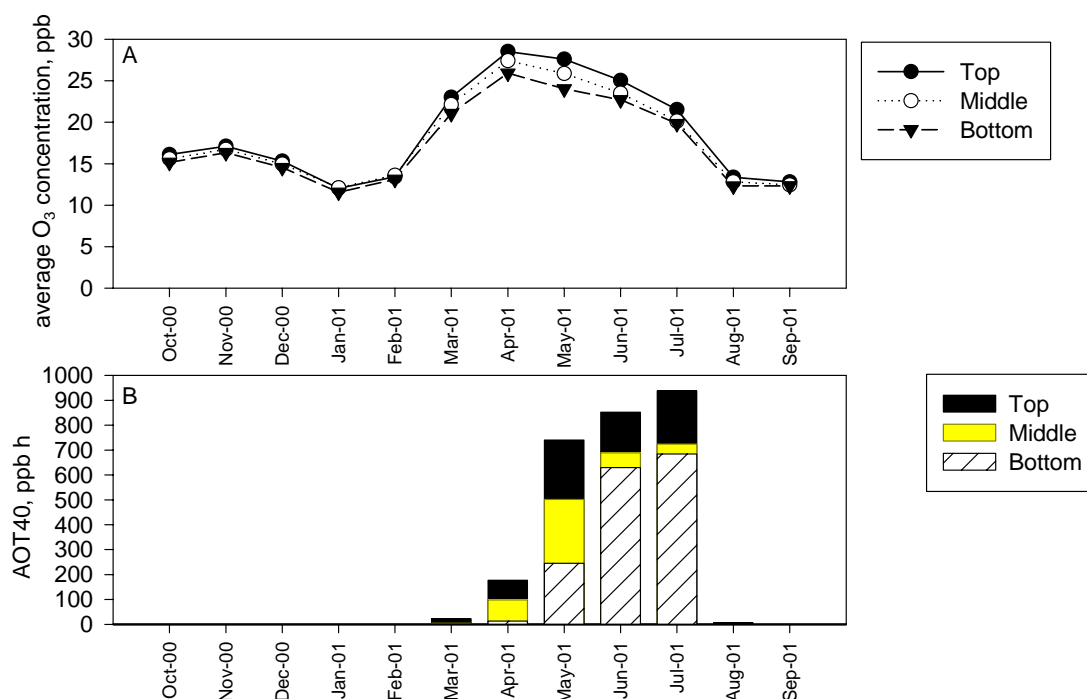

Figure 6 A. Monthly mean ozone concentration for all wind directions at each measurement height. B. Monthly total AOT40 for all wind directions at each height.

Although the stomatal flux cannot be calculated directly from the measurements for this period, the importance of this component can be inferred by considering the fluxes during daylight hours and over-night. Figure 7 shows plots of the May to July canopy resistance and ozone flux for the westerly sector, segregated in to day-time and night-time averages using a solar radiation value of 50 W m^{-2} to indicate daylight. During daylight hours the stomatal uptake provides additional ozone deposition, and so deposition is at a maximum, whereas during the night the stomata close and deposition is restricted to the external surfaces of the crop, and as a consequence, deposition is reduced. This can be seen in the very small canopy resistances measured during the day and larger values during the night (Figure 7A). The reverse is seen in the ozone flux (Figure 7b), which is large during the day and small at night. The large difference between day and night values indicates a large stomatal component to ozone fluxes at Sutton Bonnington during this period.

The AOT40 critical level for wheat is calculated for May to July daylight hours only and so the % difference between the AOT40 recorded at the top and bottom (effectively canopy height) measurement heights are plotted on Figure 7B. The greatest difference occurs in May (the bottom is only 33% of the top) when the deposition is greatest and the canopy resistance smallest during both the day and night. During June and July the differences are 74 and 73% respectively. The large variation between May, June and July is partly due to the enhanced deposition in May but there where also fewer exceedances of 40 ppb during this month, which would accentuate the difference between top and bottom. Overall the average difference between the top measurement height and the canopy is 60%, which could have a significant effect on assessments of exceedance of the AOT40 critical level, this is discussed further in final section of this report.
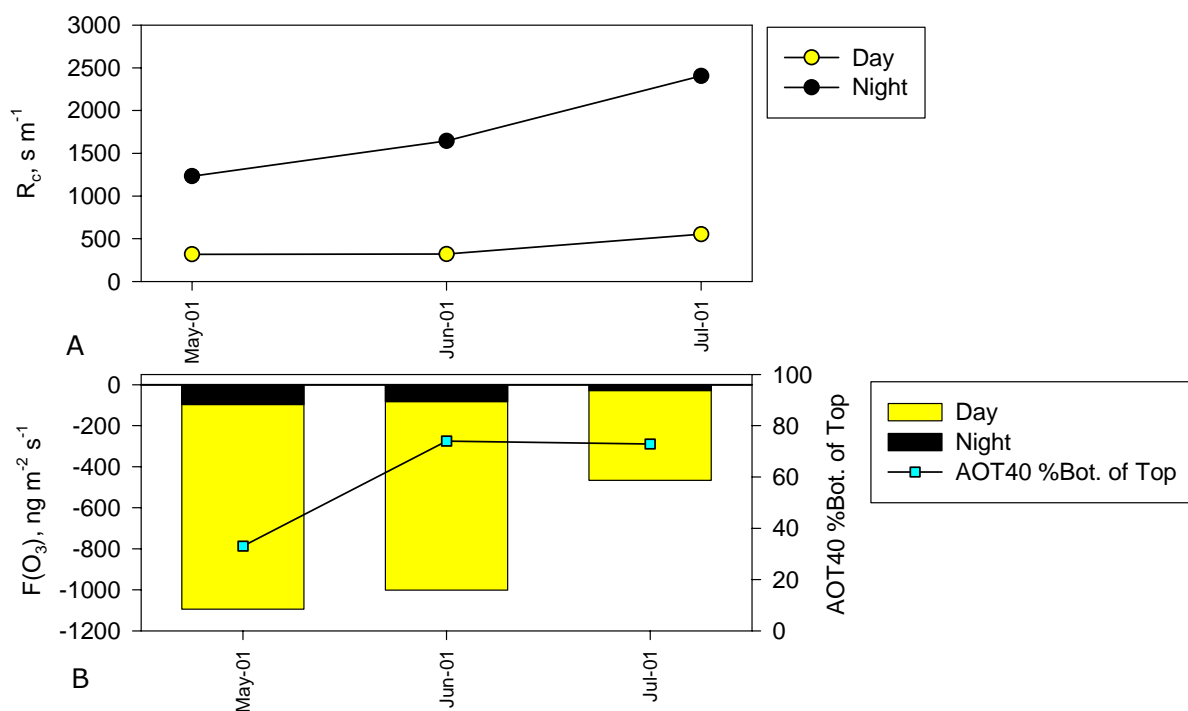

Figure 7. For the westerly fetch during May, June and July 2001: (A) canopy resistance during daylight (St ≥ 50 W m^{-2}) and night-time (St < 50 W m^{-2}) and (B) the % difference in AOT40 between the top and bottom heights, daytime and night-time ozone fluxes.

Field Measurements at Easter Bush

Field measurements have also been made during most of the growing season of 2001 (May to September) over improved grassland at a site close to CEH Edinburgh (see Figure 8A). Measurements of ozone deposition were made using the gradient method, similarly to Sutton Bonnington. Eddy-correlation measurements using a Gusten fast-ozone sonde and sonic anemometer were also obtained for some of the summer period. The site was also instrumented for CO_2 and water vapour flux measurements using an open-path LICOR, and Bowen ratio measurements using a purpose built system from Campbell Scientific. Table 3 provides the weekly data capture obtained for all the variables measured at this site. Measurements are made on the boundary of two fields that are split by a hedge and fence running roughly from the NW to SE (Figure 8B). Figure 9 shows the frequency distribution of wind directions and the sectors used in analysing the data. As the grass is the same in both fields the data from each sector are combined for the analysis.
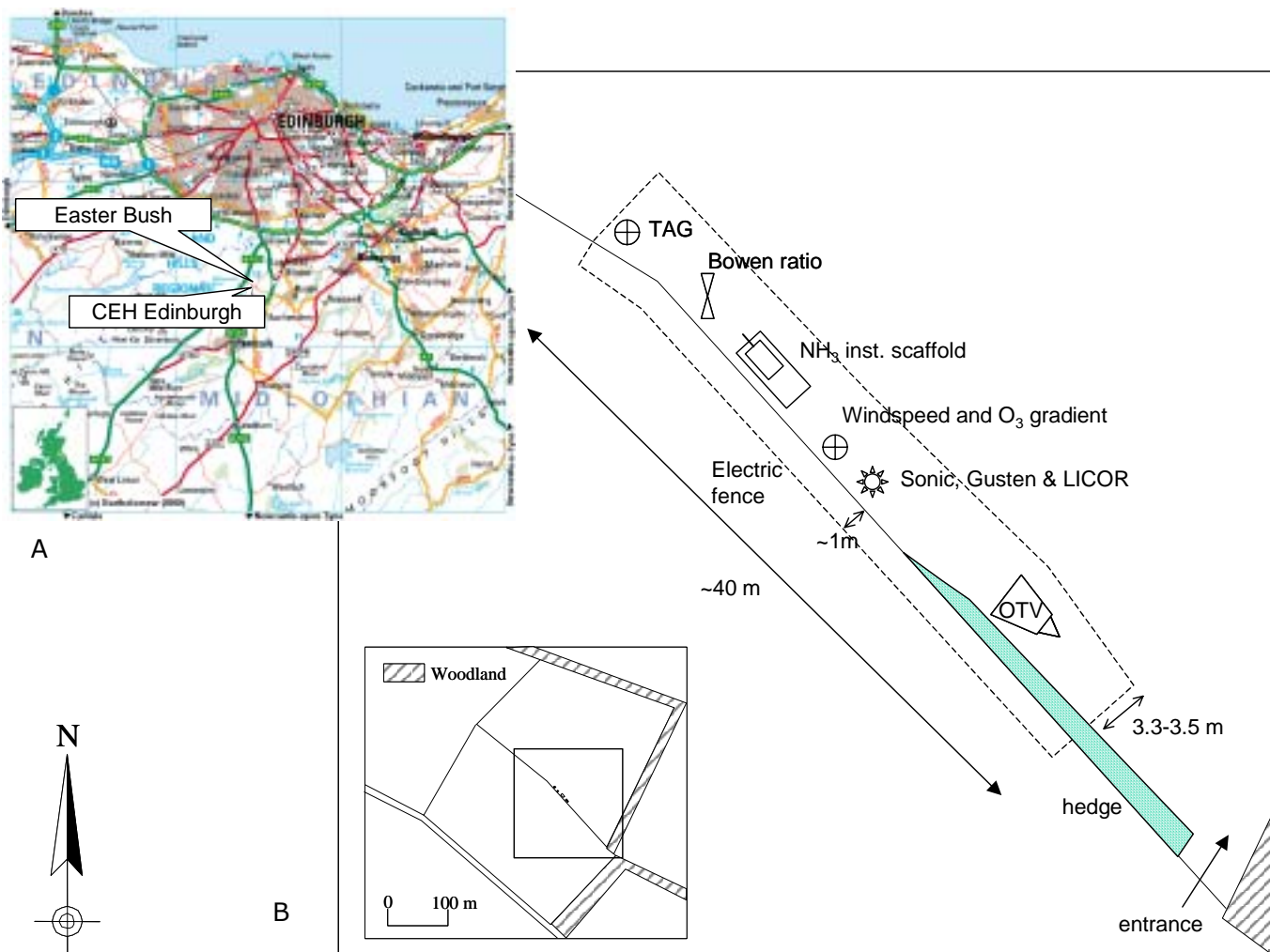

Figure 8. (A) Location of the Easter Bush field site and (B) schematic of the site configuration.
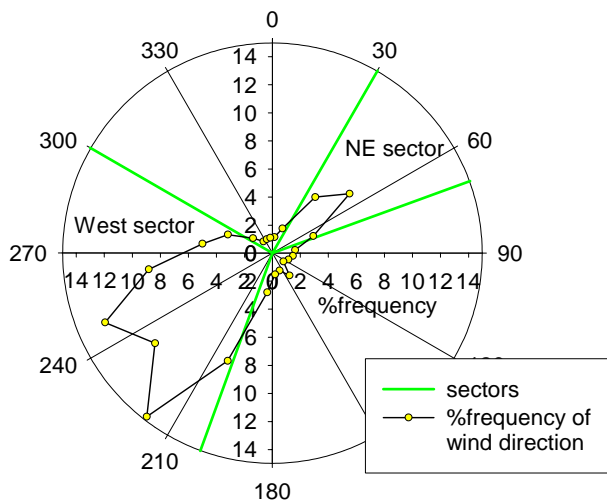
Figure 9. Wind rose showing the fetch sectors for Easter Bush and the % frequency of wind directions.

Table 3. Weekly data capture summary for the Easter Bush field site (ND refers to No Data available yet)

Week Starting	Gradient O$_3$	Gradient Dew point	Gradient u	Gradient T	Rain Gauge	Sonic	LICOR	Gusten	Bowen ratio	%In Sector
24-May	72	ND	94	91	94	ND	ND	ND	100	ND
31-May	98	ND	98	98	100	35	35	ND	100	35
07-Jun	99	ND	92	100	100	99	99	ND	99	24
14-Jun	96	ND	94	100	100	92	92	12	100	40
21-Jun	100	36	77	100	100	99	99	99	100	54
28-Jun	100	100	94	100	100	99	92	99	99	76
05-Jul	100	100	86	100	100	98	98	98	100	49
12-Jul	100	100	75	100	100	96	96	96	97	23
19-Jul	100	100	79	100	100	99	99	99	99	72
26-Jul	100	100	88	100	100	95	95	95	99	74
02-Aug	90	90	78	90	90	96	96	96	99	58
09-Aug	100	100	96	100	100	99	99	99	100	89
16-Aug	100	100	92	100	100	99	99	99	100	68
23-Aug	100	100	85	100	100	90	90	90	100	69
30-Aug	100	100	98	100	100	63	63	63	100	51
06-Sep	100	100	100	100	100	ND	ND	ND	99	ND
13-Sep	100	100	93	100	80	ND	ND	ND	100	ND
20-Sep	95	95	75	95	72	ND	ND	ND	99	ND
27-Sep	53	53	38	53	53	ND	ND	ND	99	ND

Daily summaries of the meteorological parameters measured over the season at the site are shown in Figure 10. There were frequent rainfall events and so the grassland is unlikely to have suffered from significant drought stress, but the measurements of precipitation inputs and the evaporative losses allows the water balance of the site to be followed through the season. The other parameters are typical of the location. Figure 11 shows the daily average of the 15-minute average, maximum and minimum ozone concentrations measured by the ozone analyser. In general ozone concentrations are small, typical of this location although some brief episodes with increased levels occur. The AOT40 value measured at the top height for May to July is 3.8 ppb h and 0.9 ppb h at the bottom, which corresponds approximately to the crop surface.
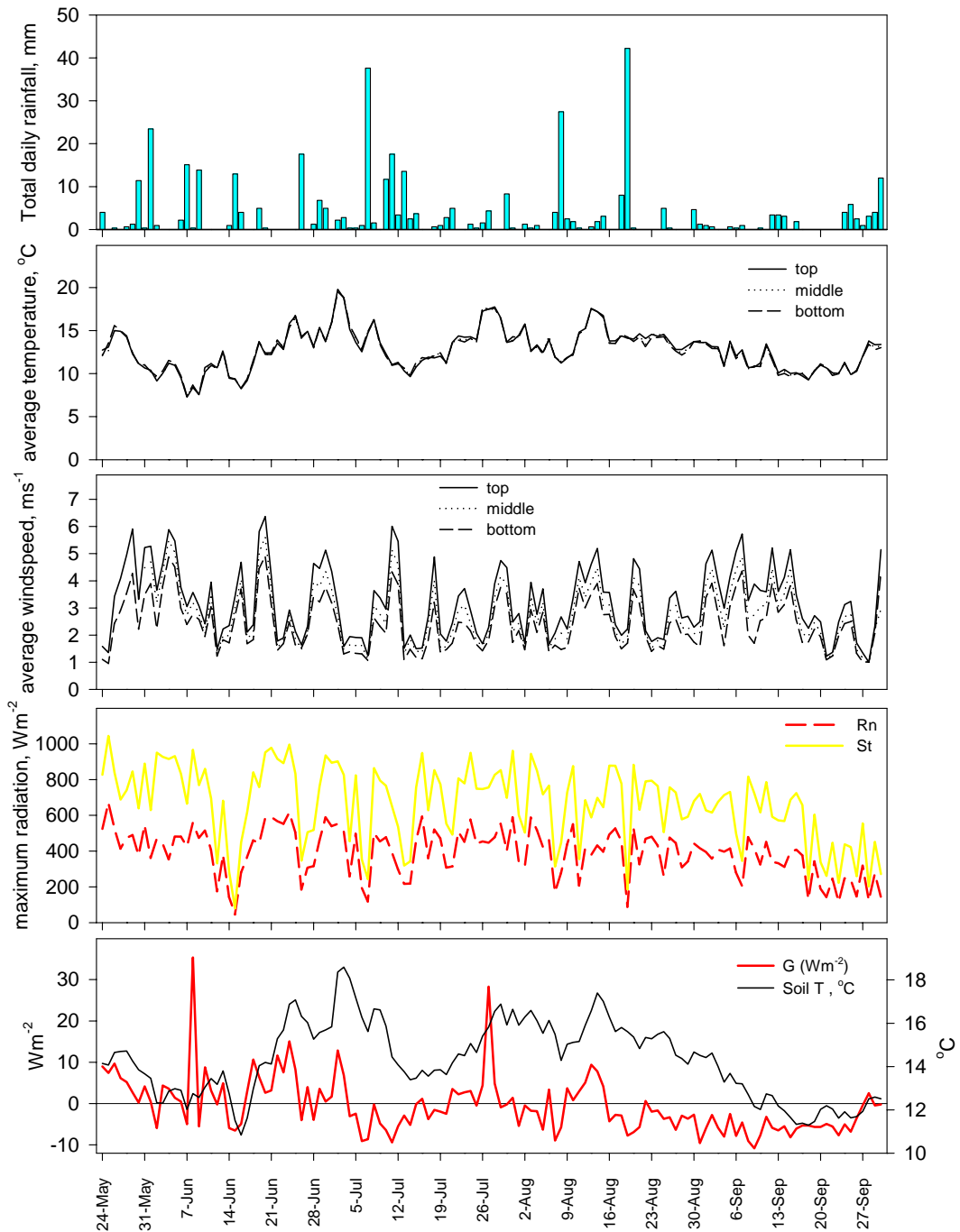
Figure 10. Daily total rainfall (mm), average temperature (^oC), average windspeed (ms^{-1}), maximum total (St) and net radiation (Rn, Wm^{-2}), average soil heat flux (G, Wm^{-2}) and soil temperature measured at Easter Bush during the summer of 2001.
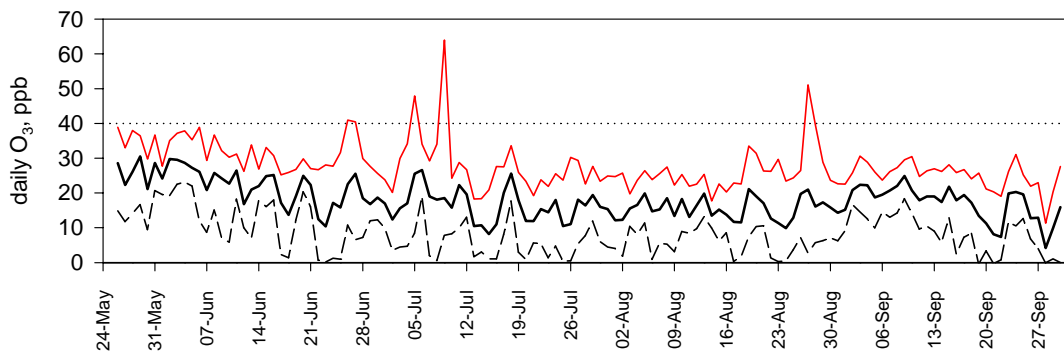

Figure 11. Daily average of the 15-minute average, maximum and minimum ozone concentration measured at all heights on the gradient mast at Easter Bush.

To illustrate the energy balance data for this measurement site, two days data are shown in figs 12A and 12B. These data show that over the day the net incoming energy (Rn) is partitioned fairly evenly into latent and sensible heat flux with a small fraction of the incoming energy left as the soil heat flux (G), generally 10% or less of Rn. The extent to which the energy balance can be closed, even at micrometeorologically ideal locations is generally limited to about 10% of Rn in the few hours either side of mid-day. These data are therefore excellent for the further processing of the data to extract the surface processes and show what regulates the surface reactions of ozone on vegetation.
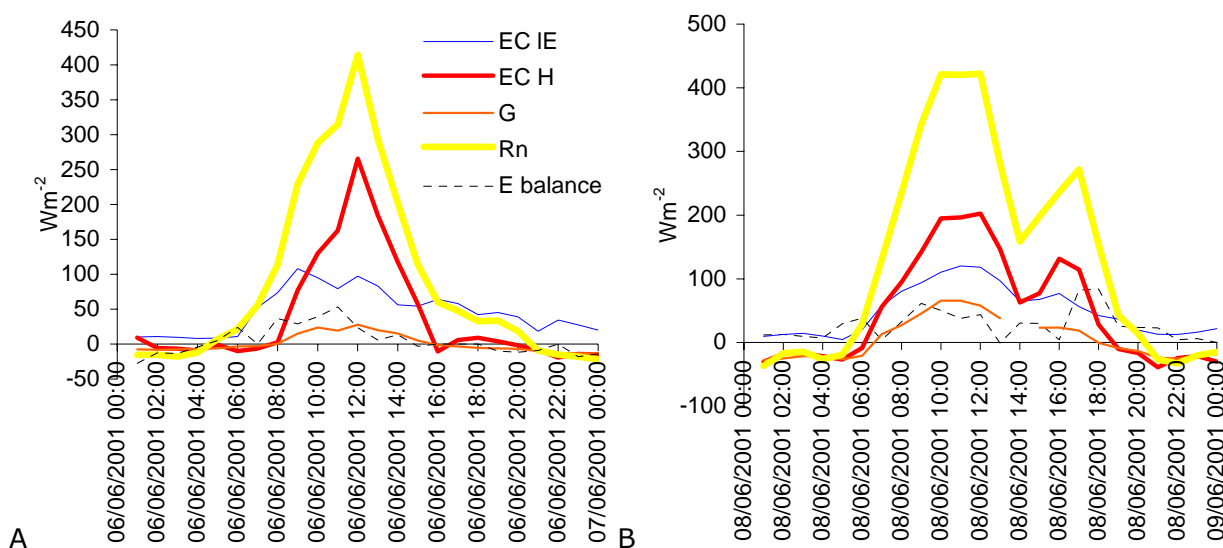
Figure 12. Illustration of the energy balance measured over grassland at Easter Bush (EC = eddy correlation measurements, lE = latent heat flux (l = λ), H = sensible heat flux, G = soil heat flux, Rn = net radiation, E balance = (H + lE) − (Rn-G)).

The grassland is managed for silage and so two cuts are made during the summer (one in early June and a second in late July) and the fields are fertilized after each cut. Following the second cut sheep and dairy cattle are allowed onto the fields to graze. The effect of these harvests can be seen in the measured fluxes, for a week or so after each cut photosynthesis is reduced and the CO_2 and latent heat fluxes (latent heat is equivalent to the water vapour flux) decline, as illustrated in Figure 13A and B for the cut on the 26th of July. The daytime latent heat flux is greatly reduced after the cut, from ~150 W m^{-2} to less than 100 W m^{-2} immediately afterwards.

The CO_2 and latent heat fluxes to do not increase again until 5 days after the cut, when the grass starts to regrow. The O_3 flux, deposition velocity and canopy resistance (Figure 13C, D & E) do not appear to be influenced by the cut. However the flux increases and the canopy resistance decreases *ca* 5 days after the cut, correlating with the re-growth of the grass.

Comparisons of different ozone flux measurement methods

The measurement of ozone flux is central to the objectives of this project and to provide both quality assurance of the flux-gradient data and to obtain fluxes in a wider range of conditions, an additional ozone-flux measuring instrument was used at the Easter Bush site. The technique is eddy covariance (also known as eddy correlation) and relies on the covariance of the deviations

about the mean ozone concentration of ozone with the vertical wind component of turbulence, providing the flux directly. However, this method requires a rapid response sensor, and a sampling frequency of about 10 Hz. The measurements at Easter Bush using eddy covariance provided an extensive data set to complement the gradient data.
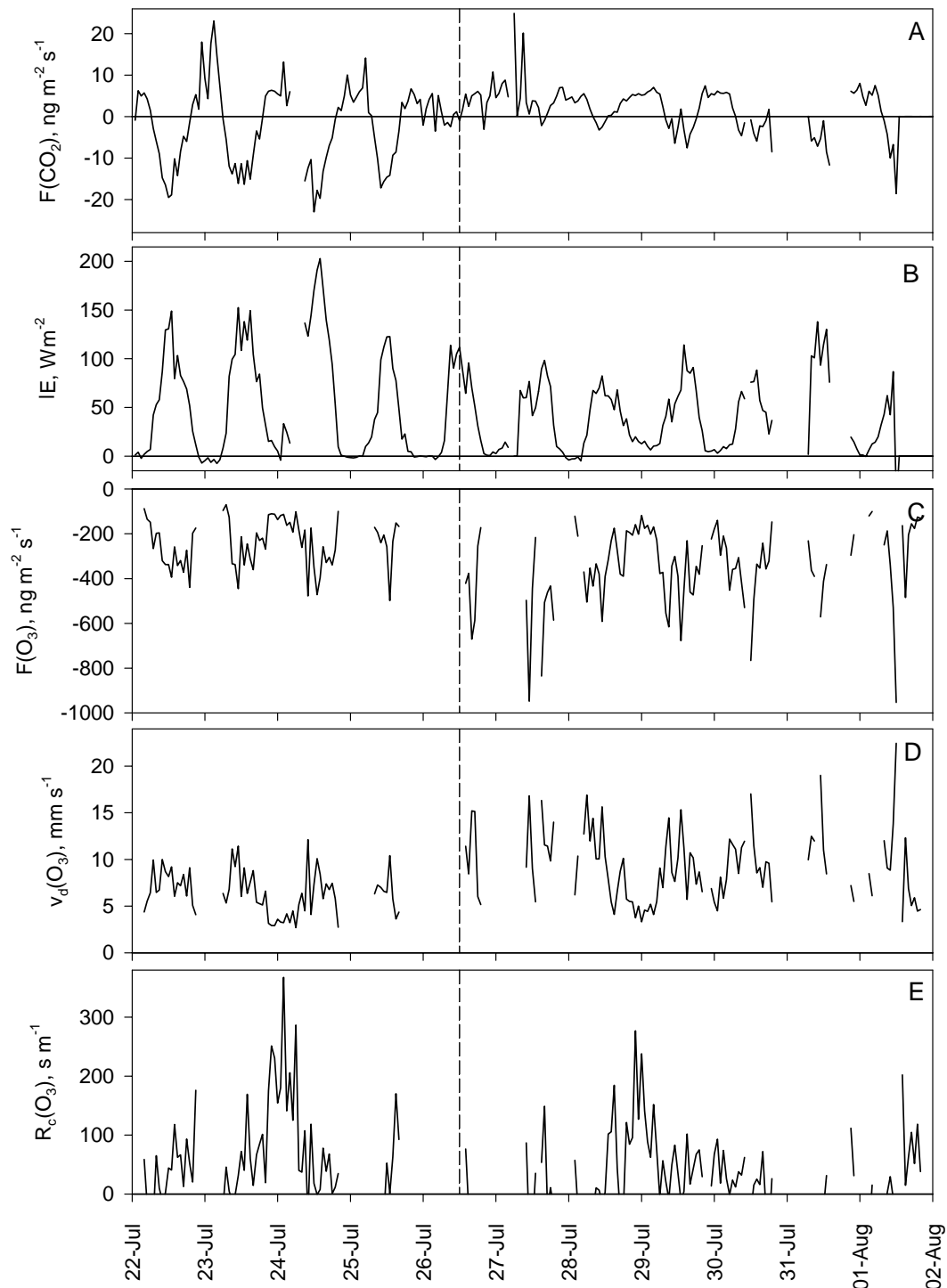

Figure 13. Hourly average (A) CO_2 flux (F(CO_2)), (B) latent heat flux (λE), (C) ozone flux (F(O_3)), (D) ozone deposition velocity (v_d) and (E) ozone canopy resistance (R_c) measured over grassland at Easter Bush over an episode of grass cutting (26th July), indicated by the dashed line.

As an example of the data obtained Figure 14A and B show 4 days of hourly flux measurements using the two methods. The methods clearly agree well on these days. The different techniques tend to favour different conditions, with the gradients becoming smaller and less ideal at higher windspeed, and the eddy covariance methods working well in these conditions. It is early in the

detailed analysis of these measurements, but the excellent data set for the summer of 2001 will allow a very detailed comparison of the different methods and an analysis of the uncertainty in each of the methods.
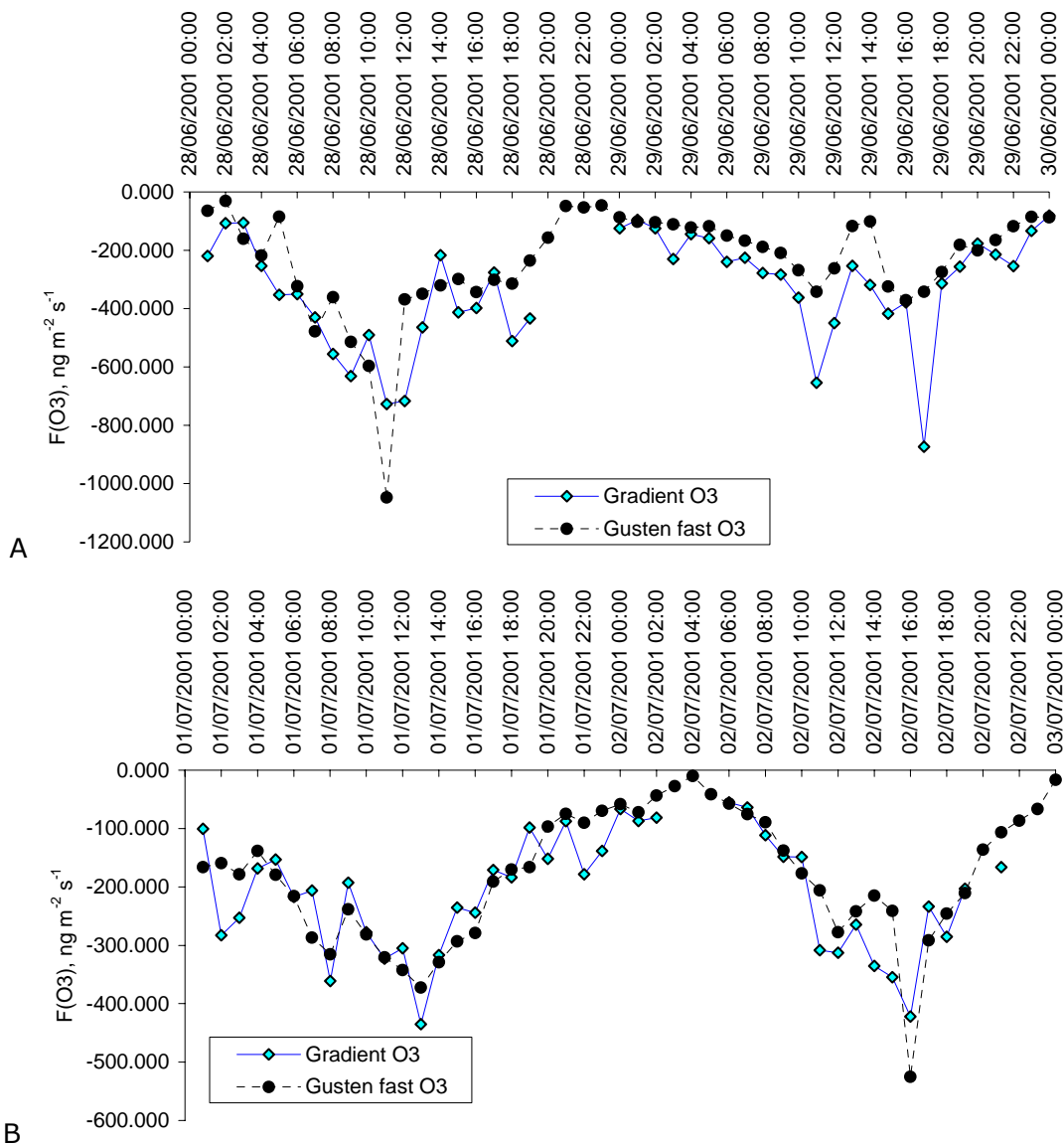

Figure 14. Comparison of ozone fluxes measured over grassland at Easter Bush using the gradient and eddy-correlation (Gusten) methods.

From the results shown in Figure 15 it appears that the stomatal flux of ozone is not as important at Easter Bush as it is as Sutton Bonnington. However, the detailed analysis is not yet complete and unlike the Sutton Bonnington data set, these measurements have extensive additional measurements of water vapour flux, so that the stomatal resistance to both water and ozone can be estimated by several different methods. The relative importance of stomatal and non-stomatal ozone deposition is illustrated further by Figure 15, which shows the monthly average day-time and night-time average flux and canopy resistance measured at Easter Bush. The day-time flux only increases by ~50% compared to the night-time whereas at Sutton Bonnington the difference averaged ~90%. The day-time canopy resistance decreases from the night-time values by ~50% whereas at Sutton Bonnington the change is ~75%.
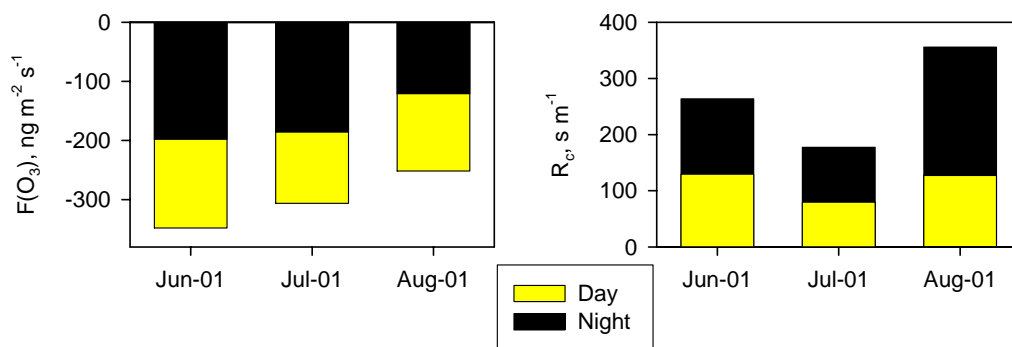
Figure 15. June, July and August average (A) ozone fluxes during daylight (St ≥ 50 W m^{-2}) and night-time (St < 50 W m^{-2}) and (B) daytime and night-time canopy resistance to ozone.

Adjusting AOT40 Measurements for the Influence of Surface Deposition

The results shown here and from earlier measurements indicate that surface deposition has a significant effect on AOT40 values measured between a height of ~3m and the top of the canopy. The measurements of the national ozone monitoring network are all made at around 3-4 m and so this effect is only significant for low vegetation, <3 m tall. Thus estimates of AOT40 forests (AOT40f) made from the network measurements will give a reasonably accurate picture of forest exposure but the AOT40 for crops and semi-natural vegetation (AOT40c) may be a significant over-estimate. It is generally accepted that its is the stomatal component of ozone deposition to vegetation that causes damage to the plants and so it would be appropriate to adjust the AOT40 forests and crops to account for this. However the data collected and analysed to date have not allowed the relationship of AOT40 to stomatal flux to be well enough established to allow this to be done. In view of the current focus on providing direct estimates of the stomatal flux and a critical level based on it, a correction to the national maps of AOT40c to account for total surface deposition only is considered in the following discussion.

The above analysis of Sutton Bonnington data focused on the summer months, May to July, as this is the period over which AOT40c is calculated. Unfortunately, at Easter Bush foot and mouth disease precautions delayed the start of the measurements until late May. At Sutton Bonnington the 2001 measurements gave a 40% reduction in the average AOT40 from the top height (3 m) to the crop height and most of this (~75%) was due to stomatal deposition. Similar proportions were measured over the cereal crops in previous years. At Easter Bush the ozone concentrations were too low to allow the calculation of an AOT40. However, we can consider the concentration difference between the top and bottom height, which averaged ~90% from the end of May to July. If we assume that the relationship between ozone concentration and AOT40 at Easter Bush is similar to that at Sutton Bonnington a reduction in the AOT40 by ~40% can be taken, however a smaller proportion of this change would be due to stomatal deposition to the grassland (~50%). The deposition of ozone to semi-natural vegetation has not been measured as part of this project, however results for a moorland in Southern Scotland (CEH unpublished data) also gave an AOT40 reduction of ~40% on average (over 1995 to 1998), although there is quite a large variation from

68% in 1998 to 25% in 1998. From these results a reduction in AOT40c of 40% for wheat (and other arable crops), grassland and moorland would be appropriate.

Figure 16A shows a map of AOT40c for 1994 to 1998, calculated using the method described in PORG (1998) and Coyle *et al*. (2001), and Figure 16B show this map reduced by 40% in the grid squares containing more than 30% crops, grassland or moorland. The land cover map used is described in Smith *et al.* (2000) and is at a 5 km x 5 km scale, so the AOT40c map has been rescaled to 5 km x 5 km (from an original 1 km x 1 km) by averaging. The uncorrected map indicates that 53% of the grid squares exceed the critical level of 3000 ppb h and this represents around 50%, 50% and 52% of arable, grassland and moorland respectively. These exceedance estimates are smaller than those normally given as they are based on a 5 km x 5 km scale map which has smoothed out some of the smaller scale variations in AOT40. The revised AOT40c gives an over all exceedance of 18% and this represents around 18%, 17% and 18% of arable, grassland and moorland respectively.

A   B.
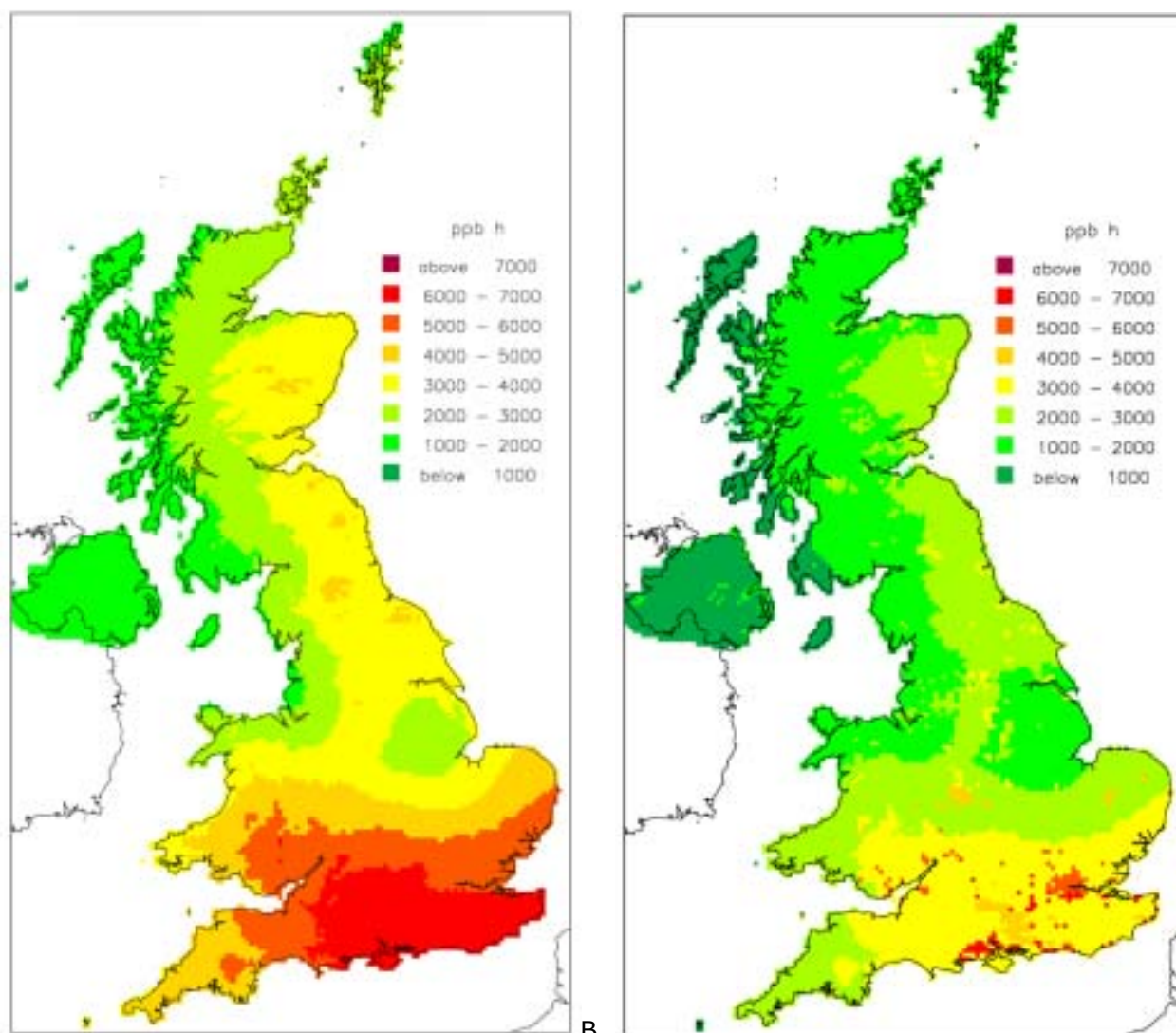
Figure 14. (A) 1994 to 1998 average AOT40 for crops and semi-natural vegetation at a 5 km x 5 km scale and (B) a map that has been revised to account for the reduction in ozone concentration with height over short vegetation.

Conclusions

1. The extensive ozone deposition fluxes at Sutton Bonnington provide ideal data to define the variability of ozone deposition velocity to wheat, sugar beet, oats and bare soil in the UK.

2. The detailed flux measurements at Easter Bush of ozone deposition to grassland, along with latent and sensible heat flux will allow the different ozone deposition models to be tested against very detailed micrometeorological data.

3. The separation of stomatal from non-stomatal deposition in the grassland data from Easter Bush will allow the process of non-stomatal deposition to be investigated and the effects of radiation and surface temperature to be separated.

4. Flux measurements have been used to correct the AOT40 from a height of 3 m to the crop surface. The correction reduces AOT40 by typically 40% and has been used to map the change in AOT40 throughout the UK resulting from this correction.

Acknowledgements

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THE SEASONAL VARIABILITY IN SENSITIVITY OF BEECH TO OZONE

(Ref. C989 0005 D4 128 003)

Final report (July 2001)

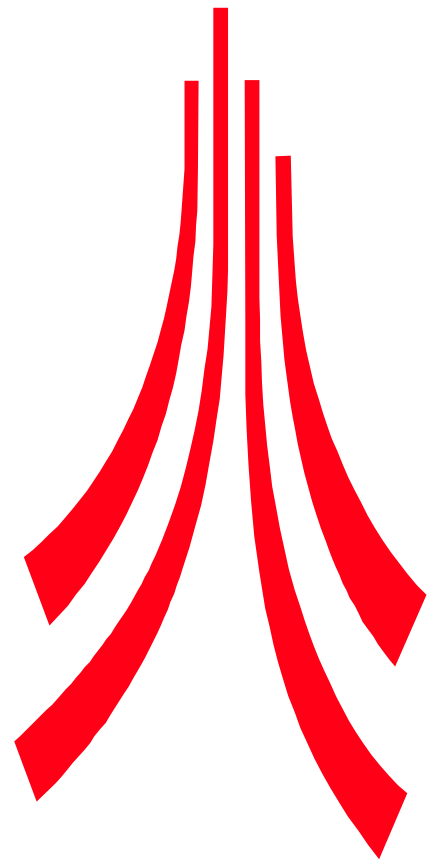Principle investigators
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EXECUTIVE SUMMARY

Critical levels of tropospheric ozone pollution are defined by the AOT40 (accumulated exposure over a threshold concentration of 40ppb ozone), calculated using total ozone from 1st April to 30th September. This project aimed to determine whether the response of beech trees to ozone varies with the timing of ozone pollution and, hence, whether AOT40 might be refined by taking account of any seasonal variation in ozone sensitivity.

- Ozone pollution early in the growing season (mid-May to mid July) significantly reduces the ability of beech to fix carbon, but comparable pollution later in the season (mid July- mid September) had no significant effect. The response to ozone in spring occurs at AOT40s less than approximately 2.0 ppmh., i.e. well below the threshold of 10 ppmh generally accepted for effects on plant growth.

- Experiments in which plants were "forced" in order to allow plants at different developmental stages to be fumigated at the same time confirmed that increased sensitivity to ozone early in the season was a function of plant developmental stage, not differences in environmental conditions between spring and summer.

- Although the mechanism of early season ozone damage remains unclear, it appears to lie in direct ozone effects on stomata rather than with photosynthetic metabolism.

- Early season ozone damage coincided with the period of maximum rates of photosynthesis, and reduced biomass increase between May and July by approximately 20%. However, in the absence of continuing ozone exposure beech was able to recovery to some extend from initial damage.

- Plants exposed to ozone throughout the growing season (mid May-mid September) were less able than unfumigated plants to fix carbon late in the season. However, carbon fixation late in the season was low compared with that in June and July, and these late-season effects of ozone have little effect on plant growth, although they may contribute to a reduction in root reserves during autumn.

- On the basis of data obtained to date, we suggest that calculation of AOT40 for ozone might be "weighted" to take account for the greater effects of pollution in late spring and early summer.

Contents

Summary

One or two year old saplings of beech (*Fagus sylvatica*) were grown in Solardome glasshouses were exposed to ozone treatments early in the growing season (mid-May to mid July) and/or late in the season (mid July- mid September). Responses were quantified using *in situ* gas analysis, and destructive harvests for plant morphology and biomass. Ozone pollution early in the season (May-July) significantly reduced net photosynthesis, transpiration and stomatal conductance in beech within one-two weeks of the start of fumigation. Comparable ozone pollution later in the season (July-September) had no significant effects. The mechanism of this response is not yet known, but seems likely to reflect a direct effect of ozone on stomata. Experiments confirmed that greater ozone response early in the season was a function of developmental stage rather environmental differences between spring and summer. The early season response occurs at cumulative ozone concentrations well below the current critical AOT40 of 10 ppmh. In plants exposed to ozone throughout the season, photosynthesis during August and September was significantly inhibited compared with controls. This response was associated with a significant increase in internal CO_2 concentration, but not with any change in stomatal conductance. However, photosynthetic rates late in the season were low compared with those in June and July, and these reductions in late-season carbon fixation due to long-term ozone fumigation do not have major effects on biomass production, although it may have contributed to a significant reduction in root:shoot ratio during autumn. Data obtained during this project data suggest that the current critical level criteria might be improved by "weighting" ozone episodes according to season, with greater weight being given to episodes early in the year.

1. Introduction

The concept of critical levels for tropospheric ozone has been substantially refined over the past ten years. The recognition that potential ozone damage to plants is best described by the cumulative ozone concentration above a defined threshold has led to the adoption of AOT40 (accumulated exposure over a threshold concentration of 40ppb ozone) as a standard measure of ozone pollution. The critical AOT40 for forest trees is set at 10 ppmh for daylight hours over a six month growing period based on an expected response of a 10% reduction in biomass (Anon, 1997). However, it is recognised that the current definition of critical levels is limited in some respects. It does not account for the differences between species and, for example, recent evidence suggests that beech (*Fagus sylvatica*), widely used as a model of the response of woody plants to ozone, may be more sensitive than many conifers (Landolt *et al.*, 2000) but less sensitive than many other woody species (Van der Heyden, *et al.*, 2001; Bortier *et al.*, 2000). In addition, current critical levels do not take account of interactions between ozone and other environmental factors, such as soil moisture content, temperature, humidity and CO_2 concentration (e.g. Broadmeadow *et al.*, 1999; Broadmeadow, 1998). Such environmental factors may affect the uptake and/or physiological effects of ozone, and hence modify the damage that is caused. A further constraint on current critical levels is that the effects of cumulative ozone dose are assumed to be independent of plant developmental age or time of year. However, there are a number of reports that suggest that plant responses to ozone are significantly affected by the timing of ozone episodes (e.g. Hawes, 1998; Reich, 1983; Pääkkönen *et al.*, 1995a and 1995b). Studies of beech (*Fagus sylvatica*) conducted at Lancaster under a previous NERC/DETR contract showed that response varied with season. Beech saplings exposed to ozone in late spring-early summer showed significant reductions in net photosynthesis, transpiration and stomatal conductance, but no such responses occurred in saplings exposed in mid-summer (Hawes, 1998). The aim of the current contract is to corroborate and refine these observations and hence to establish whether the response of beech to a given AOT40 varies with the timing of ozone episodes.

2. Materials and Methods

All experiments were carried-out using four Solardome glasshouses (Rosedale Engineering, Filey, UK), as described before (Lucas *et al.*, 1987). At any time ozone was supplied to two of the four Solardomes. Strictly, since ozone treatments were imposed on Solardomes, not plants, there were two true replicates (Solardomes) per treatment. Analysis of the growth of beech in 1998, when no ozone treatment was imposed, showed no significant between-dome variation (data not presented). However, to minimise the effect of any remaining "between dome" differences, ambient and ozone treatments were re-allocated between domes at intervals throughout the study period (Appendix 1). Clearly, plants of a particular treatment were moved between domes and, at the same time, plants within treatments were re-randomised between and within domes.

2.1   Ozone treatments

Ozone fumigation experiments were conducted in 1999, 2000 and 2001. Control plants received unfiltered air in all three years. In 1999 and 2000 ozone treatments commenced in early-May and continued until late September, while in 2001 fumigation ended in mid-July, at the end of the contract (Appendix 2).

2.1.1 1999 experiment

In 1999 ozone fumigation was divided in to two periods of nine weeks: "Early" (16th May –18th July) and "Late" (19th July – 19th September), and plants were exposed to ozone according to a fully factorial design, i.e.:

No ozone: "Control"
Early ozone only   "Early ozone"
Late ozone only   "Late ozone"

Early and late ozone "Continuous ozone"

Within each period, ozone was provided as a defined series of episodes, each of which lasted one week. Within each episode peak daily ozone concentration was increased from the Monday to Wednesday, then decreased to Friday. No ozone was added on Saturday or Sundays. Peak ozone concentration was 60, 80, or 120 ppm ozone, based on the range of ozone treatments provided in previous seasons. However, ozone treatments given during previous experiments with beech were based on prevailing weather conditions (e.g. Hawes, 1998). Clearly, coupling treatment to weather would not necessarily produce comparable "Early" and "Late" treatments (see Hawes, 1998 for example), so in this experiment ozone treatments were independent of weather. The target ozone episodes were designed to give an AOT40 of 8.4 ppmh, just below the accepted threshold for damage. Clearly, the "Continuous ozone" treatment provided an AOT40 of 16.8 ppmh, well above the critical limit.

Details of the ozone treatments actually achieved during the 1999 experiment are presented in Appendix 2.

2.1.2 2000 and 2001 experiments

In 2000 ozone fumigation were provided May-July and/or July-September, i.e. treatments were comparable to the "early", "late" and "continuous" ozone fumigations in 1999 (see Appendix 2). In 2000 ozone treatments provided AOT40s of 10.8, 11.1 and 21.9 ppmh respectively for early, late and continuous ozone treatments (Appendix 2). In 2001, fumigation was limited to the "early" (May-July) period, providing an AOT40 of 12.3 ppmh (Appendix 2). In both years, ozone was provided as a defined series of weekly episodes, with target peak ozone concentrations of 60, 80, or 120 ppm ozone, as in 1999.

2.2   Ozone generation and control

Ozone was generated using an ozone generator (Ozonia CFS-1) supplied with compressed dried air. Ozone was passed through water to remove impurities, in particular N_2O_5 (Bytnerowicz *et al.*, 1995) before being added to the Solardome air supply. Ozone concentration was monitored using a PC based data acquisition and control systems (Lancaster Data Analysis System Version 1.5.2-B6: LanDACS) and the desired concentration was maintained by regular adjustment of the voltage supplied to the ozone generator. LanDACS was also used record temperature, humidity and solar radiation in the domes.

2.3   Plant material

2.3.1   1999 experiment

Three hundred two year old, similar sized beech trees (*Fagus sylvatica* L.), planted in 10 litre polythene pots using a mixture of sterilized loam, grit and peat (5:3:3 by volume) to which slow release fertiliser (Osmocote: 16:8:12 NPK) was added at 1.8g per 100 l compost. The final compost was in the range pH 5-6. In early May, the trees were allocated randomly between the four ozone treatments (see section 2.1.1) and placed in the Solardomes. The plants were watered two-three times a day, using an automatic dripper system, controlled by a timer.

2.3.2   2000 and 2001 experiments

For the shorter term experiments conducted in 2000 and 2001, one year old ozone trees were used, planted in 4 litre pots. Other cultivation methods were as in 1999.

2.4 Shoot and root pruning

Preliminary experiments during 1998 (Appendix 3) showed that leaf expansion was largely completed by June-July, with only a small additional increase attributable to "lammas growth" later in the season, while root growth was minimal prior to July, but rapid from July until the end of growth. This temporal separation of leaf and root growth relates to one possible mechanism for seasonal variation in ozone response i.e. that ozone early in the season creates an imbalance between water loss (transpiration from the rapidly expanding foliage) and water uptake (by a limited root system).

Thus, in 2000, root:shoot ratios were manipulated by selective pruning either roots or shoots. Foliage area was reduced by removing approx. 50% of lateral shoots using secateurs, remaining laterals were not pruned. Root pruning was achieved by inserting a sharp, long bladed knife into the compost, and drawing it across the pot, slightly to one side of the beech stem, which was planted centrally in the pot. Clearly, it was not possible in this way to remove a precise proportion of the root system, but destructive sampling of a small group of plants immediately after pruning confirmed that an average of approximately 50% of the root system had been removed.

2.5. Forcing treatments to produce beech at contrasting developmental stages at the same time.

In 2000 and 2001, plants at different developmental stages at the start of the growing season were produced as follows:-

i)   Unforced. Plants were retained in the field until 7th May, then transferred to the Solardomes prior to the start of ozone fumigation (9th May). This is the same regime as used in previous seasons.

ii)   Forced. Plants transferred to a Solardome at the beginning of March, and after two weeks transferred to an heated glasshouse with a 16h day provided by supplementary lighting (400W SON-T) giving approximately 150 μmole m^{-2} s^{-1} PAR at plant height. Plants remained in the warm glasshouse for five days and were then returned to the Solardome, where they remained until 7th May, after which they were treated as for the unforced plants.

At the start of fumigation (early May), leaves of plants forced in the cold glasshouse were fully pigmented and hardened, but there was no sign of the second flush. In unforced plants first flush leaves were still expanding at the start of fumigation, and these leaves remained pale green and unhardened for the first 3-4 weeks of the experiment.

2.6   Gas exchange

In each experiment, leaf gas exchanges in forty marked trees, ten per ozone treatment, were measured at intervals of approximately one week . The youngest fully expanded leaf of the leading shoot was selected prior to the start of fumigation, and marked by loosely tying a loop of 1 mm electrical wire around the leaf petiole. Gas exchanges were measured using a portable infrared gas analysis system and Parkinson leaf cuvette (CIRAS-1, PP Systems, Hitchin, Hertfordshire). All gas exchanges measurements were made with CO_2 concentration within the leaf cuvette set at 360 ppm $\pm$ 10) and PAR (provided by the CIRAS cuvette illumination system) set at 1500 μmole m^{-2} s^{-1}. It was not possible to measure all trees in one day. Therefore, each week measurements were made on successive days. Twenty plants selected at random from all treatments and Solardomes, were recorded on the first day, and remaining plants were on the following day. All measurements were made between the hours of 10.00 and 13.00 BST. Data for net photosynthetic rate (A), internal CO_2 concentration (c_i), transpiration rate (E) and stomatal conductance (G) were recorded.

2.7 Sap flow measurements

Total plant water use was quantified using sapflow gauges (Dynagage, Model sga10, Dynamax Inc., Houston, Texas). Sapflow gauges were fitted to the stems of 24 plants (6 per treatment) at the start of the growing season. Since a study by Wiltshire *et al*. (1995) showed that the silicone grease used with the gauges caused stem damage, only the minimum amount was applied to the heater strip and interior of the sensor. After fitting each gauge, a triple layer of foil was wrapped around the entire gauge assembly, and also the stem sections above and below the gauge, to prevent excessive temperature gradients. Operation of the sapflow gauges was carried out using a Delta-T, DL2 datalogger according to the manufacturer's instructions (application note: SFS-AN-1.1, Delta-T Devices Ltd., Burwell, Cambridge, UK). Output from each gauge was monitored every minute and averaged every 10 minutes throughout the daylight hours. The data were downloaded on a daily basis to a computer.

Between one hour after sunset and one hour before sunrise, no power was supplied to the gauges to prevent heat damage to the stems during the night when sapflow ceased. Apart from short periods when they were refitted to allow for stem radial growth, the gauges remained on the trees throughout the growing season.

2.8   Destructive harvests

2.8.1 1999 experiment

Trees were harvested at intervals throughout the season. Each harvest included twenty trees per treatment, ten from each of the two domes that provided ambient or elevated ozone. At harvest, all leaves were removed and total leaf area measured before drying to constant weight at 80°C. The root system was excised at soil level, washed and divided into fine and coarse roots. The total lengths of these two root fractions were measured. In half of the trees, a small sub-sample of fine roots were taken for estimation of root hydraulic conductance. Remaining roots were then dried at 80°C. The stem was oven dried at 80°C. The dry weights of all components were recorded, and used for growth analysis.

2.8.2 2000 and 2001 experiments

In the shorter term experiments, destructive harvests were carried out as in 1999, but confined to one immediately before the start of fumigation and a second at the end of fumigation (mid July).

2.9   Statistical Analysis

As noted above, ozone treatments were re-randomised between Solardomes, and trees between Solardomes within treatment, allowing plants to be treated as replicates. On this basis there were 20 per treatment (10 trees per dome with two domes) at each harvest date for destructive harvests, and ten trees per treatment (5 per dome) for gas analysis. Statistical analysis was carried out using a range of analyses of variance (ANOVA) using SPSS statistical software.

3. Effects of ozone fumigation "early" and "late" in the season

3.1 Results

3.1.1 Gas exchanges

3.1.1a Responses during "early ozone" treatment, prior to "cross-over" (16th May – 18th July) Net photosynthesis increased over the initial period of exposure in both control and fumigated trees (significant effect of time, p< 0.001, no significant time x treatment interaction). The main effect of ozone treatment was also significant (p<0.01), since net photosynthesis was consistently lower in fumigated and control trees (Figure 3.1). The magnitude of this reduction in net photosynthesis varied (2-29% compared with controls), and there was a tendency for the greatest effects to occur early in the course of ozone treatment. For example, photosynthesis was 15% lower in fumigated than control trees after one week of ozone fumigation. Changes in stomatal conductance during "early ozone" (Figure 3.2) were broadly similar to those in photosynthesis. Thus, conductance increased with time, independent of treatment (main effect of time significant at p<0.001, no significant time x treatment interaction) and was significantly (p<0.01) lower in ozone treated than control trees. Despite the significant main effect of fumigation, the effects of ozone on conductance varied markedly from week to week, but it was notable that a large response occurred within one-two weeks of treatment.
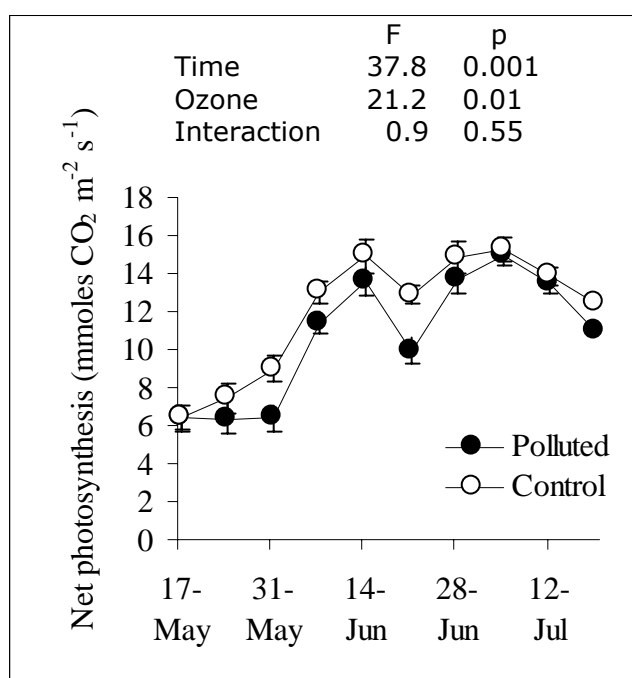

Figure 3.1 Net photosynthesis during the "Early ozone" period (16th May – 18th July) in control (open symbols) and ozone-fumigated plants (filled symbols). Data are the mean of 40 replicate plants $\pm$ 1SE. The results of two-way ANOVA (time x ozone treatment, with time treated by repeated measures) are summarised.

Figure 3.2 Stomatal conductance during the "Early ozone" period (16th May – 18th July) in control (open symbols) and ozone-fumigated plants (filled symbols). Data are the mean of 40 replicate plants $\pm$ 1SE. The results of two-way ANOVA (time x ozone treatment, with time treated by repeated measures) are summarised.
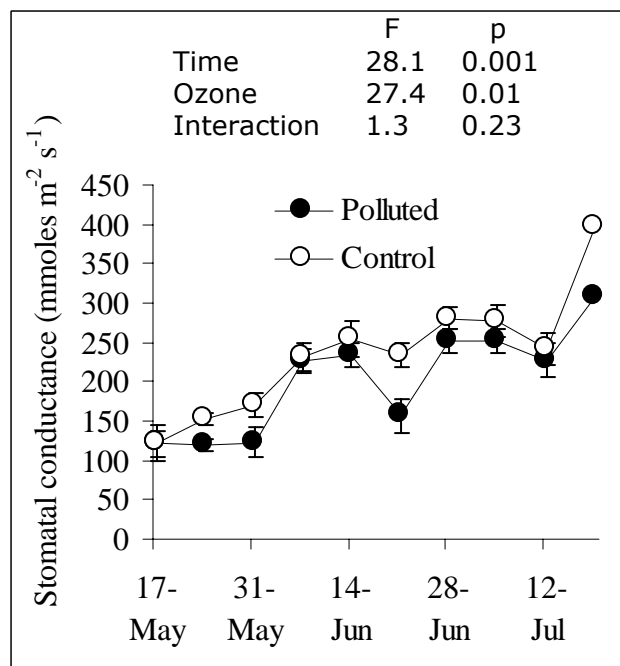
By contrast with photosynthesis and stomatal conductance, internal CO_2 concentration (Ci) was not significantly affected by ozone fumigation, nor was there a significant ozone x time interaction (Figure 3.3). The main effect of time of internal CO_2 concentration was significant

(p<0.01), reflecting a gradual decline in Ci until late June, followed by an increase for the remainder of this early part of the season. Transpiration rate fluctuated during the early part of the season (main effect of time significant at p<0.001, no significant time x treatment interaction) but, in broad terms, tended to increase with time between mid-May and mid July (Figure 3.4). The transpiration rate of ozone-fumigated trees was significantly lower than that of controls (main effect of treatment: p<0.01) although the magnitude of this effect varied, with minimal differences between treatments at some sample dates (Figure 3.4).

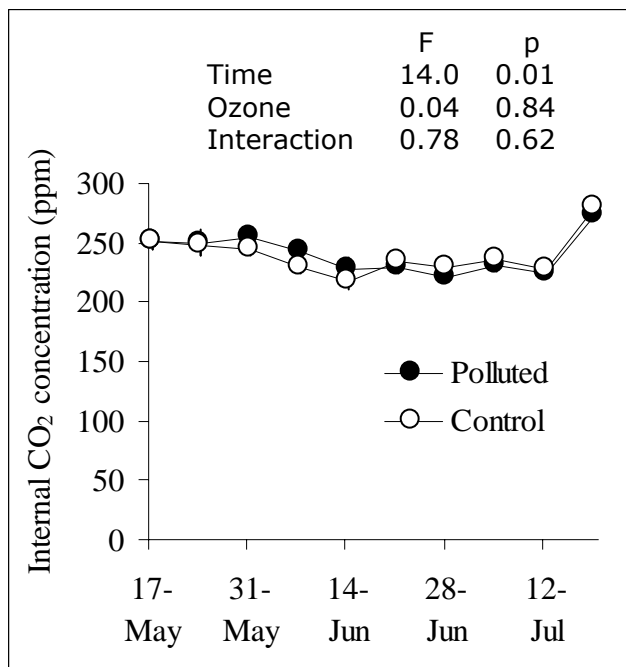|	F	p
Time	14.0	0.01
Ozone	0.04	0.84
Interaction	0.78	0.62

Figure 3.3　Internal CO_2 concentration during the "Early ozone" period (16th May – 18th July) in control (open symbols) and ozone-fumigated plants (filled symbols). Data are the mean of 40 replicate plants $\pm$ 1SE. The results of two-way ANOVA (time x ozone treatment, with time treated by repeated measures) are summarised.

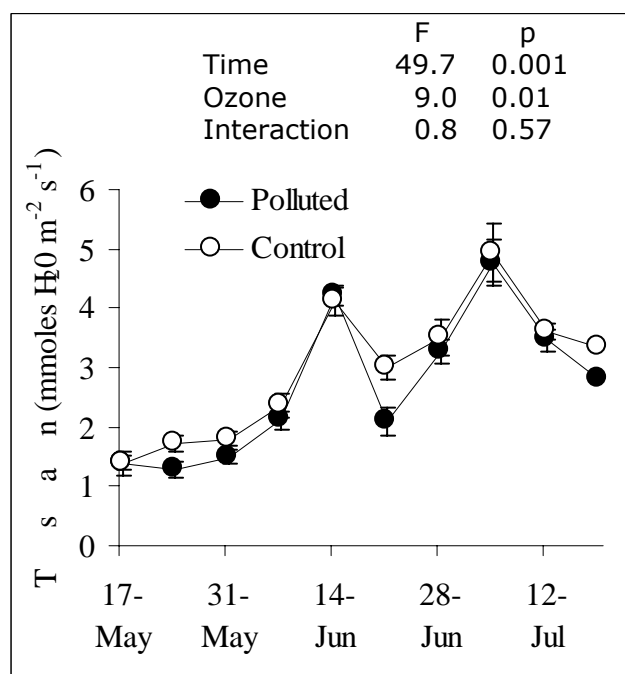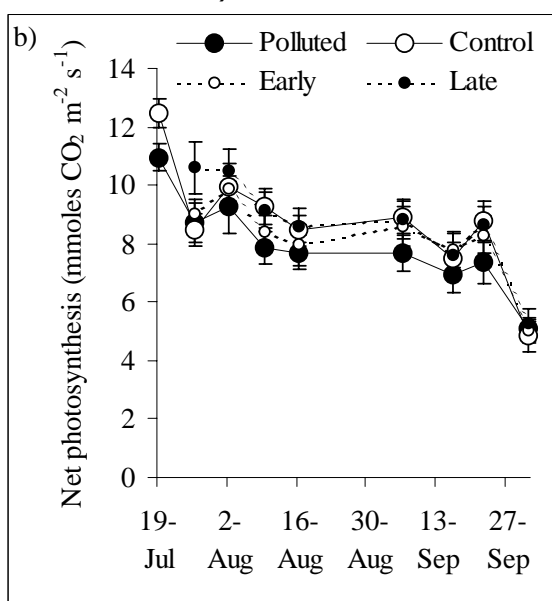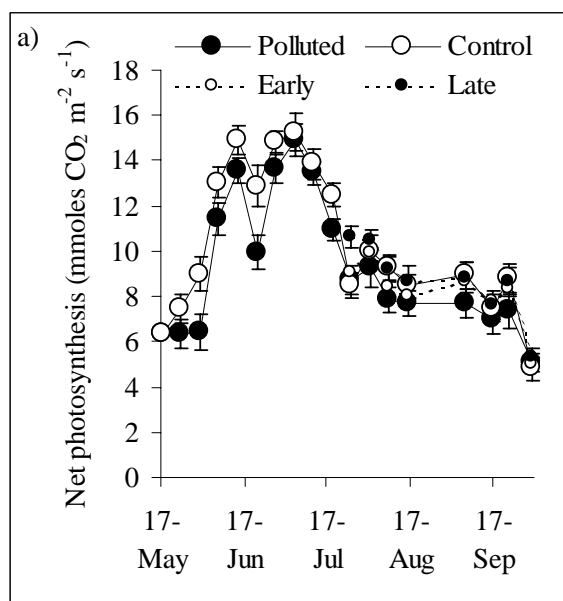|	F	p
Time	49.7	0.001
Ozone	9.0	0.01
Interaction	0.8	0.57

Figure 3.4　Transpiration rate during the "Early ozone" period (16th May – 18th July) in control (open symbols) and ozone-fumigated plants (filled symbols). Data are the mean of 40 replicate plants $\pm$ 1SE. The results of two-way ANOVA (time x ozone treatment, with time treated by repeated measures) are summarised.

Source	F	p
Time	26.2	<0.001
Early ozone	5.7	0.02
Late ozone	0.69	0.41
Early x late ozone	5.4	0.03

Figure 3.5 Net photosynthesis over the whole season, before and after the "cross-over" between early and late ozone treatments (19th July). Data are the mean of 10 replicate plants $\pm$ 1SE. Summary table of the results of three-way ANOVA (early ozone x late ozone x time, with time treated by repeated measures). Only significant terms are shown.

3.1.1b Responses during following the "cross-over" (19th July-)

The decline in photosynthesis apparent in all plants during the latter part of the "early" part of the season continued throughout the "late" period (Figure 3.5a). The main effect of time during the late period was significant ($p < 0.001$) but there were no significant interactions between time and the effects of ozone treatments. "Late ozone" had no significant effect on net photosynthesis and while the effect of "early ozone" was significant ($p < 0.05$) there was also a significant interaction between early and late ozone ($p < 0.05$). This significant interaction occurred since the only consistent effect of ozone treatments on net photosynthesis was a reduction in plants that had been fumigated during <u>both</u> the early and late treatments (Figure 3.5b). Net photosynthesis in plants that had been exposed to ozone only during the "early" period was not significantly different from that in controls.

The significant decline in photosynthesis during the latter part of the season was associated with a significant increase in internal CO_2 concentration (main effect of time significant at $p < 0.001$: Figure 3.6a). There were no significant interactions between the effects of time and those of ozone treatments. However, the main effects of both "early ozone" and "late ozone" treatments, and their interaction were all significant ($p < 0.05$, $p < 0.05$, and $p < 0.01$ respectively). These ANOVA results were a function of a small but significant increase in Ci in plants that had been exposed to ozone throughout the season (Figure 3.6b). By contrast Ci in plants exposed to ozone only during the "early" or "late" periods was not significantly different from that in controls. Although the main effects of time on stomatal conductance was statistically significant ($p < 0.001$) there was no systemic change after the transient high conductance observed at the time of cross-over in mid-July (Figure 3.7a). Conductance was in the range 200-300 mmole m^{-2} s^{-1} in all treatments and there were no significant effects of "early" or "late" ozone treatments (Figure 3.7b). As in the early part of the season, transpiration after cross-over fluctuated markedly (Figure 3.8a). The only factor to significantly affect transpiration in the post cross-over period was time (main effect of time significant at $p < 0.001$, all other factors and interaction $p > 0.05$).
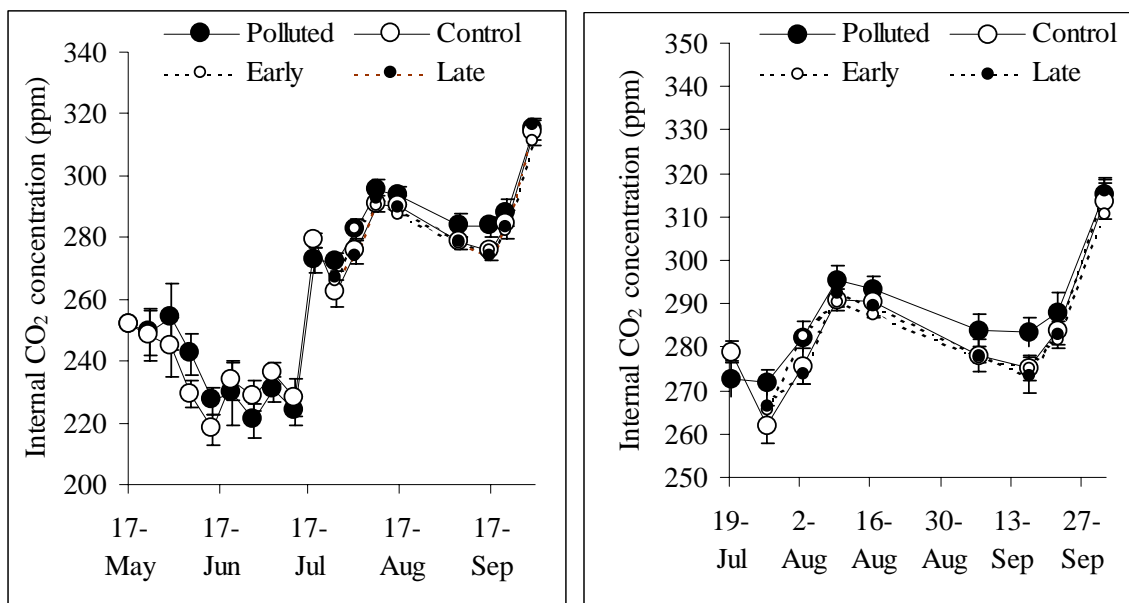
Source	F	p
Time	51.4	<0.001
Early ozone	5.6	0.023
Late ozone	6.42	0.016
Early x late ozone	9.16	0.005

Figure 3.6 Internal CO_2 concentration over the whole season, before and after the "cross-over" between early and late ozone treatments (19th July). Data are the mean of 10 replicate plants $\pm$ 1SE. Summary table of the results of three-way ANOVA (early ozone x late ozone x time, with time treated by repeated measures). <u>Only significant terms are shown.</u>

Figure 3.7 Stomatal conductance over the whole season, before and after the "cross-over" "cross-over" between early and late ozone treatments (19th July). Data are the mean of 10 replicate plants ± 1SE. Three-way ANOVA (early ozone x late ozone x time, with time treated by repeated measures) showed that the only significant factor affecting stomatal conductance during the "late period" was time ($p < 0.001$).
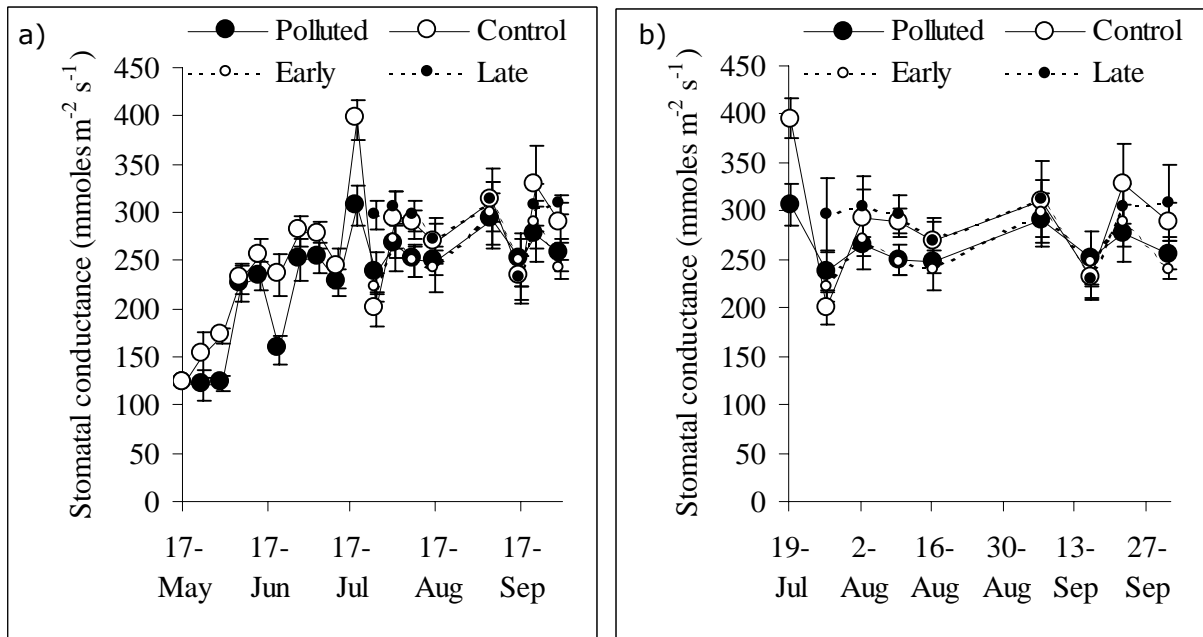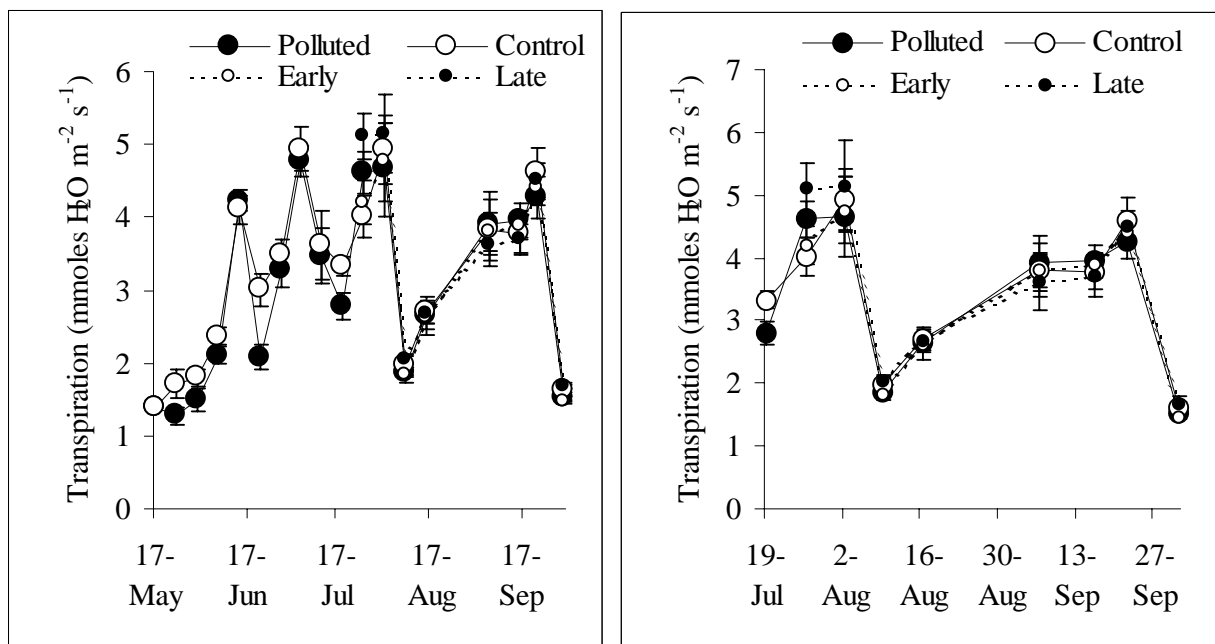

Figure 3.8 Transpiration over the whole season, before and after the "cross-over" between early and late ozone treatments (19th July). Data are the mean of 10 replicate plants ± 1SE. Three-way ANOVA (early ozone x late ozone x time, with time treated by repeated measures) showed that the only significant factor affecting stomatal conductance during the "late period" was time ($p < 0.001$).

3.1.2 Destructive harvests

From the time of bud-burst (mid-late April) leaf expansion was rapid until mid-May, but then slowed substantially (Figure 3.9a). Leaf dry weight increased rapidly between April and June but, unlike leaf area, continued to increase until September (Figure 3.9b). Unlike the initial flush of leaf growth, root growth was limited early in the season but both root length and root weight increased rapidly between May and September (Figure 3.9c-d), after which fine root length, but not dry weight declined substantially (Figure 3.9c,d). Stem dry weight (Figure 3.9e) increased significantly only after May. Root:shoot ratio (R:S) declined rapidly from approx. 1.7 at the start of growth, to 0.75 in June, but then increased for the remainder of the growing season (Figure 3.9f). Total plant dry weight also increased little before mid-May, after which it there was a progressive, near linear increase until September (Figure 3.10).

Of the dry weight components, leaf areas or root lengths that were measured, and the parameters derived from these harvest data, only R:S was significantly affected by ozone treatments. The effects of ozone treatments on R:S were significant only at the final two harvests (September and November) when R:S was significantly lower in plants exposed to ozone throughout the season than in controls (Figure 3.9f). R:S in trees exposed to either "early" or "late" ozone also tended to be lower than that in controls, but these effects were not significant.

The hydraulic conductivity of fine roots increased after May, perhaps reflecting the production of new roots, but was not significantly affected by ozone fumigation (data not presented)
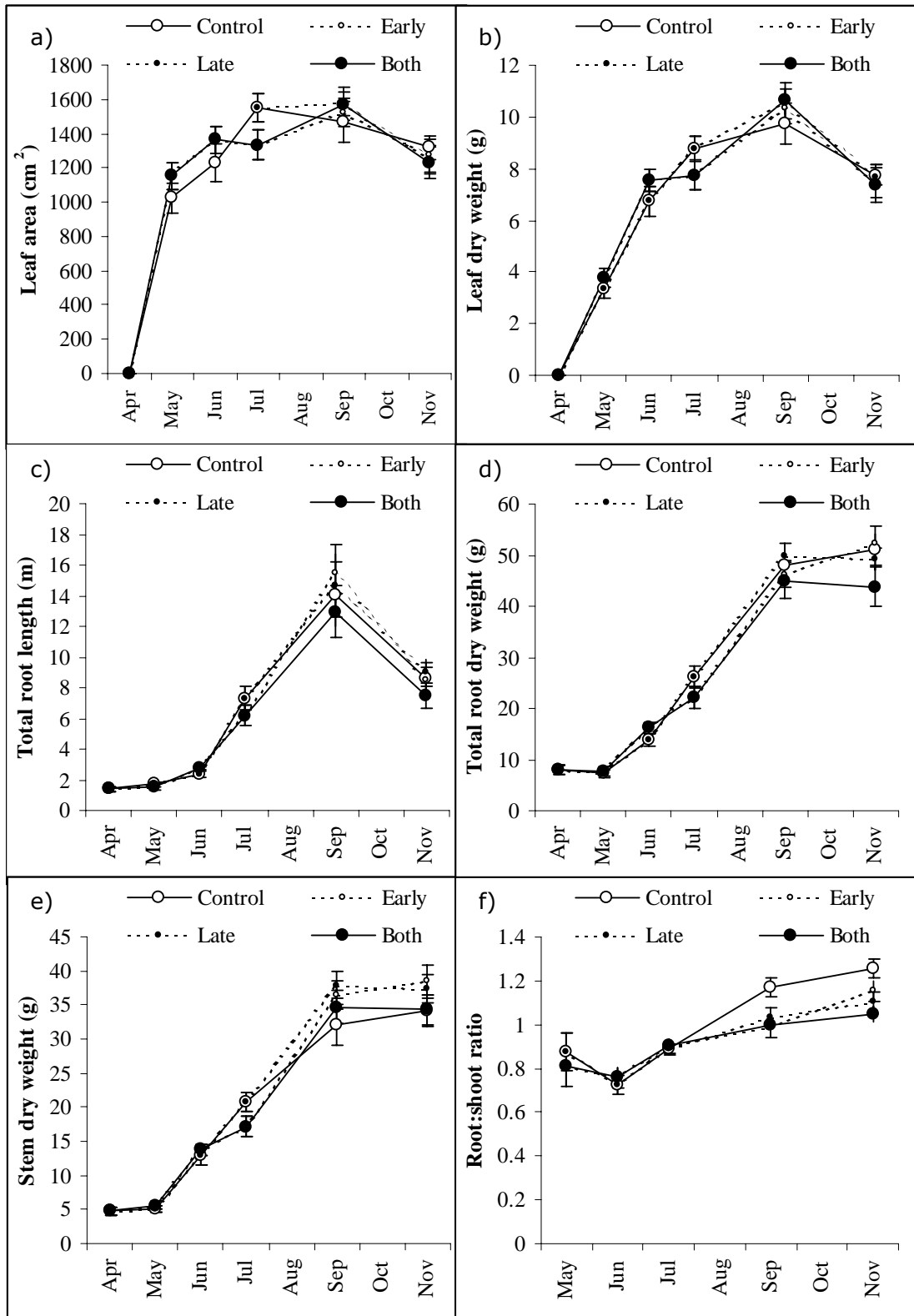
Figure 3.9 Area and dry weight of leaves, length and dry weight of coarse and fine roots of beech saplings exposed to ambient ozone (open symbols) or elevated ozone concentrations (filled symbols). Data are the mean of 20 replicate plants $\pm$ 1SE. Only root:shoot ratio was significantly affected by ozone treatments. The results of ANOVA for R:S were as follows: Time $P<0.001$, Early ozone $p>0.05$, Late ozone $p>0.05$, Early x late ozone $p<0.01$.
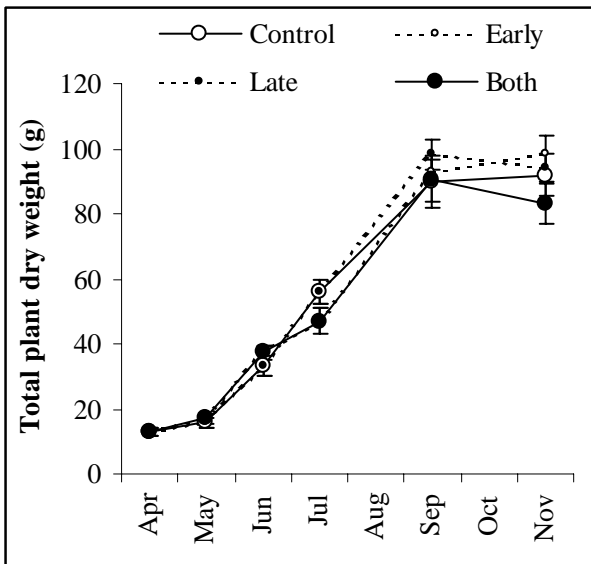
Figure 3.10 Total plant dry weight of beech saplings exposed to ambient ozone (open symbols) or elevated ozone concentrations (filled symbols). Data are the mean of 20 replicate plants $\pm$ 1SE.

3.1.3 Sap flow.

Sap-flow gauges measured plant water use continuously from the time of bud-burst until leaf senescence was complete. This produced an extremely large data set, with the potential to be analysed on a range of time scales, from within single days, to daily water use over the season, to longer-term integrals of water use. Here, only summary data for the effects of ozone treatments on daily and cumulative water loss are presented. As expected, water use fluctuated substantially according to prevailing weather conditions, especially irradiance, but expressing data relative to water use in control plants removes such day-to-day variation and clarifies the effects of ozone treatments.
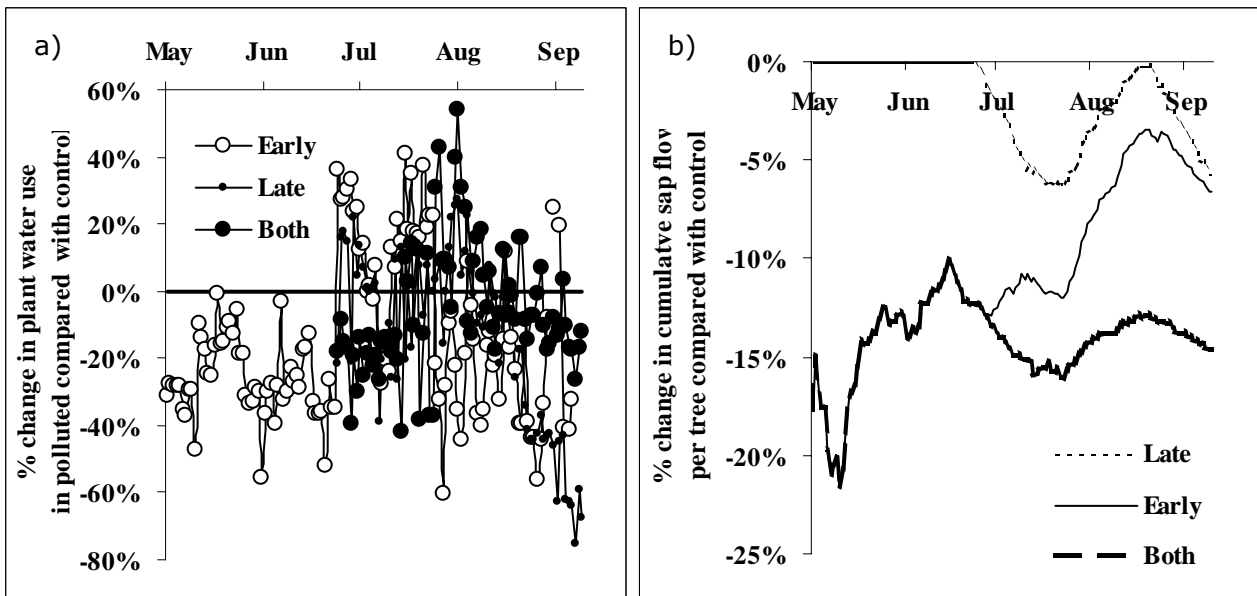
Figure 3.11. Sapflow in beech saplings exposed to early and/or late ozone treatments. Data are a) daily water use and b) cumulative sap flow over the season. In both cases, data for the three ozone treatments are summarised here as percent change compared with control plants.

Daily water use was substantially reduced by ozone during "early" fumigation treatment, with reductions averaging 25$\pm$ 2% over first month of this treatment (Figure 3.11a). Reductions became less marked as the "early" fumigation continued, and by the time of the cross-over, cumulative water use by "early" fumigated trees was approximately 12% less than in controls (Figure 3.11b). By contrast, "late" ozone fumigation had only small and variable effects on daily water use, with the average change during the first month of this treatment being 2 ± 3% (Figure 3.11a). However, again in contrast to the early fumigation, the effect of "late" ozone increased as fumigation continued (Figure 3.11a), so by the end of the season, both "early" and

"late" fumigations had reduced cumulative water use by approximately 6%. Tress exposed to ozone throughout both the "early" and "late" fumigations showed a persistent reduction in water use, with cumulative water use over the whole season being some 15% lower than in control trees (Figure 3.11b).

3.2 Discussion

In the early part of the season exposure to environmentally relevant ozone concentration resulted in a significant reduction in net photosynthesis within seven days (Figure 3.1). This detailed programme of gas exchange measurement carried out during 1999 confirmed the initial observation of Hawes (1998) that ozone treatment had greater effects on leaf gas exchange early in the season. This response occurs at a very low cumulative ozone dose (AOT40 < 1ppmh) and persisted throughout the "early ozone" treatment (mean reduction 13 $\pm$ 2%), although there was a tendency for the magnitude of the reduction to decline with time (Figures 3.1 and 3.12a). However, there was little evidence that this effect persisted in plants that were removed from ozone treatment since "early ozone" treatment had no significant effect on conductance after the cross-over (Figure 3.7 and 3.12b). This rapid response to ozone did not occur in plants exposed when their leaves were fully expanded: the mean reduction in stomatal conductance due to the "late ozone" treatment was 3 $\pm$ 2% (Figure 3.7 and 3.12b). With "early ozone" the rapid decrease in photosynthesis in the first 1-2 weeks of exposure was associated with significant decreases in stomatal conductance (Figures 3.2) but internal CO_2 concentration was not significantly affected by ozone during this period (Figures 3.3). Thus, it seems likely that the observed reduction in photosynthesis was the result of ozone induced stomatal closure.
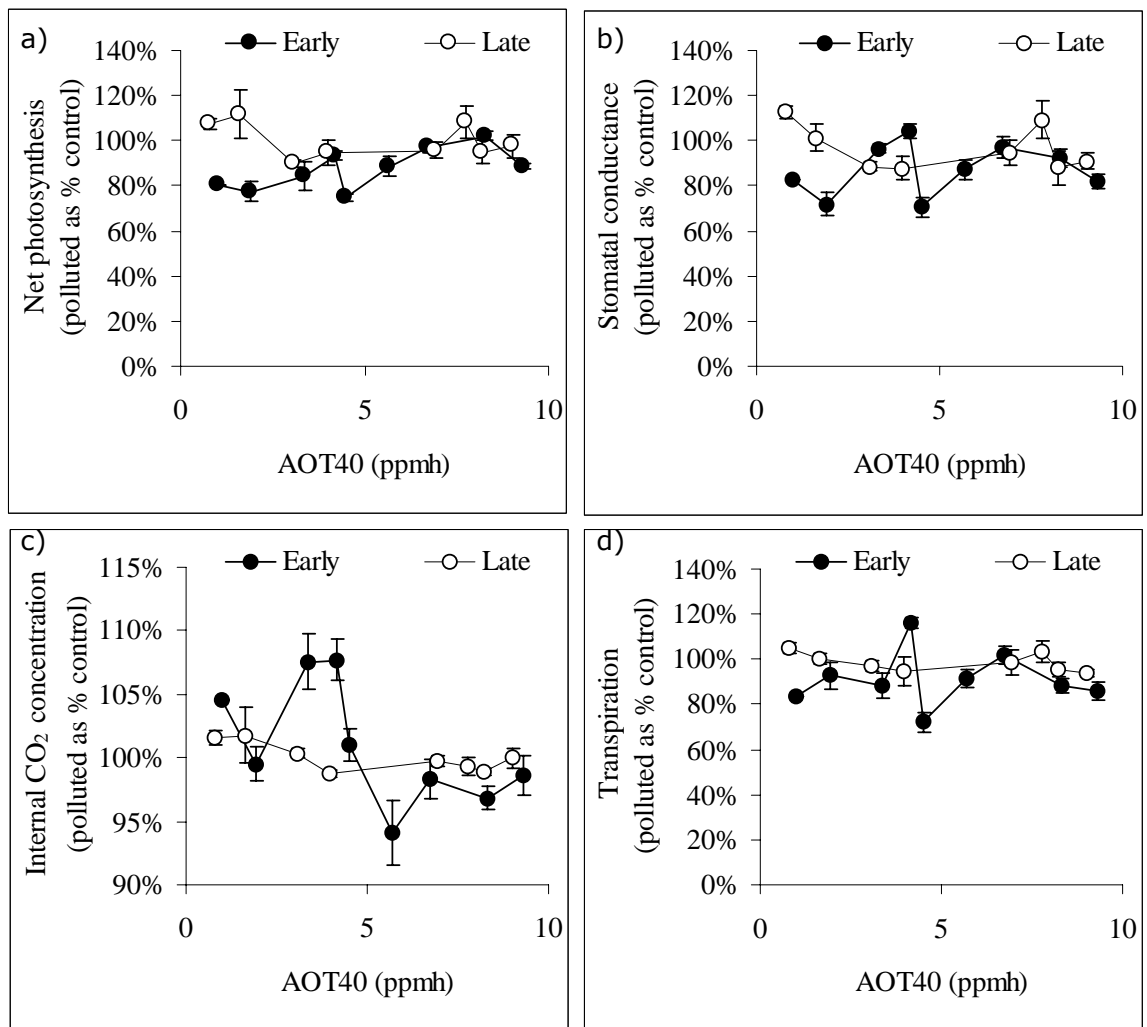

Figure 3.12 Contrasting effects of ozone on a) net photosynthesis, b) stomatal conductance, c) internal CO_2 concentration and d) transpiration in plants exposed to ozone either from May-July ("early") or July-September ("late"). Data ozone fumigated plants are expressed as a percentage of control values.

While plants that were exposed to ozone only during the late period showed no significant physiological responses, marked effects occurred in trees that were fumigated during <u>both</u> the "early" and "late" treatments. In these trees there was a progressive reduction in photosynthesis compared with control plants from late July (Figures 3.5 and 3.13). After this time photosynthesis in fumigated plants declined to around 80% of that in control plants for the remainder of the season (Figure 3.13a). It was notable that this "long-term" effect on photosynthesis occurred above a threshold AOT40 of approx. 10ppmh, i.e. close to the accepted limit for marked ozone effects on biomass production. This photosynthetic response was not correlated with any effect of ozone on stomatal conductance, which was not significantly different in control and ozone fumigated plants during this period (Figures 3.7 and 3.13b). However internal CO_2 concentration was slightly but significantly increased in fumigated plants (Figures 3.8 and 3.13c) suggesting that the long-term effect of ozone on photosynthesis in beech is the result of changes in the inherent capacity of leaf tissues to fix carbon. Although the underlying mechanism(s) of this response remains unclear, it may be consistent with a more rapid onset of senescence in fumigated trees, which is a common response to ozone in many species, including beech (Braun & Fluckiger, 1994; Krause & Hockel, 1995; Langebartels et al., 1997).
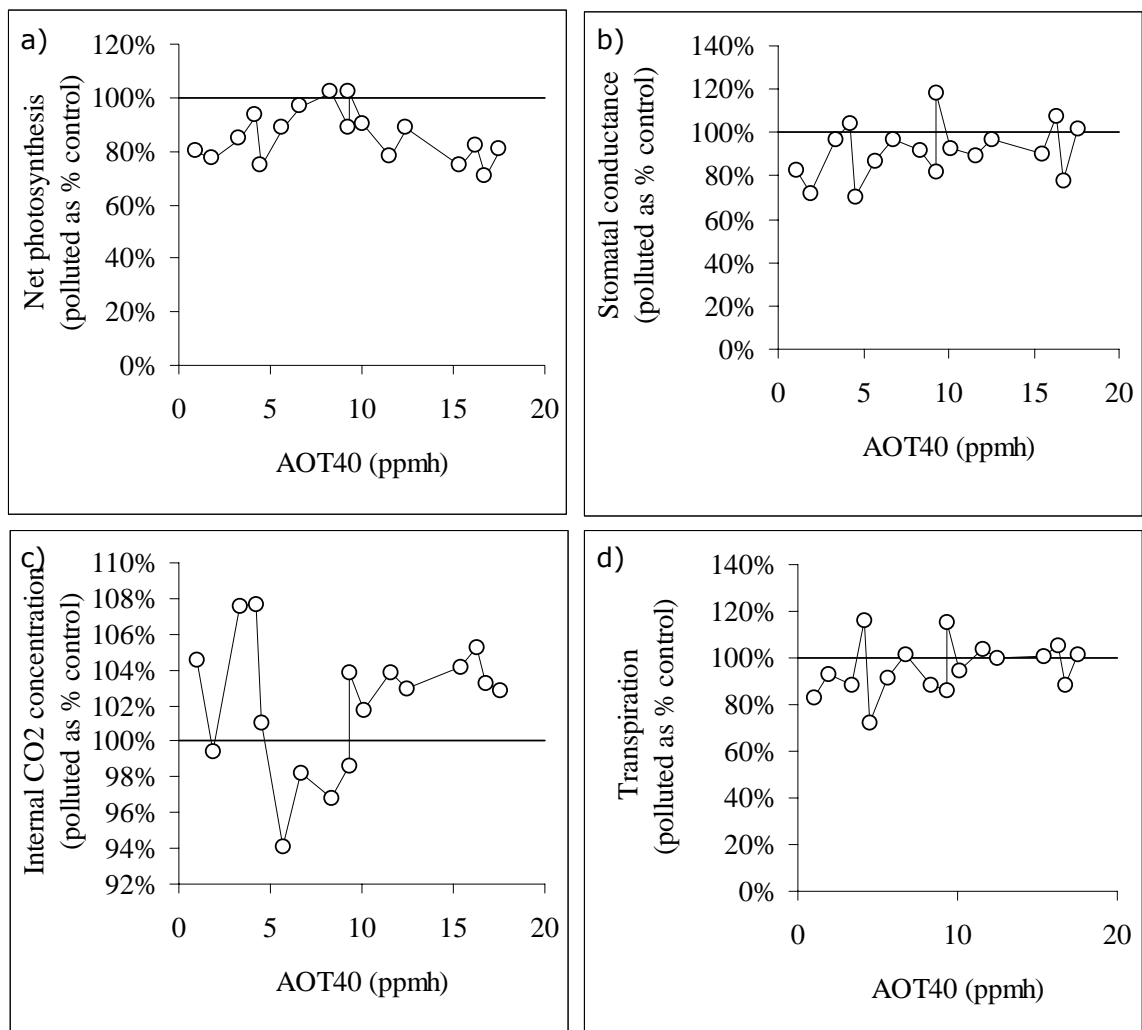
Figure 3.13 a) net photosynthesis, b) stomatal conductance, c) internal CO_2 concentration and d) transpiration in ozone fumigated plants expressed as a percentage of control values. Note that data refer to plants exposed to ozone during <u>both</u> the "early ozone" and "late ozone" treatments.

In general, rates of photosynthesis during increased during the period May to July, but then declined for the remainder of the season (see Figure 3.5 for example), so that rates during the "early" period were substantially greater than those observed later in the season. Thus, reductions due to "early ozone" might have long-term effects on total biomass gain over the whole season, even though photosynthesis later in the season was not significantly affected by previous exposure to ozone (Figure 3.5). The progressive decline in the rate of photosynthesis

later in the season (Figures 3.5 and 3.13a) may have wider implications. On the basis of the cross-over experiment, photosynthesis measured between mid-May and mid-July was estimated to be approximately 60% of the potential whole season carbon fixation. This pattern is clearly subject to modification by changes in environmental conditions, notably PAR, over the course of the season. However, at least on the basis of potential photosynthesis, the reduction in late-season photosynthesis in plants exposed to both early and late ozone, although marked and significant, may not result in large effects on biomass production. Although caution is required in extrapolating from *in situ* gas exchanges measured on small areas of foliage, this seems to be consistent with the growth data obtained. Cumulative photosynthesis in plants exposed to ozone throughout the season was estimated to be around 11% lower than in controls, while the measured reduction in biomass increment over the season was 10% (Figure 3.10). Cumulative photosynthesis during "early" fumigation was estimated to be reduced by approximately 11% by ozone fumigation while the increment in plant dry weight was 20% lower in fumigated plants than controls. By contrast, in plants subjected only to late ozone, cumulative photosynthesis was estimated to be changes by less than one percent compared with control, and the measured increment in plant dry weight was also little changed (3% increase over control). Overall, while the reduction in photosynthesis resulting from exposure to "early" ozone may not in itself, be sufficient to cause significant reductions in biomass at the end of the season, such early ozone effects may be far more important than comparable exposure late in the season.

4. Effects of manipulating root:shoot ratio on the response of beech to ozone

4.1 Results

4.1.1 Responses to ozone in beech subjected to root or shoot pruning in May

In contrast to previous seasons, there were no rapid changes in leaf gas exchanges in response to ozone fumigation early in the season during 2000, regardless of pruning treatments. In unpruned trees, photosynthesis was significantly (up to 30%) inhibited by ozone fumigation late in the season (Figure 4.1) and, as before, this change was associated with an increase in internal CO_2 concentration, but no change in leaf conductance (data not presented). Shoot pruning in May had no short-term effect on the response to photosynthesis to ozone, but appeared to delay or limit this late season inhibition of photosynthesis (Figure 4.1a).   By contrast, the inhibition of late - season photosynthesis by ozone was greater in trees that had been root pruned in May, although photosynthesis during May and June was increased transiently in ozone fumigated trees that had been root pruned (Figure 4.1b).
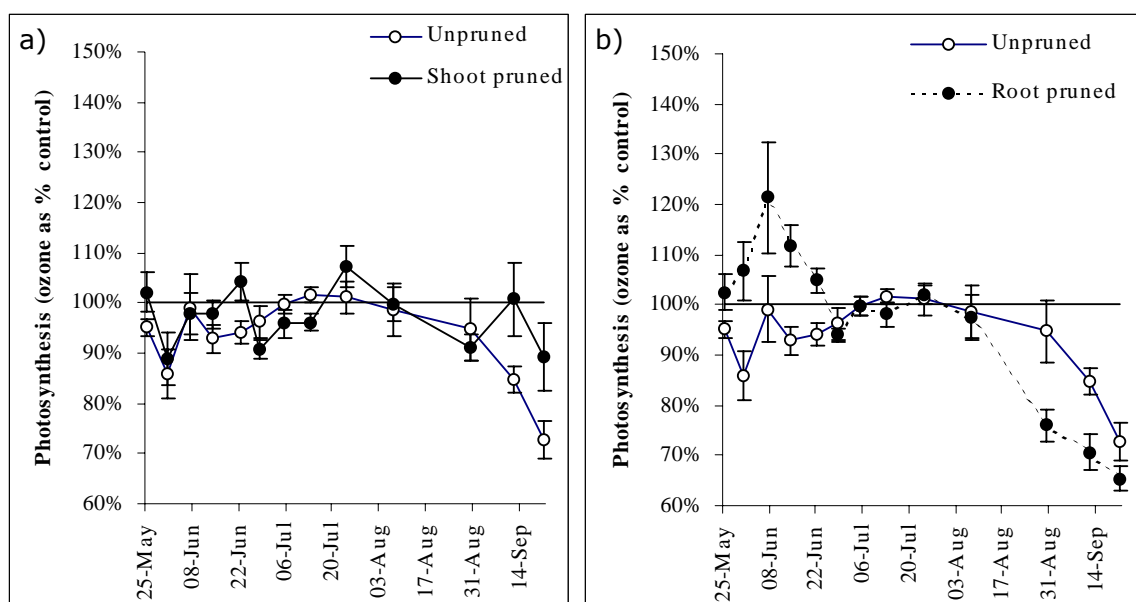

Figure 4.1 The effects of ozone fumigation on net photosynthesis of beech trees subjected to either a) shoot or b) root pruning in mid-May. All data are means of 10 replicates $\pm$ 1SE.

4.1.2 Responses to ozone in beech subjected to root or shoot pruning in July

There were no rapid changes in leaf gas exchanges in response to ozone fumigation in July. In unpruned trees, and those subjected to root pruning, there was also evidence that "late" ozone reduced photosynthesis at the end of the growing season (Figure 4.2). However, in plants that had been shoot-pruned in July photosynthesis was significantly inhibited by ozone from early September (Figure 4.2a).
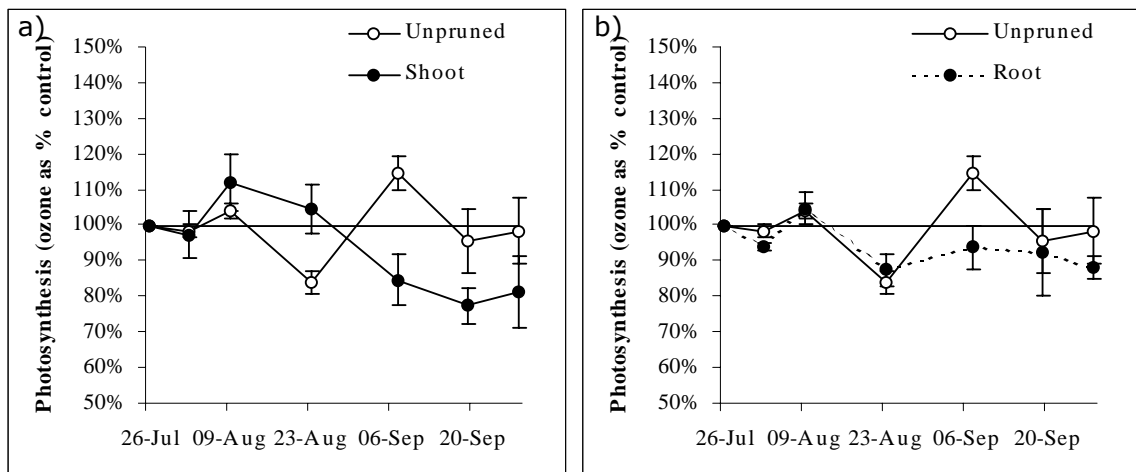

Figure 4.2 The effects of ozone fumigation on net photosynthesis of beech trees subjected to either shoot or root pruning in mid-July. All data are means of 10 replicates $\pm$ 1SE.

4.2 Discussion

In the absence of any significant effect of ozone fumigation on any treatment, it is not possible to draw any conclusions concerning the effect of root:shoot ratio on ozone response in beech. The reason for the lack of significant early response is not clear as there were no clear-cut differences between this growing season and 1997,1999 and 2001, when the responses was observed.

Photosynthesis late in the season, broadly after late-August to early-September (when AOT40 was approximately 15 ppmh), was significantly inhibited in trees that had been subjected to ozone throughout the season (Figure 4.1) and, depending on pruning treatment, also in those exposed to ozone only after July (Figure 4.2). These effects of ozone are long-term and occur at AOT40s well above 10 ppmh, and so are distinct from the short-term responses that were this experiment was designed to investigate. However, it was notable that the effect of ozone on late season photosynthesis was influenced by pruning treatments.

There was a tendency for this response to be reduced by shoot pruning and exaggerated by root pruning. This is consistent with the hypothesis that trees with high leaf area to root length ratios are more vulnerable to suffer photosynthetic damage from ozone fumigation. In particular, shoot pruning in May altered the ration of fine root length to leaf area to values near those in unpruned trees in July, and also protected the plant from ozone damage. However, shoot pruning also significantly increased stomatal conductance for the first four-five weeks following pruning, i.e. during the period of "early" ozone treatment. This increase in conductance might have been expected to increase ozone uptake, and so exaggerate the damaging long-term effects of the "early" ozone treatment, the opposite of the response actually observed. Thus, the mechanism by which shoot pruning in spring exaggerates long-term effects of early season ozone fumigation remains unknown, and merits further investigation.

5. Forcing experiment: responses to ozone in beech of contrasting developmental age.

5.1 Results
As with the pruning experiment conducted in the spring of 2000, the forcing experiment conducted at the same time showed no significant short-term effects of ozone fumigation on

beech trees at any developmental stage. The reasons for this lack of response remain unknown (see above), and when the experiment has repeated in 2001 significant responses were observed (see below). Therefore, the 2000 forcing experiment will not be considered further here.

The main effects of time and forcing treatment were both significant as was the time x forcing interaction (all p<0.001: Figure 5.1a). There were no consistent or significant effects of ozone fumigation on rates of net photosynthesis in forced trees. (Figure 5.1a). By contrast, net photosynthesis in unforced trees was substantially and significantly inhibited (21%, p<0.001) after one week of fumigation (Figure 5.1a). This inhibition reached a maximum (36%, p<0.001) after two weeks of ozone treatment but remained significant (p<0.05) until the fifth week of fumigation (Figure 5.1a). Thereafter, ozone had no significant effect on net photosynthesis in unforced trees. These changes in net photosynthesis broadly matched those in stomatal conductance. The main effects of time and forcing treatment on conductance were both significant as was the time x forcing interaction (all p<0.001: Figure 5.1b). As with photosynthesis, there were no significant effects of ozone on conductance in forced trees, while in unforced trees, conductance was significantly inhibited throughout the first five weeks of fumigation (until mid June), with a maximum reduction (26%) after two weeks of ozone treatment (Figure 5.1b). The effects of ozone on internal CO_2 concentration (Figure 5.1c) were not significant, and changes were smaller and less consistent than those on net photosynthesis and conductance (Figure 5.1a,b). Changes in transpiration were closely related to those in conductance, i.e. the main effects of both ozone and time, and their interaction were all significant at p<0.001, with significant effects of ozone confined to the unforced trees (Figure 5.1d).
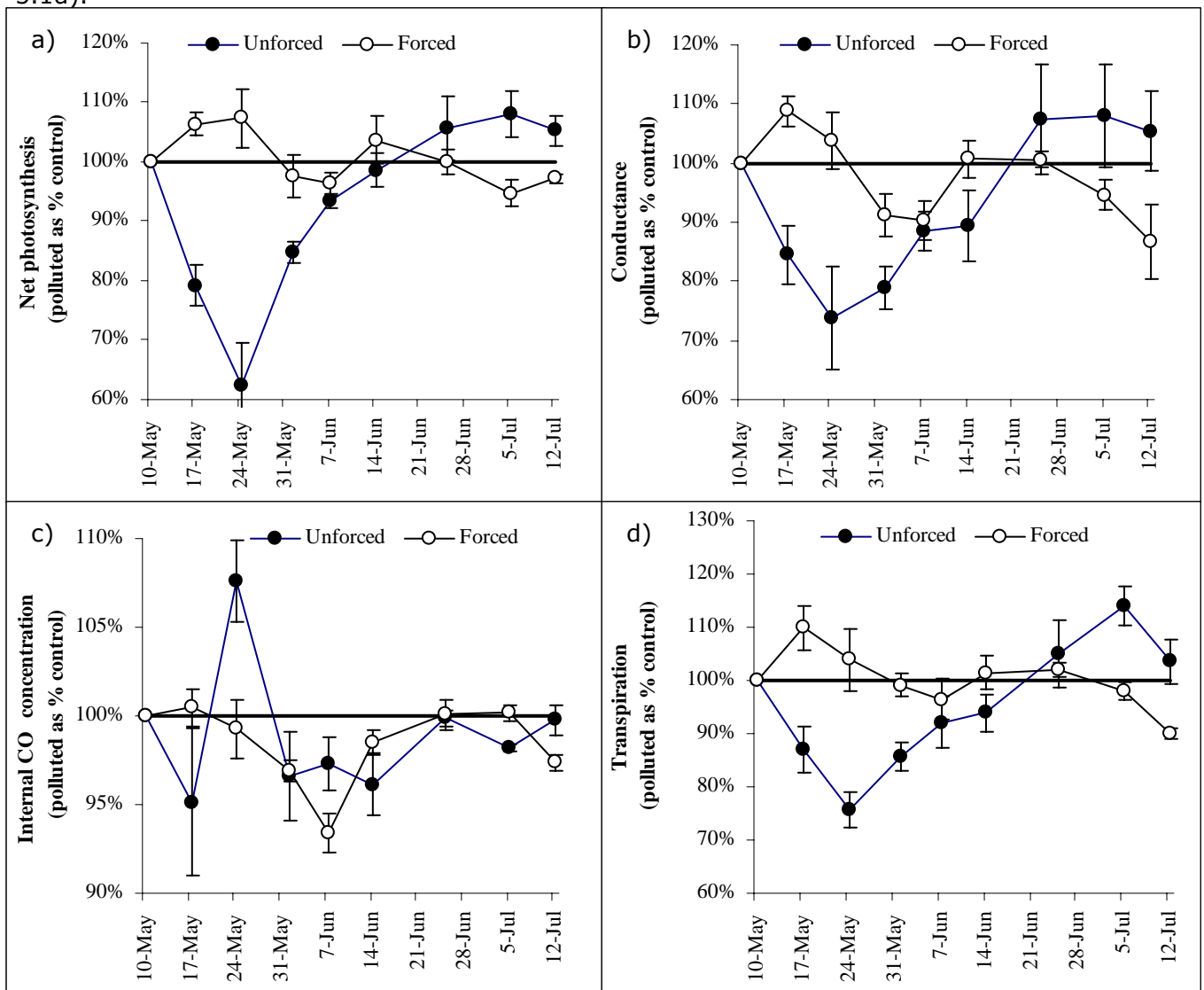

Figure 5.1 The effects of ozone fumigation on net photosynthesis of unforced and forced beech trees (see text for more detail of forcing treatments). All data are means of 10 replicates ± 1SE.

5.2 Discussion

The different responses of leaf gas exchanges in forced and unforced trees exposed to ozone early in the season were very similar to the contrasting responses to early and late fumigation observed in previous experiments (e.g. see Figure 3.14). Comparing the forcing experiment with the early and late fumigations carried-out in 1999 reveal the following points of similarity.

- Net photosynthesis inhibited rapidly (1-2 weeks after the start of fumigation) followed by a gradual recovery over the remainder of the fumigation period.

- Changes in net photosynthesis closely correlated with changes in stomatal conductance.

- Effects of ozone on internal CO_2 concentration generally small, and less marked than those in net photosynthesis and conductance, although there was a transient significant increase in Ci early in fumigation in both years (after 3-4 weeks in 1999, after 2 weeks in 2001).

As in the early-late fumigation, the rapid initial effect of ozone on net photosynthesis in trees still undergoing leaf expansion is explained by ozone-induced stomatal closure. This response occurs at very low AOT40s, <2.0 ppmh in both years. The forcing experiment confirms that this response is a function of plant developmental stage, not to any differences in environmental conditions between early and late fumigations in previous experiments.

6.   Overall conclusions

Experiments conducted during the course of this contract have confirmed that beech shows particular sensitivity to environmentally relevant ozone concentrations early in the growing season, and that this response occurs at AOT40s well below the currently accepted threshold of 10 ppmh. Experiments using plants "forced" to provide different developmental stages at the same time confirmed that the contrasting response of beech to early and late ozone is a function of plant developmental age rather than differences in environmental conditions that occurred during the two periods. The mechanism(s) underlying increased ozone sensitivity of early season beech have not been fully resolved. Early season inhibition of photosynthesis is highly correlated with ozone induced reductions in stomatal conductance, while internal CO_2 concentration was generally little affected. We conclude that reduced carbon fixation was due to changes in stomatal conductance during ozone exposure, rather than direct effects of ozone on photosynthetic metabolism. Unfortunately, since the root and shoot pruning experiments in 2000 did not clarify whether changing root: shoot ratio affected short-term ozone response, it remains unclear whether contrasting morphology of plants during the "early" and "late" periods contributed to seasonal variation in ozone response. However, since there was no evidence that ozone influenced root:shoot ratio or root hydraulic conductivity during the "early ozone" treatment, a direct effect on stomata seems most likely, and is consistent with previous studies (Dobson *et al.*, 1990; Pearson & Mansfield, 1993; Thiec *et al.*, 1994). This conclusion, though tentative, focuses attention on mechanisms that might increase ozone effects on the stomata of expanding leaves. One point of interest is that early season sensitivity to ozone occurs even though stomatal conductance in early season trees (typically 100-150 mmole m^{-2} s^{-1}) is significantly lower than that in more mature leaves during "late" fumigation (250-300 mmole m^{-2} s^{-1}). It is generally accepted that ozone uptake into the leaf is proportional to stomatal conductance (e.g. Bortier *et al*, 2001; Emberson *et al.*, 2000; Kollist, *et al.*, 2000; Martin *et al.*, 2000) and both genetic and external environmental factors that reduce stomatal conductance are known to protect trees from ozone injury (e.g. Kouterick *et al.*, 2000; Broadmeadow *et al.*, 1999). Thus, lower stomatal conductance might have been expected to reduce ozone uptake by plants during "early" fumigations and so tend to protect trees from ozone damage, the opposite of the response observed experimentally. The implications of this apparent contradiction have yet to be explored fully, but one possibility is that in expanding leaves ozone damage results from its direct action on the epidermis and stomatal guard cells, but this remains far from clear.

Ozone fumigation after mid-July caused no short-term effect on gas exchanges in beech in any of the experiments conducted during this project, even though fumigation during this period exceeded the threshold AOT40 of 10ppmh. However, in trees subjected to "late" fumigation" as well as "early", there was a consistent reduction in photosynthesis late in the season, broadly

after late-August to early-September, that were distinct from the rapid "early" season effects of ozone. It is not clear whether this is due to any specific effects of "late" fumigation per se, or simply of the far greater accumulated dose provided by season-long ozone treatment. This response was observed in all experiments where ozone treatments were maintained through the season, and occurred at AOT40s > 10ppmh. This late season inhibition of photosynthesis was not associated with any gross, visible change in pigmentation, but may be consistent with an earlier onset of leaf senescence in ozone fumigated tree. Physiological changes during leaf ageing in woody plants are complex (Bond, 2000), but recent studies of beech have shown little chlorophyll synthesis after July, resulting in a progressive decline in chlorophyll content which may be compounded by ozone injury (Garcia-Plazaola & Becerril 2001). Late season inhibition of photosynthesis by ozone has been observed previously in several tree species, including beech (Bortier, *et al.*, 2001; Paludun Muller *et al.*, 1999). In the current experiments, this late reduction in photosynthesis, though quite marked (20-30%) did not appear to contribute to any reduction in whole plant biomass increment (see section 4.2). This reflects the relatively small contribution of late season photosynthesis to the total potential carbon fixation over the whole season. In general, rates of photosynthesis during increased during the period May to July, but then declined for the remainder of the season, so that rates during the "early" period were substantially greater than those observed later in the season. On the basis of our gas exchange and growth analyses (from 1998, 1999 and 2000), we estimate that carbon fixation after the start of September contributes less than 15% of the total over the growing season. Even the 20-30% reduction in photosynthesis during this period resulting from long-term ozone injury might be expected to have only small effects on total biomass increment (probably in the order of 3%). By contrast, from gas exchange and growth data, we calculate the photosynthesis during the "early" fumigation period contributes approximately 60% of the whole season biomass increment. The reduction in photosynthesis due to ozone averaged over this "early" period was 13% in 1999 and 11% in 2001 (unforced trees), potentially reducing whole season biomass increase by 6.5%-8%. Thus, reductions in carbon fixation due to "early ozone" might have long-term effects on total biomass gain over the whole season, even though photosynthesis later in the season was not significantly affected by previous exposure to ozone.

However, "early" ozone alone may not be sufficient to produce reductions in biomass in beech at the end of the season since there is clearly capacity for some recovery if plants are not exposed to ozone later in the year (Section 2). It is arguable that the main effect of late-season ozone in beech is to prevent such recovery from earlier damage, with inhibition of photosynthesis as senescence begins being of relatively minor importance. However, this late inhibition of photosynthesis may have significance beyond any effect on total plant biomass, since it coincides with the period when R:S increases markedly, presumably reflecting storage in the roots prior to overwintering. It is noticeable that trees exposed to both "early" and "late" ozone show both a significant reductions in R:S in September-November, and significant inhibition in photosynthesis late in the season. It may be, therefore, that these two responses are connected, with late season photosynthetic damage inhibiting the usual deposition of reserves in the roots as autumn progresses. Reduced root:shoot ratio appears to be a common response to ozone fumigation in several trees species (Oksanen & Rousi, , 2001; Landolt, *et al.*, 2000; Kouterick *et al.*, 2000; Paludan-Muller *et al.*, 1999) Changes in root storage during autumn may have effects lasting through the winter in to the following season, and may contribute to the "carry-over" of ozone effects from one season to the next and, thus, to cumulative effects of multi-season ozone exposure.

In conclusion, the current method of calculating AOT40 uses cumulative ozone exposure between 1st April and 30th September. The gas exchange and growth data obtained here show that late season ozone (broadly from mid July) has little or no effect on beech, in terms of gas exchange or biomass increment. Hence, in considering refinements of AOT40, one possibility may be to calculate cumulative ozone only between 1st April and, say, mid August.

Acknowledgements

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Appendix 1

1999

Date	Treatment			
	Control 1	**Control 2**	**Ozone 1**	**Ozone 2**
11/05/99	Dome 5	Dome 8	Dome 7	Dome 6
14/06/99	Dome 6	Dome 5	Dome 8	Dome 7
19/07/99	Dome 7	Dome 6	Dome 5	Dome 8

Table A1.1

Assigned of treatments to the four Solardomes over the course of the 1999 experiment.

2000

Date	Treatment			
	Control 1	**Control 2**	**Ozone 1**	**Ozone 2**
15/0500	Dome 5	Dome 8	Dome 6	Dome 7
19/06/00	Dome 8	Dome 5	Dome 7	Dome 6
19/08/00	Dome 5	Dome 8	Dome 6	Dome 7

Table A1.2

Assigned of treatments to the four Solardomes over the course of the 2000 experiment.

2001

Date	Treatment			
	Control 1	**Control 2**	**Ozone 1**	**Ozone 2**
09/05/01	Dome 5	Dome 8	Dome 6	Dome 7

Table A1.2

Assigned of treatments to the four Solardomes over the course of the 2001 experiment.

Appendix 2   Ozone Pollution Regimes

Dates of the start and end of ozone fumigation in the three experimental seasons

Growing season	Start of fumigation	End of fumigation
1999*	16th May	19th September
2000	15th May	22nd September
2001	9th May	12th July (determined by end of contract)

- See text for details of "early" and "late" ozone treatments in 1999.
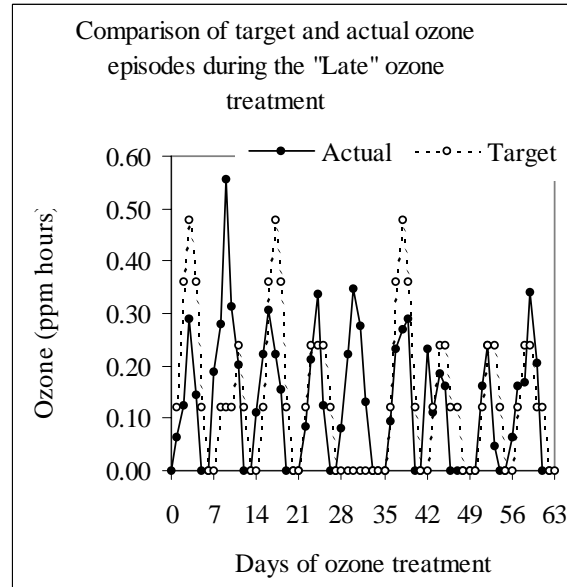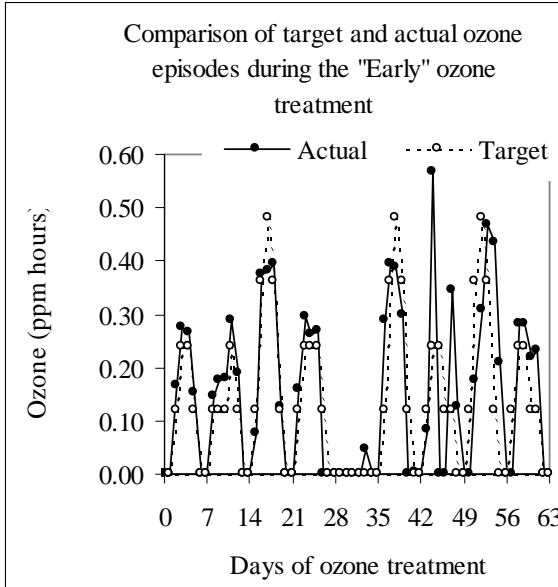
A2.1 1999 Experiment

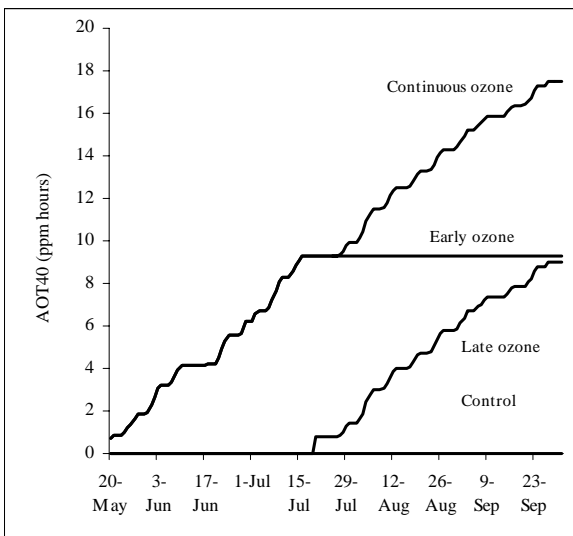Figure A2.1 Ozone episodes over the study period in 1999.

Figure A2.2 Cumulative ozone concentration across the four treatments provided during 1999, expressed as AOT40s. Note the separation of fumigation in to "early" and "late" treatments: see text for more details.

In terms of the planned series of ozone episodes, the treatment provided during the early period was generally close to the target (Figure A2.1a). During the "late ozone" treatment (Figure A2.1b, there was greater deviation from the intended pattern of exposure, due to sporadic problems with the ozone generator, which led to periods when treatments were adjusted to compensate for earlier problems. The maximum ozone concentration was 121 ppb in the early part of the season, and 133 ppb in the late period. In comparison with the target AOT40s (8.4 ppmh for both early and late ozone), the actual ozone treatment provided slightly more ozone: AOT40 was 9.3 ppmh for the "Early ozone" treatment and 9.0 ppmh for the "Late ozone" treatment (Figure A2.2). The total AOT40 for the continuous ozone treatment was 18.3ppmh (compared with the target of 16.8 ppmh).

A2.2 2000 Experiment
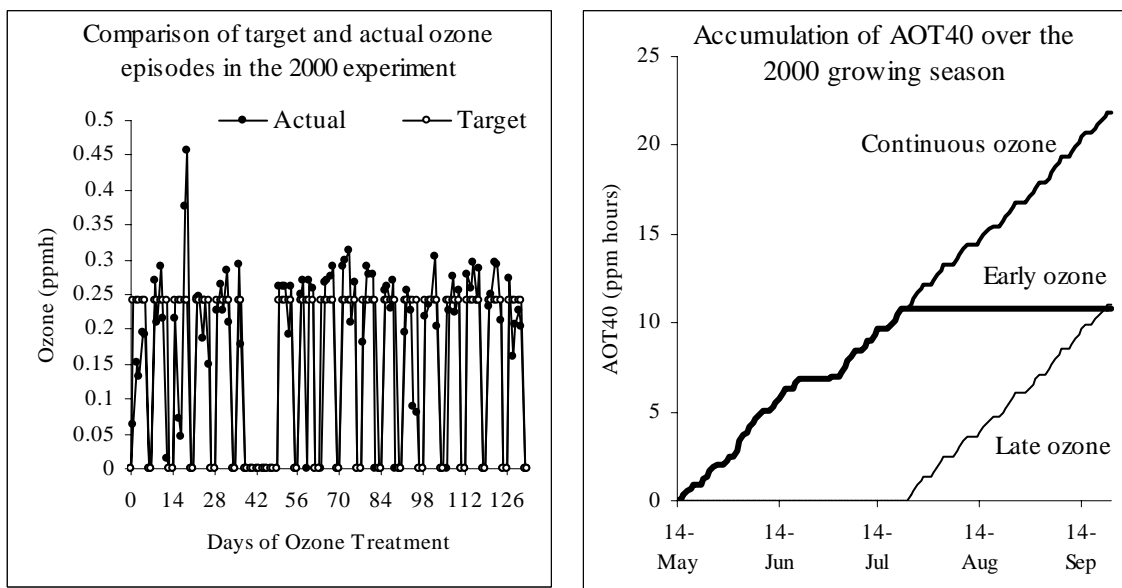
Relative to the planned series of ozone episodes, the treatments provided were close to the target with the exception of a short period (21st-30th June) during the "early" treatment when an equipment fault prevented ozone treatments from being provided (Figure A2.3a). The maximum ozone concentration was 117 ppb in the early part of the season, but rather lower (105 ppb) in the late period. In comparison with the target AOT40s (10.5 ppmh for both early and late ozone), the actual AOT40s were 10.8 ppmh for the "Early ozone" treatment and 11.1 ppmh for the "Late ozone" treatment (Figure A2.2b).

A2.3 2001 Experiment
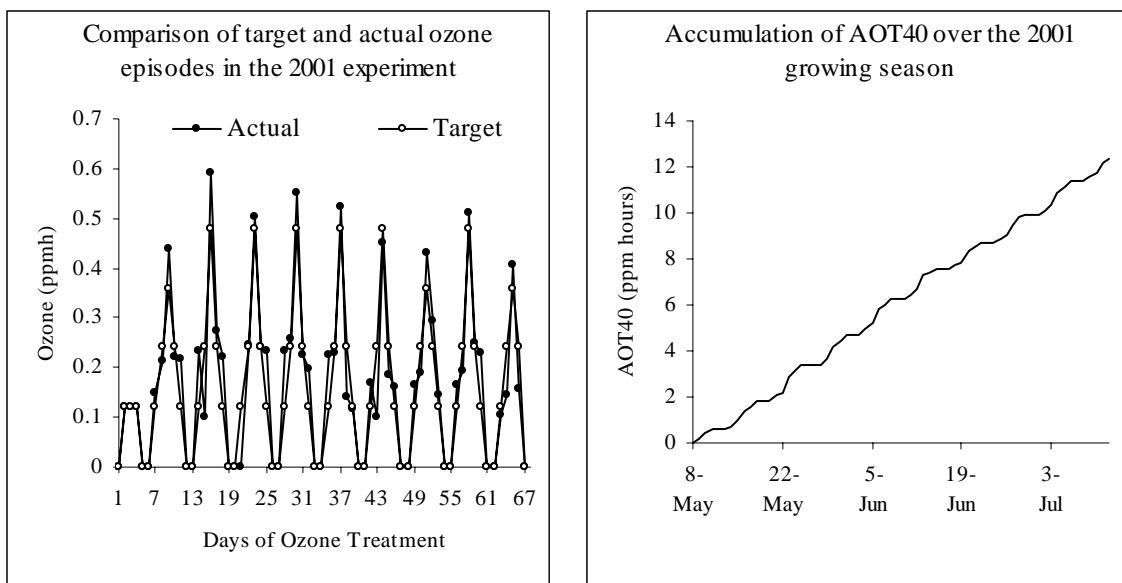
The ozone treatments provided were close to the target series of ozone episodes, with a maximum ozone concentration was 136 ppb. In comparison with the target AOT40s (10.5 ppmh), the actual AOT40 was 12.3 ppmh (Figure A2.2b).

Appendix 3. Preliminary studies

Since funding in 1998 was not confirmed until mid-summer it was not possible to complete a full programme of research during the 1998 growing season. However, resources allowed a preliminary study designed to clarify the pattern of growth of beech saplings over the course of a season. The increase in total plant dry weight with time was broadly log-linear over the season April-October (Figure A3.1a). However, leaf expansion was largely completed by June-July, with only a small additional increase attributable to "lammas growth" later in the season (Figure A3.1b). By contrast, root growth was minimal prior to July, but rapid from July until the end of growth (Figure A3.1b). This demonstration that leaf and root growth were temporally separate provided a useful context for the "early" and "late" ozone treatments used previously (Hawes, 1998). In that experiment the early treatment ozone was provided during a period of rapidly increasing leaf area and rapidly decreasing root:shoot ratio (Figure A3.1c). By contrast, in the late ozone treatment leaf expansion was minimal but root growth was rapid and root:shoot ratio increased substantially (Figure A3.1c). This also relates to one possible mechanism for seasonal variation in ozone response i.e. that ozone early in the season creates an imbalance between water loss (transpiration from the rapidly expanding foliage) and water uptake (by a limited root system). Thus, one priority of the experiment during 1999 was to complement gas exchange data with measurements of root growth and hydraulic conductivity.
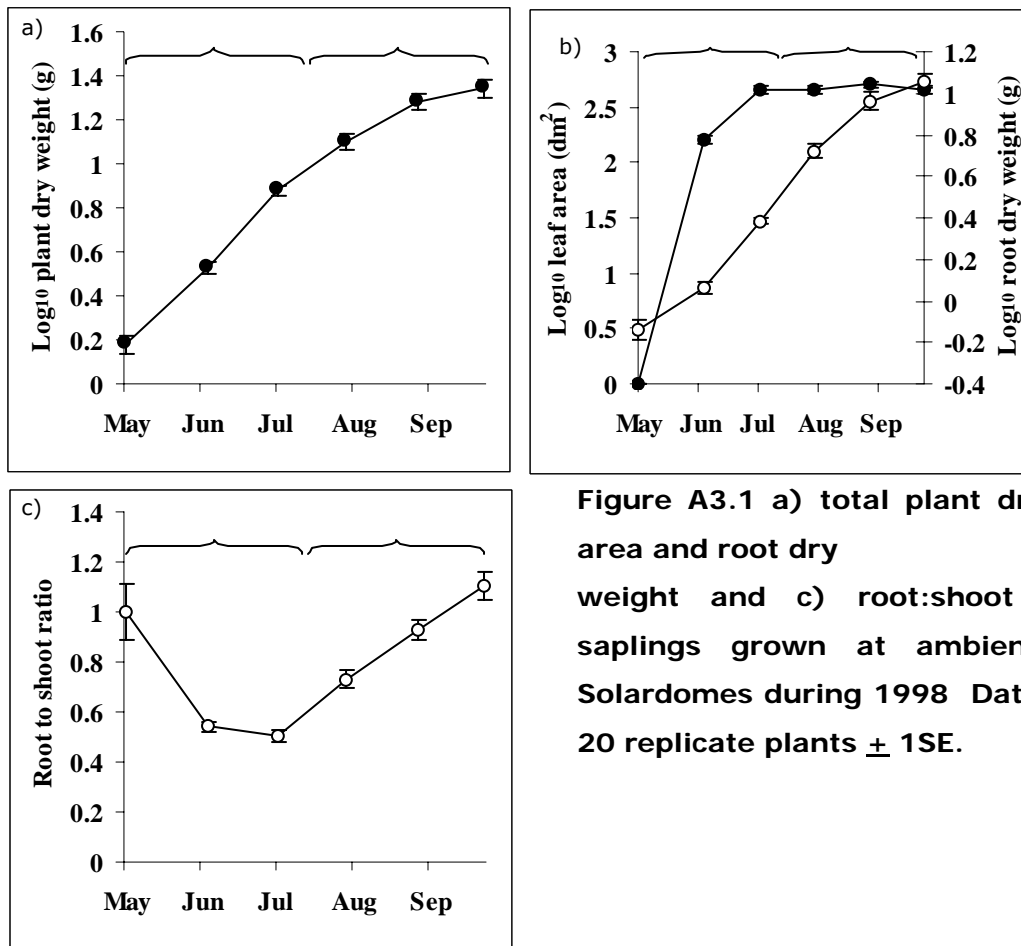
Figure A3.1 a) total plant dry weight, b) leaf area and root dry weight and c) root:shoot ratio of beech saplings grown at ambient ozone in the Solardomes during 1998 Data are the mean of 20 replicate plants $\pm$ 1SE.

Assessment of the Relative Sensitivity of Wetland Plant Species to Ozone.

Final Report

Contract EPG 1/3/121

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Executive Summary

Ozone effects are more closely related to flux into the leaves than to ambient concentrations. Wetland plants, which typically have a high stomatal conductance and suffer less water stress in ozone episodes, maybe thus be particularly sensitive to this pollutant.

The objectives of this research were to determine the sensitivity of wetland plant species to ozone and to identify physiological or ecological characteristics which are associated with high sensitivity to ozone.

The effects of ozone on wetland plant species were investigated in season-long fumigation in open-top chambers and in shorter-term fumigation in closed chambers within a controlled environment.

The effects of ozone on a group of damp woodland species and a group of rare species were also investigated in the shorter-term fumigation experiments.

The eight wetland species in the longer-term experiment received AOT40 exposures in the range 9-11,000 ppb.h over about two months. The results from this study were combined with those from a study in the previous season, using AOT40 values in the range 11-15,000 ppb.h.

Eight of the twelve species studied in the two seasons showed visible leaf injury in ozone, but only three species had a significant reduction in above-ground biomass.

Root/shoot ratios were generally lower in ozone, with three of the twelve species showing a significant reduction.

The shorter-term experiments, which lasted three weeks, provided an AOT40 exposure of about 3500 ppb.h, close to the current provisional critical level for semi-natural vegetation.

Only two of the sixteen wetland and damp woodland species showed characteristic visible leaf injury in ozone. However, seven species showed a significant adverse effect of ozone on their relative growth rate. Two species showed a significant reduction in root-shoot ratio

Nine of the more sensitive species were selected for a further exposure-response experiment. Estimates of the AOT40 value required to produce a 10% reduction in relative growth rate (RGR) were made for eight species.

Two of these eight species showed a threshold AOT40 of about 3000 ppb.h for above-ground relative growth rate, with three further species having values of 5-6000 ppb.h.

When threshold AOT40 values were calculated for root relative growth rates, lower values were found for most species. For two species, the threshold values were in the range 1000-1500 ppb.h.

There was a significant positive correlation between the size of the reduction in RGR caused by ozone and the stomatal conductance of the species in the short-term experiments. Similarly, there was a significant positive correlation between stomatal conductance and the reduction in biomass below-ground, but not above-ground, in the longer-term experiments.

The most sensitive species in the short-term experiments were characterised by high relative growth rates, a high stomatal conductance and a high specific leaf area. However, the correlation coefficients between reduction in RGR in ozone and both growth rate and specific leaf area were not significant.

Rare species as a group showed little evidence of adverse effects of ozone. This group had a lower mean stomatal conductance and relative growth rate than wetland plants, a finding which is consistent with the trends in the data for wetland species.

There was no significant association between Ellenberg ecological indicator values for moisture, light, pH and fertility and ozone sensitivity in the short-term experiments. However, this may reflect the low range of values between the species.

Overall, the experimental results clearly demonstrate the sensitivity of wetland species to ozone and the significance of a high stomatal conductance in enhancing sensitivity. They provide strong support for the current critical level for semi-natural vegetation of 3000 ppb.h, and indeed suggest that lower critical levels may be needed to prevent adverse effects on the most sensitive UK wetland species.

Analysis was made of a database of previous experimental studies of ozone effects on semi-natural species, primarily of grasslands. As for the experimental data, no significant association was found with Ellenberg indicator values. However the analysis suggested that there was a high proportion of sensitive species in groups associated with woodlands and woodland edge habitats.

New data on changes in frequency of individual species in the Countryside Vegetation Survey over the period 1978-2000 was analysed. There was no evidence that species declining in frequency are more sensitive to ozone. However, this analysis is crude and needs to be further developed to provide a more rigorous test of the hypothesis.

An attempt was made to compare the possible impacts of ozone on the range of grassland communities identified in the National Vegetation Classification. The approach used the ozone exposure, levels of soil moisture deficit and the proportion of dominant or frequent species which are ozone-sensitive. The results showed that mesotrophic grasslands were overall at higher risk than calcareous grasslands. Acid grasslands proved impossible to quanitify because of the low proportion of dominant species which had been studied.

1. Introduction

There is a vast literature concerning the effects of ozone on crops and forest trees, whereas the effects on semi-natural vegetation have been given less attention. Only one published study exists on the effects on species of wetland plant communities (Franzaring *et al*, 2000). Wetland plants may be particularly sensitive to ozone because they grow in permanently damp environments; therefore they maintain high stomatal conductances in summer months when ozone concentrations are high. Also, many wetland plants have high relative growth rates, and research on the impact of ozone on grasslands has shown that the faster growing species tend to be more sensitive.

Preliminary work into the effect of ozone on wetland plants was conducted at Imperial College (DETR contract report for the period April 1997 to May 1998). Five species were investigated using a system of open-top chambers, whereby plants were exposed to O_3 (90ppb) for 9hrs daily when climatic conditions permitted, over a 2-month growing season. All species were affected by ozone, with four of the five being negatively affected in terms of either visible injury or detrimental effects on growth or physiology. The AOT40 values of 11600-14300ppb.h were within the range reported for the UK, suggesting these communities to be at risk.

The work described in this report employs a wider range of species, encompassing wetland, but also some damp woodland and rare species, to examine the impact of ozone. Three different experimental approaches were used. The first involved the fumigation of a number of wetland species over a growing season using open-top chambers. The second approach involved shorter-term experiments, in a controlled environment, with a larger number of plant species in order to determine their relative sensitivities to ozone and explore factors associated with sensitivity. The third approach was to conduct exposure-response experiments using sensitive species to assess the appropriateness of the current critical levels for semi-natural vegetation. Finally, the interactions between ozone sensitivity and water availability were examined for one sensitive species.

Current critical levels of ozone for semi-natural vegetation are set at the same level as for crops (AOT40 of 3000ppb h accumulated over 3 consecutive months during the growing season). This level was set due to the limited data available (mostly from herbaceous plant communities, especially grasslands) showing that some semi-natural species are as sensitive as the most sensitive crop species (Ashmore & Davison, 1996). However, more data is required to confirm this conclusion. Since only a limited range of species have been studied experimentally, there is also a need to develop methods to predict which species or communities may be of high sensitivity to ozone.

The objectives of this research were thus to determine the sensitivity of wetland plant species to ozone, to assess if the current critical level was appropriate for these species, and to identify physiological or ecological characteristics which are associated with high sensitivity to ozone.

2. Methods

2.1. Long-term Studies

Data analysis from the experiment carried out in the summer of 1998 to investigate the responses of eight species characteristic of fen and fen-meadow communities (NVC community types S24, S25, S26, S27, M22, M24) was carried out, together with that from an experiment carried out at Imperial College in 1997 under an earlier contract to investigate the responses of five species of similar communities. The species used are summarised in Table 2.1. Plants were exposed in a system of 8 open top chambers at Imperial College's field station in Ascot, Berkshire. Half of the chambers were ventilated with charcoal filtered air, while the other half received charcoal filtered air with additional ozone. Ozone was delivered to the chambers between 09.00 and 18.00 daily, with target concentrations of 90 nl l^{-1}. Ozone exposure was restricted to dry, still days, on which maximum temperatures exceeded 17°C.

Regular measurements of both stomatal conductance and photosynthetic rate were made in both summers. Reproductive development was monitored at weekly intervals and plants were checked for development of visible injury. Destructive harvests of above and below ground biomass were carried out at the end of each experiment. Plant species were harvested sequentially, giving slightly different AOT40 exposures for each species (Table 2.1). The seasonal exposures were significantly lower in 1998 than 1997, due to the cooler weather allowing less days for fumigation.

Table 2.1. Cumulative ozone exposures for each plant species in long-term experiments.

	AOT 40
1997:	
Symphytum officinale	11,600 ppb.h
Valeriana officinalis	13, 200 ppb.h
Iris pseudacorus	13,200 ppb.h
Lythrum salicaria	13,900 ppb.h
Mentha aquatica	14,300 ppb.h
1998:	
Vicia cracca	9,200 ppb.h
Lathyrus pratensis	9,200 ppb.h
Rumex acetosa	9,200 ppb.h
Cirsium arvense	10,000 ppb.h
Lychnis flos-cuculi	9,300 ppb.h
Lotus uliginosus	10,100 ppb.h
Filipendula ulmaria	10,600 ppb.h
Valeriana officinalis	10,300 ppb.h

2.2. Short-term experiments

The species used in these experiments were chosen primarily using the vegetation groups distinguished by the Countryside Vegetation System (CVS) (Bunce et al., 1999). The groups chosen were primarily those found at water edges or in marshy habitats. However, a decision was also made to select species found in woodland or woodland edge habitats. This was because analysis of factors associated with sensitivity of semi-natural species (see Section 5.1) showed that there was an unusually high proportion of sensitive species in these habitats. Species for fumigation within these groups were selected due to their seed availability and germination success. The following species groups were used:-

6: Water edge plants on wet alluvial soils
10: Maritime saline or freshwater edge plants on gleyed brown earths
14: Wood or wood edge plants on calcareous or neutral brown earths
16: River edge or aquatic plants on wet alluvial soils
17 Wood or wood edge plants on brown earth soils
20: Wood or wood edge plants on damp fertile brown earths
23 Marsh, wood edge or woodland plants on wet gleyed brown earths
24 Marsh or water edge plants on soil water gleys
25 Wood or wood edge plants on damp fertile brown earths

As an additional exercise, a number of rare species were also tested, since populations of these species may be particularly vulnerable to additional environmental stresses. Little attention has been paid to date to the effects of ozone on rare species, although Thwaites (1996) showed that certain rare species were very sensitive to ozone.

Screening experiments were conducted using a system of 8 closed chambers 0.8m x 0.8m x 0.8m, arranged in pairs. One of each chamber pair received ozone at a target concentration of 70ppb for 8hr day^{-1}, 5 day $week^{-1}$ over a 21 day exposure period. The remaining 4 chambers received air filtered using a mixture of charcoal and purifil. All experiments were conducted at controlled levels of temperature ($20^{o}C$ day, $10^{o}C$ night) and humidity (50%RH day, 40% RH night). A 16hr daylight cycle was used, with photon flux density levels ranging from 320-411 μmol m^{-2} s^{-1}. In all, five screening experiments were conducted in order to screen 24 plant species (12 wetland, 8 rare, 4 woodland). The plant species used, together with the AOT40 values calculated for each species, are shown in Table 2.2.

All plants were grown from commercially obtained seed (Chiltern Seeds / Emorsgate) with the exception of *Typha latifolia* and *Phragmitis australis*, which were grown from rhizomes obtained locally. Plants for fumigation were aged 3-4 weeks.

Initial growth measurements (leaf counts, plant height, leaf area, estimates of initial biomass) were conducted immediately prior to fumigation. Assessment of plant growth, photosynthetic rate and stomatal conductance were made both

during and at the end of each experiment. Plants were also monitored for first signs of visible injury.

Table 2.2. Cumulative ozone exposure and category for each plant species in short-term experiments.

Species	Category	AOT40 value (ppb h)	Species	Category	AOT40 value (ppb h)
Phragmitis australis	Wet	3493	*Silene noctiflora*	Rare	3477
Myosotis scorpioides	Wet	3493	*Stachys germanica*	Rare	3477
Lychnis flos-cucculi	Wet	3493	*Cirsium eriophorum*	Rare	3605
Typha latifolia	Wet	3439	*Digitalis purperea*	Wood	3605
Geum rivale (Geum 1)	Wet	3439	*Geranium robertianum*	Wood	3605
Scrophularia auriculata	Wet	3439	*Potentilla palustris*	Wet	3605
Stachys palustris	Wet	3439	*Sedum roseum*	Rare	3605
Epilobium hirsutum	Wet	3477	*Primula farinosa*	Rare	3510
Eupatorium cannabinum	Wet	3477	*Geum urbanum* (Geum2)	Wood	3510
Valeriana officionalis	Wet	3477	*Viola riviniana*	Wood	3510
Verbascum lychnitis	Rare	3477	*Filipendula ulmaria*	Wet	3510
Lychnis viscaria	Rare	3477	*Pulsatilla vulgaris*	Rare	3510

At the end of each experiment, plant height, and the number of main leaves, side leaves and side shoots were determined. Leaves were removed and their area was determined using a Leaf Area Analyser. Above and below ground biomass were determined following drying at $80^\circ C$ for 48 hours.

2.3. Exposure-response Experiments

The short-term experiments revealed a number of species to be sensitive to ozone. Three exposure-response studies were then conducted to examine these sensitive species in more detail. These experiments used the same chambers under the same conditions as for the short-term studies with the exception that two chambers received ozone at a target concentration of 80ppb, three received ozone at 55ppb and the remaining three received charcoal filtered air. Species used in these studies, together with the range of AOT40 values, are shown in Table 2.3. Growth and gas exchange measurements were made as described earlier.

Table 2.3. Exposure-response experiments - species used and range of AOT40values.

Experiment	Species	AOT4O ppb h
1	*Epilobium hirsutum*	1618-4955
	Filipendula ulmaria	1384-5186
	Scrophularia auriculata	1384-5186
2	*Eupatorium cannabinum*	1538-5104
	Geum rivale	1538-5104
	Typha latifolia	1271-5081
3	*Valeriana officionalis*	1472-4362
	Verbascum lychnitis	1564-4410
	Digitalis purpurea	1564-4410

2.4. Drought Experiment

One droughting study was conducted using *Eupatorium cannabinum*. The fumigation set-up was identical to that of the initial experiments whereby four chambers received ozone at a target concentration of 70ppb, whilst the remaining four chambers received filtered air. Two drought treatments were explored. In the first treatment, plants were subjected to 3 drying cycles during the course of the fumigation. Plants were re-watered and left in standing water during each successive weekend before the next drought cycle began. A control treatment, whereby plants were kept in standing water for the duration of the study, was also used. Stomatal conductance measurements were made once per week (to coincide with end of each droughting cycle).

The second treatment tested drought-sensitivity following O_3 exposure. Plants in ozone and filtered air were left in standing water for the duration of the fumigation. After 21 days, water was withdrawn. Leaf water potential measurements were conducted on leaf 3 of both sets of plants, on all subsequent days until the leaves had wilted.

3. Results

3.1. Long-term experiments

Recognisable ozone injury was observed on eight of the 12 species, with the first signs of injury being recorded on *V. cracca* following an ozone exposure of only 1,950 ppb.h. The development of visible injury in relation to ozone exposure is illustrated in Figure 3.1.
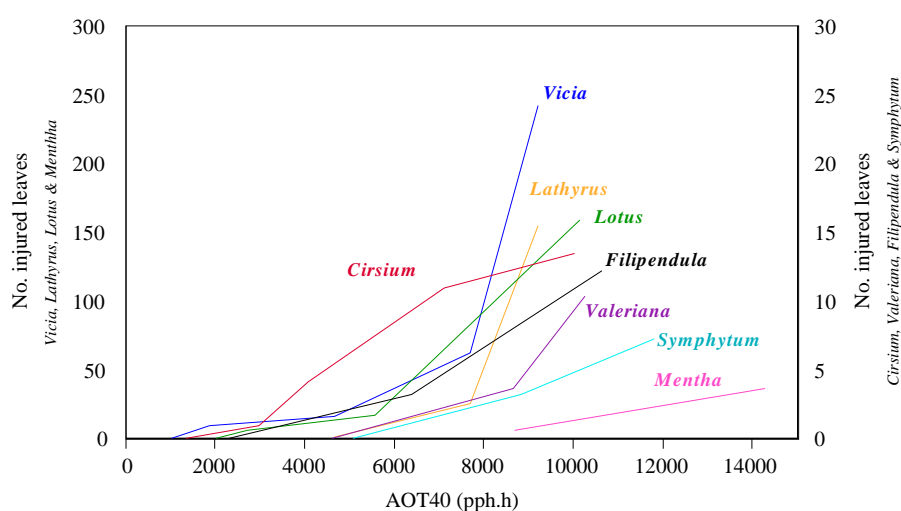

Figure 3.1. Development of visible injury in relation to ozone exposure

Table 3.1. Above- and below-ground biomass (g dry weight) and root:shoot ratios for all species. Live (leaf+stem) weights exclude senescent/dead leaves (and flower stalks for *L. salicaria* and *M. aquatica*) */** represent significant treatment effects at the P<0.05/0.01 level of probability

	Live (leaf + stem) weight			Root weight			Root:shoot ratio		
	O_3	FA	F ratio	O_3	FA	F ratio	O_3	FA	F ratio
S. officinale	**18.3**	**23.7**	**9.82***	42.7	54.2	3.56	1.71	1.85	0.24
V. officinalis	**14.3**	**18.2**	**6.50***	**25.0**	**36.5**	**54.6***	**1.58**	**1.95**	**10.0***
L. salicaria	48.3	52.4	0.96	45.8	52.1	3.41	0.89	0.92	0.25
I. pseudacorus	7.2	7.4	0.06	7.8	8.8	4.41	1.04	1.16	3.91
M. aquatica	**13.4**	**8.5**	**11.12***	3.9	3.4	1.28	0.27	0.36	4.48
R. acetosa	6.8	7.1	0.36	4.3	4.4	0.08	0.62	0.61	0.01
V. cracca	2.0	2.5	1.69	1.1	1.5	3.09	0.58	0.63	2.12
L. pratensis	1.4	1.3	0.43	**0.9**	**1.1**	**7.22***	**0.66**	**0.83**	**14.6***
C. arvense	2.7	4.0	5.48	**0.9**	**2.2**	**7.12***	**0.33**	**0.52**	**17.1***
L. flos-cuculi	4.2	3.6	3.78	1.2	1.2	0.48	0.30	0.30	0.13
L. uliginosus	4.3	3.8	1.90	1.1	1.2	0.22	0.26	0.33	2.15
F. ulmaria	**1.1**	**1.4**	**6.13***	0.8	0.8	4.82	**0.71**	**0.65**	**6.57***

Table 3.1. summarises the effects of ozone on above- and below-ground biomass, and on root-shoot ratios. It was noticeable that not all of the species showing visible injury had any significant effect of ozone on biomass. Indeed,

Mentha aquatica, which had some limited visible injury, had a greater biomass in ozone that in filtered air at the end of the season. Three species had significant adverse effects of ozone on above-ground biomass, all of which (*Symphytum, Valeriana* and *Filipendula*) also showed significant levels of visible injury.

Three species, including *Valeriana*, showed a significant reduction in root weight. In general, below-ground biomass was more affected by ozone than above-ground, as is reflected in the trend for lower root:shoot ratios in ten of the twelve species examined. The greater weights recorded for the first five species listed reflects the longer experimental period in 1997 compared with 1998.

Exposure to ozone resulted in large and significant reductions in photosynthetic rate, at some stage of the experiment, for five species. For three species, *(V. officinalis, I. pseudacorus* and *S. officinale*), the effect of ozone on photosynthetic rate increased over the course of the experiment . The pattern of response to ozone differed in the other species, which showed an initial stimulation followed by a reduction in photosynthesis (*M. aquatica*), and a significant reduction from the first measurement period (*C. arvense*). Figure 3.2 illustrates these differences in temporal changes in plant photosynthetic response to ozone exposure, for three species.

Stomatal conductance values for the species used in this experiment were relatively high, compared with grassland species used in earlier studies at Imperial College. Mean values of 0.323 mmol m^{-2} s^{-1} were obtained in filtered air for the wetland species, compared with values of 0.231, 0.194 and 0.200 mmol m^{-2} s^{-1} for acidic, calcareous and mesic grassland species, respectively, in recent greenhouse experiments. There was a very weak relationship between stomatal conductance and reductions in above-ground weight in this experiment, (r^2 = 0.059), but the relationship between stomatal conductance and reductions in ozone of both root weight (r^2= 0.409) and root:shoot ratio (r^2= 0.344) was stronger. This suggests that species with a higher stomatal conductance were more sensitive to ozone.
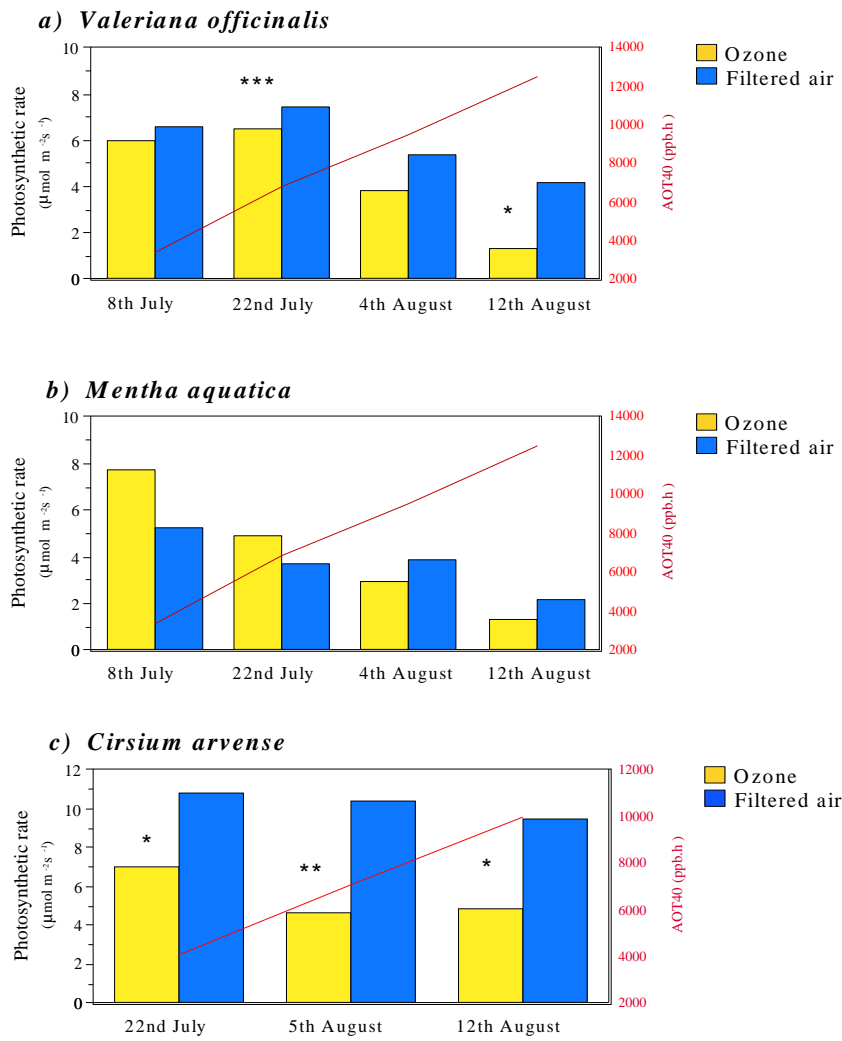
Figure 3.2. Relationship between AOT40 exposure and effects on plant photosynthetic rate for a) *V. officinalis*, b) *M. aquatica* and c) *C. arvense*

3.2. Short-term experiments

3.2.1. Visible injury

Ozone injury was observed on two plant species - *Eupatorium cannabinum* and *Stachys palustris*. In both cases, injury appeared on the upper surfaces only, and was greater on older leaves that had been exposed to ozone for longer. Extensive leaf curling was found in the ozone treatment in *Epilobium hirsutum*. Accelerated senescence was not noted for any species during the three-week exposure period.

3.2.2. Gas exchange

Stomatal conductance and photosynthesis measurements were made where possible on the first and third fully expanded leaves per plant. In general, effects were greater on leaf 3 (the older leaf which had been exposed to ozone for longer) than leaf one and stomatal conductance and photosynthesis were generally reduced in plants exposed to ozone. Of the wet and woodland species tested, 4 of the 13 species (Fig. 3.3) showed significant reductions following exposure. Significant reductions ranged from 25% (*E. cannabinum*) to 47% (in *Epilobium hirsutum*).

Of the eight rare species, only three (*S. germanica, V. lychnitis* and *C. eriophorum*) were used for gas exchange measurements. The leaves for all other species remained too small for testing. *Verbascum* was the only species to show an effect, with a 37% reduction in stomatal conductance on day 12 and a 65% reduction on day 21.

Significant reductions in photosynthesis were found for four of the 13 wet and woodland species tested (Fig. 3.3). Of these, 2 species also showed significant reductions in stomatal conductance. Significant reductions in photosynthetic rate ranged from 29% (*S. palustris*) to 34% (*E. hirsutum*). Of the three rare species tested, only *C. eriophorum* showed an effect. A 17% reduction in photosynthetic rate was recorded on day 11. No measurement for this species was made on day 21.
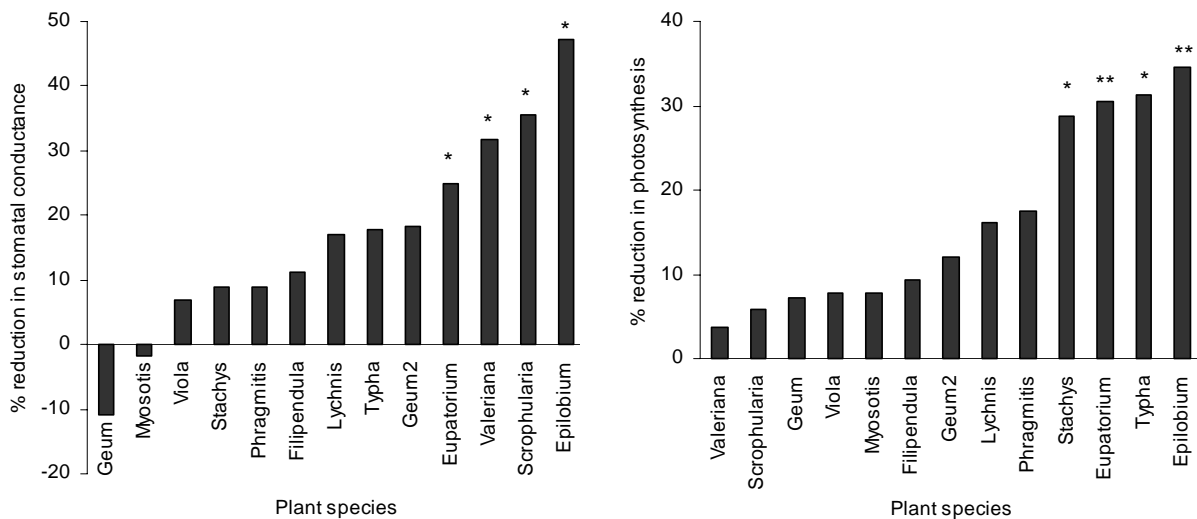
Figure 3.3 % reduction in a) stomatal conductance and b) photosynthetic rate in O_3 relative to FA for leaf 3, day 21 only. *p<0.05; **p<0.01.

3.2.3. Relative Growth Rates

Data for the above-ground, below-ground and total biomass were converted to a relative growth rate to take account of any differences in initial plant size. In general, ozone treatment resulted in a reduction in growth rate. Six of the 14 wet and woodland species tested (Fig.3.4) showed significant reductions in RGR (total biomass). These reductions ranged from 7% in *S. auriculata* to 16% in *V. riviniana*. Of the rare species, only *V. lychnitis* showed a significant effect, with total RGR reduced by 17%.

Five of the seven species showing reductions in total RGR also showed reductions in above ground RGR. Three of the seven species (*E. hirsutum, E. cannabinum* and *Stachys palustris*) also showed significant reductions in below ground RGR. Reductions in below ground RGR ranged from 6.6% in *S. palustris* to 18.9% in *E. hirsutum.*
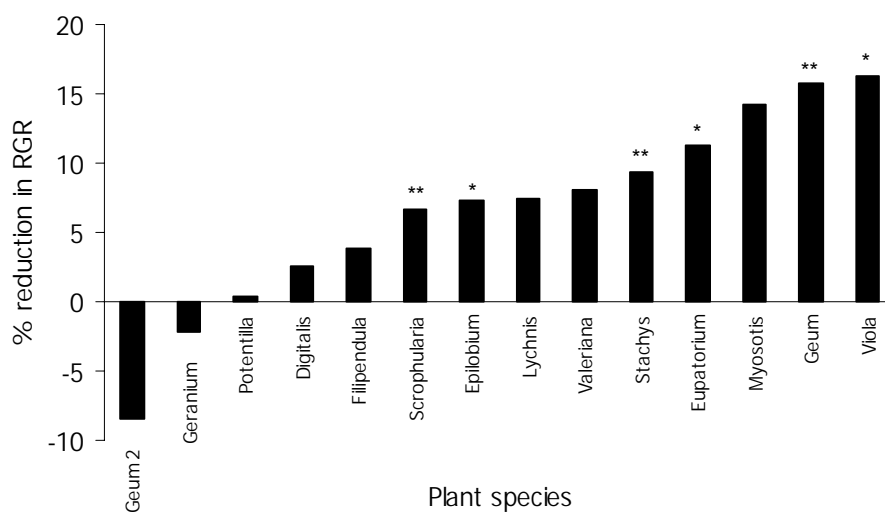

Figure 3.4.   % reduction in total RGR in O_3 relative to FA, *p<0.05; **p<0.01.

3.2.4. Root:shoot ratios

Two species showed an effect of ozone on partitioning of resources between above and below-ground components and although effects were large, they were only significant at the 5% level, indicating large variation between plants. *E. hirsutum* and *V. lychnitis* both showed a decrease in RSR in ozone (42% and 11% decrease, respectively).

3.2.5. Plant height

E. hirsutum showed a 20% increase in plant height (p<0.01) in ozone. The number of leaves remained similar regardless of treatment, but greater internodal distances were observed in ozone.

3.2.6. Specific leaf area

Specific leaf area (SLA) varied widely between species in FA. *E. cannabinum, S. palustris and E. hirsutum* were shown to have the highest SLA, whilst *T. latifolia* had the lowest SLA of those species tested (n=11). Plants of *E. cannabinum* and *Digitalis purpurea* showed a significant increase in SLA when exposed to ozone.

3.2.7. Factors associated with sensitivity

Relationships between ozone sensitivity and stomatal conductance, leaf thickness, growth rate and ecological niche were explored. Fig. 3.5 shows a significant (P<0.05) positive correlation between stomatal conductance and reduction in total RGR for wet and woodland species combined. No significant correlation however, was found between the total RGR in FA and the reduction in total RGR when exposed to O_3 (Figure 3.6). Fig.3.7 shows a positive, but non-significant association between SLA and reduction in total RGR for wet and woodland species combined.

No significant correlation was found between stomatal conductance (leaf 3) and SLA, although high values of both were obtained for *Epilobium hirsutum* and *Eupatorium cannabinum*.
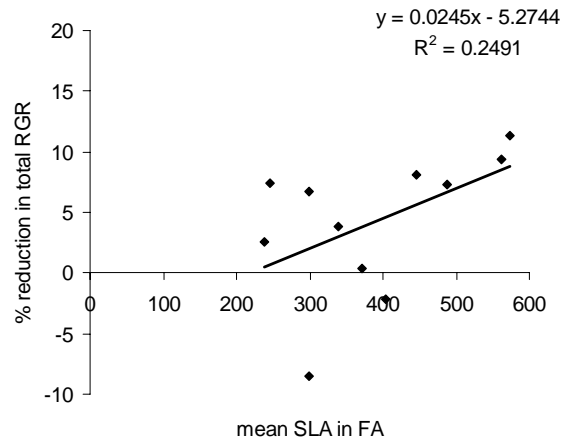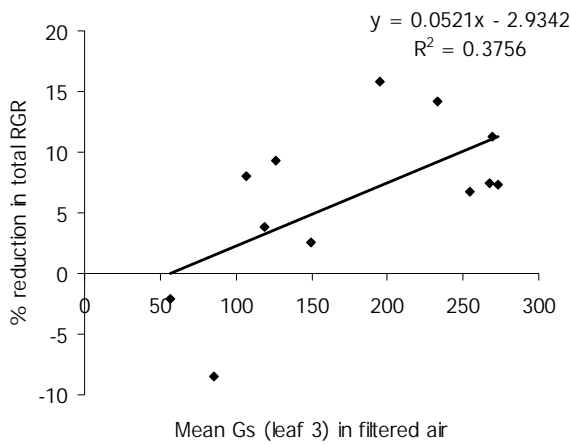
y = 0.0521x - 2.9342
R² = 0.3756

% reduction in total RGR

Mean Gs (leaf 3) in filtered air

y = 0.0245x - 5.2744
R² = 0.2491

% reduction in total RGR

mean SLA in FA

Fig.3.5 Relationship between leaf 3 stomatal conductance (mmol m^2 s^{-1}) and reduction in total RGR.

Fig.3.6 Relationship between SLA and reduction in total RGR.

y = 58.612x - 1.1402
R² = 0.1124

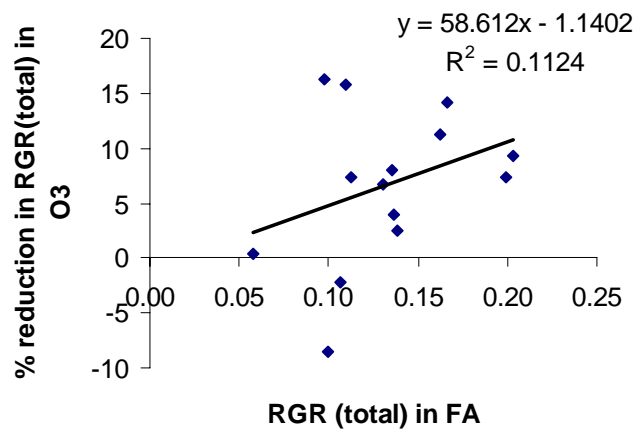% reduction in RGR(total) in O3

RGR (total) in FA

Figure 3.7. Relationship between the mean RGR (total biomass) and % reduction in mean RGR (total) in ozone.

Ecological indicator values (Ellenberg index) were used to investigate links between ecological niche and ozone sensitivity. Ellenberg values for light, moisture, reaction (acidity) and nitrogen for all species were obtained (Hill et al., 1999). No significant correlation coefficients were found with any index. The test species tended to have a small range of values within each index and this may have prevented any relationships being detected. However, Franzaring *et al.* (1999) also failed to show any relationship between Ellenburg indices and ozone sensitivity, although their study used a much wider range of species, and hence Ellenberg values.

Links between C-S-R functional types and ozone sensitivity were also explored. The C-S-R functional types were quantified using the scheme reported by Ashenden *et al* (1996). Relationships were explored separately for the C,S and R co-ordinates, regressed against the % reduction in RGR. None of the regressions were significant.

3.3. Exposure-response experiments

Data for all species were subjected to statistical analysis using both analysis of variance (ANOVA) and regression analysis. The significance of differences between individual ozone treatments was assessed using an LSD-test.

3.3.1. Relative growth rate

Relative growth rates were calculated for all species except *Typha latifolia* for which only gas exchange measurements were made. There were significant differences between treatments for five out of the eight species: *E. hirsutum*, *Scrophularia auriculata, Geum rivale, Eupatorium cannabinum* and *Digitalis purpurea*. *E. hirsutum, E. cannabinum* and *D. purpurea* showed significant differences between treatments for above, below and total biomass RGR. In all cases, the highest treatment was significantly different to the FA treatment (Fig. 3.8). In *E. hirsutum,* the below ground RGR in the 55ppb treatment was significantly reduced compared to the FA treatment.
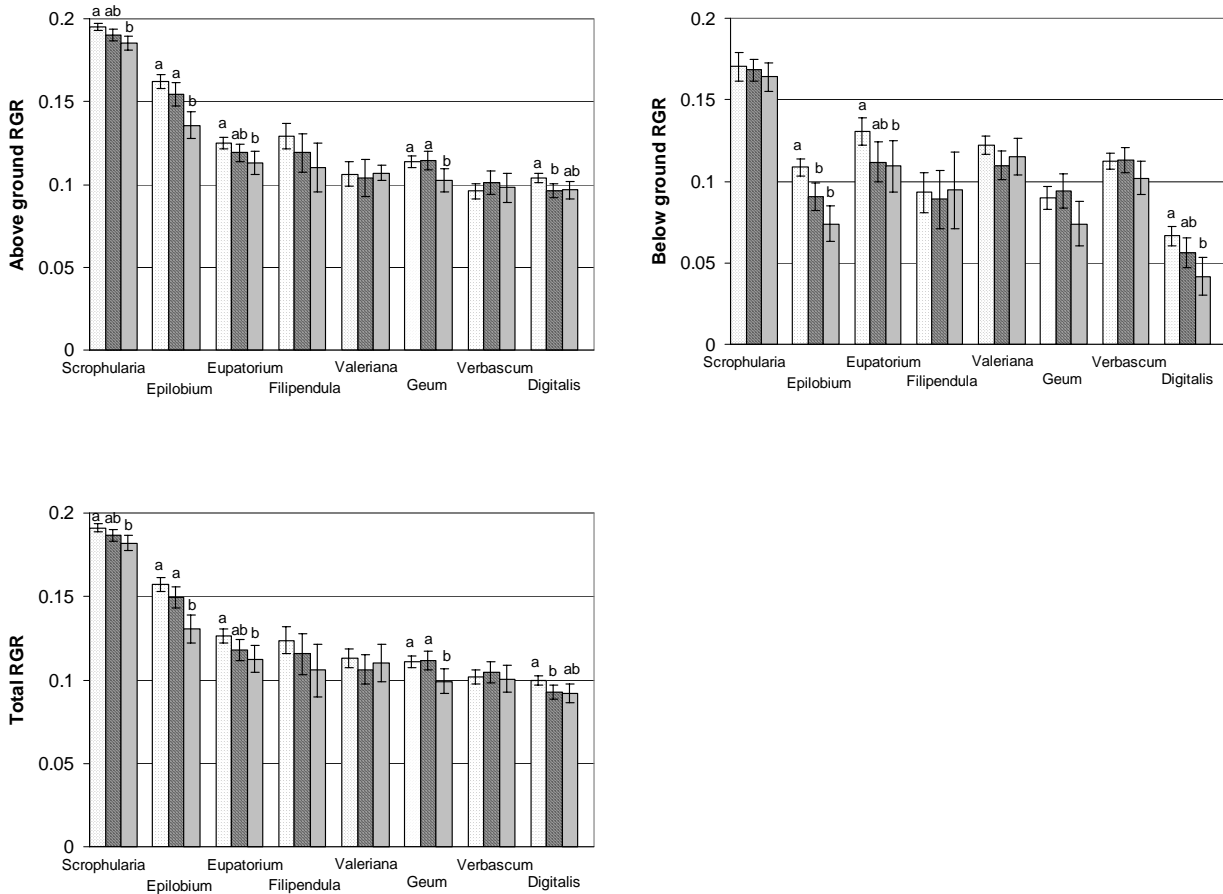
Figure 3.8.   Mean RGR ($^+$/- LSD bar) for each plant species in the different treatments:
☐ FA (filtered air);   ▨   55ppb O$_3$;   ▥ 80ppb O$_3$. Different letters indicate significant differences between treatments at P=0.05..

All species showed a negative relationship between the RGR (above, below and total biomass) and increasing AOT40, with six species showing a statistically significant relationship in regression analysis (*Epilobium hirsutum, Scrophularia*

auriculata, Eupatorium cannabinum, Geum rivale, Digitalis purperea and *Filipendula ulmaria*). Table 3.2 summarises the threshold AOT40 values derived for a 10% reduction in total, above-ground and below-ground RGR from the regression equations for each species.

Five of the eight species tested showed a significant regression coefficient for above-ground RGR. Two species (*Epilobium hirsutum* and *Filipendula ulmaria*) showed a threshold AOT40 value of 3200 ppb.h., close to the current critical level, while three other species showed threshold AOT40 values in the range 5-6000 ppb.h.

The results for below-ground RGR showed fewer significant regression coefficients, but this may reflect the greater variability of root data. The threshold values of AOT40 were in fact lower than those for above-ground RGR in six of the eight species. Two of the species (*Epilobium hirsutum* and *Digitalis purpurea*) showed a 10% reduction in biomass at AOT40 values (1000-1500 ppb.h) well below the current critical level. whilst *Filipendula ulmaria* showed reductions in RGR at values approximating to the current critical levels.

Table 3.2, Linear regressions for the relationship between plant biomass RGR and ozone dose (AOT40 ppb h). For each equation, the critical AOT40 values (ppb.h) derived for a 10% reduction in RGR are shown.

Species	RGR (total)	AOT40	RGR (above)	AOT40	RGR (below)	AOT40
Epilobium hirsutum	$y = -0.0055x + 0.158$ $r^2 = 0.87$**	2640	$y = -0.0055x + 0.163$ $r^2 = 0.86$**	3262	$y = -0.0068x + 0.106$ $r^2 = 0.79$**	1519
Scrophularia auriculata	$y = -0.0019x + 0.191$ $r^2 = 0.73$**	9545	$y = -0.002x + 0.195$ $r^2 = 0.74$**	9730	$y = -0.0013x + 0.170$ $r^2 = 0.22$	17040
Filipendula ulmaria	$y = -0.0038x + 0.124$ $r^2 = 0.45$	3090	$y = -0.004x + 0.129$ $r^2 = 0.50$*	3213	$y = 9E^{-5}x + 0.092$ $r^2 = 0.00$	102000
Geum rivale	$y = -0.0024x + 0.113$ $r^2 = 0.48$*	5635	$y = -0.0023x + 0.116$ $r^2 = 0.48$*	5780	$y = -0.0032x + 0.094$ $r^2 = 0.31$	3120
Eupatorium cannabinum	$y = -0.0028x + 0.125$ $r^2 = 0.591$*	4409	$y = -0.0025x + 0.124$ $r^2 = 0.596$*	5041	$y = -0.0044x + 0.126$ $r^2 = 0.41$	2888
Verbascum lychnitis	$y = -0.0005x + 0.103$ $r^2 = 0.03$	20660	$y = 0.0005x + 0.097$ $r^2 = 0.03$	19500	$y = -0.0025x + 0.114$ $r^2 = 0.31$	5705
Valeriana officionalis	$y = -0.0005x + 0.111$ $r^2 = 0.02$	22120	$y = 0.0002x + 0.110$ $r^2 = 0.00$	52500	$y = -0.0014x + 0.118$ $r^2 = 0.09$	11810
Digitalis purpurea	$y = -0.0018x + 0.100$ $r^2 = 0.41$	4900	$y = -0.0016x + 0.102$ $r^2 = 0.34$	5085	$y = -0.0057x + 0.066$ $r^2 = 0.70$*	1097

Figure 3.9 shows the relationship between total RGR and ozone dose for species for which the regression coefficient was significant. Both *Scrophularia auriculata* and *Epilobium hirsutum* showed highly significant relationships, but whereas the growth of *E. hirsutum* shows a sharp decline with increased O_3 dose, *S. auriculata* shows only small, but significant reductions.
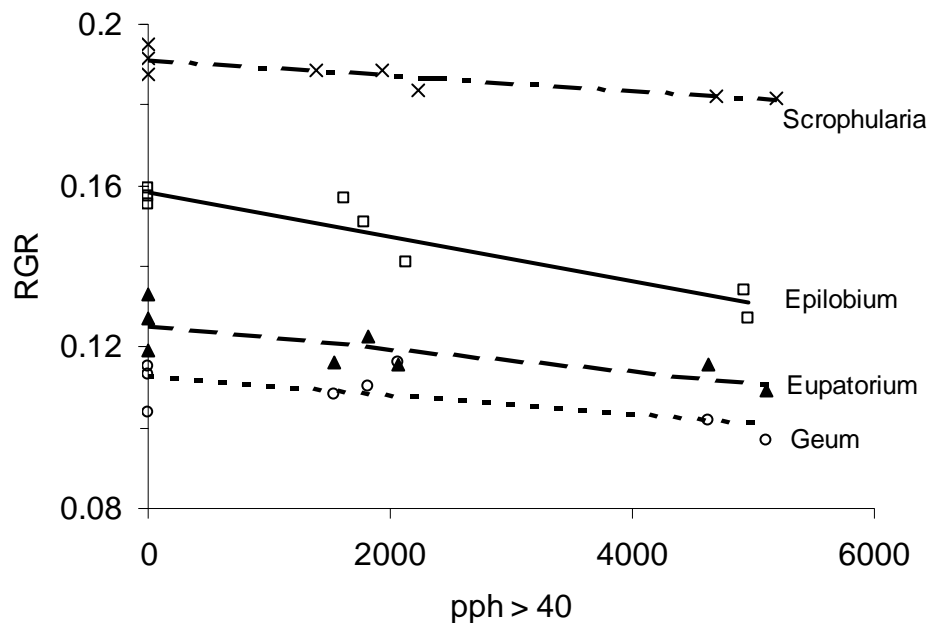

Figure 3.9   Relationships between total RGR and ozone dose (AOT40).

3.3.2. Gas exchange

Significant differences between treatments for either photosynthesic rate or stomatal conductance were found in ANOVA in leaf 3 for five of the nine species: *Epilobium hirsutum*, *Typha latifolia, Geum rivale*, *Eupatorium cannabinum* and *Verbascum lychnitis*. On all occasions when leaf 1 was measured, no significant effect was recorded. In general, those plants exposed to 80ppb ozone showed reduced rates of gas exchange (Fig. 3.10). Plants of *E. hirsutum,* and *T. latifolia* showed reduced rates of photosynthesis at 55ppb as well as at 80ppb. Plants of *E. hirsutum* and *E. cannabinum* showed reduced stomatal conductance at 55ppb.

Three of the nine species tested (*Epilobium hirsutum, Scrophularia auriculata* and *Digitalis purpurea*) showed significant negative relationships between the AOT40 value and stomatal conductance measurements. Three species (*E. hirsutum, Typha latifolia* and *Geum rivale*) showed significant negative relationships between AOT40 and photosynthetic measurements (Figure 3.11).
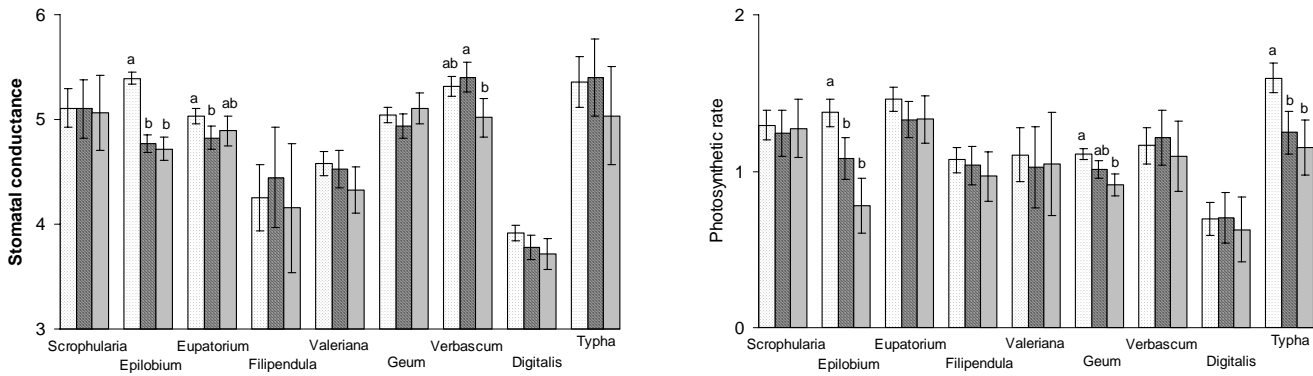
Figure 3.10 Stomatal conductance and photosynthetic rates measured in each treatment:

$\boxed{\because}$ FA (filtered air); $\boxed{\text{\tiny \\\\\\}}$ 55ppb O_3; $\boxed{}$ 80ppb O_3. Data are ln-transformed. Stomatal conductance was measured in mmol m^{-2} s^{-1}, photosynthesis was measured in μmol m^{-2} s^{-1}. Columns with different letters are significantly different at P=0.05.
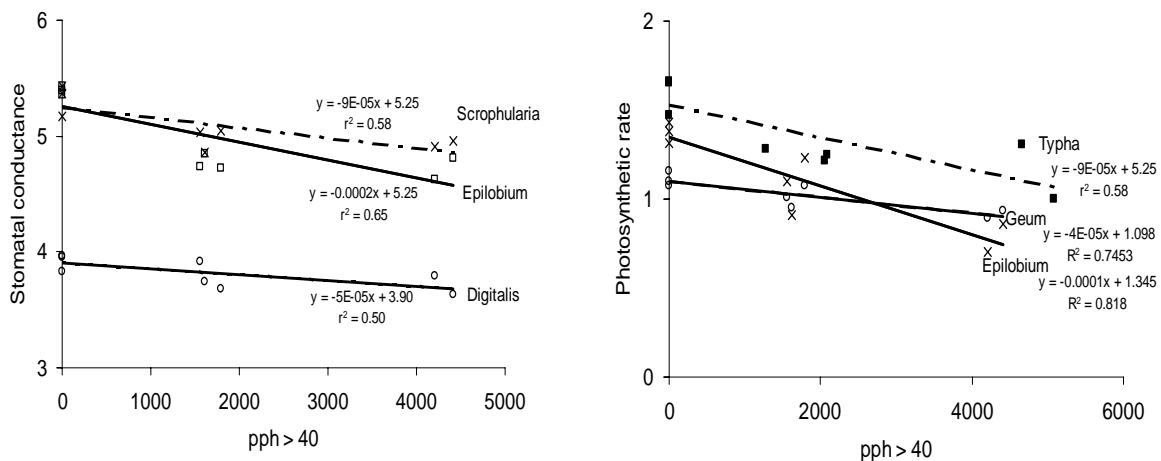
Figure 3.11. Relationship between ozone dose and a) stomatal conductance (mmol m^{-2} s^{-1}) and b) photosynthesis (μmol m^{-2} s^{-1}); for species showing a significant regression. Data are ln-transformed.

3.3.3. .SLA and root:shoot ratio

The only significant effect of ozone found was for the root:shoot ratios of *V. lychnitis* and *D. purpurea*. In *D. purpurea,* root:shoot ratios were reduced in the 80ppb O_3 treatment (25.5% reduction) but not in the 55ppb treatment. In *Verbascum lychnitis* root:shoot ratios were increased at 55ppb although the effect was not significant. However, the 80ppb treatment was significantly lower than the 55ppb treatment, but not significantly different from FA. No significant relationships were found using regression analysis.

3.4. Drought experiment

3.4.1. Water stress during ozone exposure

The proportion of ozone-damaged to total leaf weight in ozone was tested, using arc-sin transformed data. The results of ANOVA showed significantly reduced visible injury caused by ozone on the droughted plants, compared to well-watered plants.

Gas exchange measurements were made on the third fully expanded leaf on each plant at the end of each drought cycle (day 8, 15 and 21). Photosynthetic rate showed no significant effect of ozone or drought treatments at any measurement period. In contrast, stomatal conductance showed significant differences for each measurement day. On day 8 stomatal conductance was 15.4% lower in ozone than filtered air, but drought had no significant effect. On days 15 and 21 significant ozone -water-stress interactions were found (Figure 3.12). On day 15 the well-watered plants in FA had significantly higher stomatal conductances than all other ozone / water treatments. On Day 21 plants from the FA drought and O_3 control treatments showed the lowest stomatal conductances. On both days, ozone significantly reduced stomatal conductance in the control treatment but not in the droughted plants.
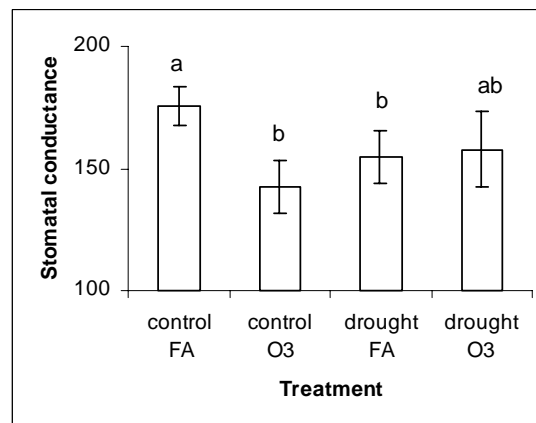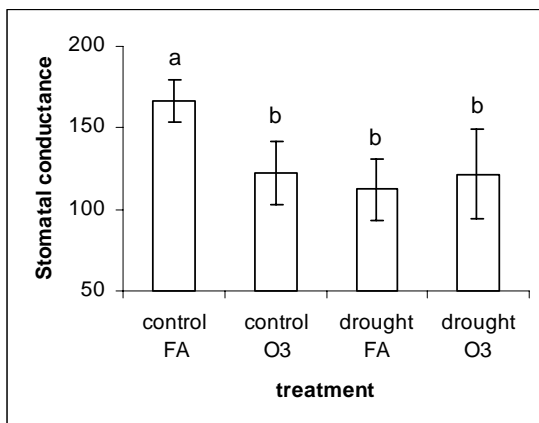
Figure 3.12a. Stomatal conductance (mmol m^{-2} s^{-1}) day 15.

Figure 3.12b. Stomatal conductance (mmol m^{-2} s^{-1}) day 21.

Ozone and drought treatments had no detectable effect on the RGR of either total or below ground biomass of *Eupatorium cannabinum.* However, the above ground biomass RGR was significantly reduced (by 4.6%) in the drought treated plants. Drought treated plants also showed a 7.3% reduction in Leaf area RGR and a 9.1% increase in RSR. SLA showed significant reductions in both the drought (8.0%) and ozone (7.5%) treatments, but the ozone-water stress interaction was not significant. Plants had thinner leaves following exposure to drought and/or ozone.

3.4.2. Water-stress following ozone fumigation

Water potential measurements were made on day 1, 2,3, 5, 7 and 9 for all plants that had not wilted. Complete sets of data were collated until day 5, thereafter plants began to wilt. No significant differences in water potential were found between ozone treatments on any of these days. At the beginning of the droughting period, plants from the ozone chambers tended to be smaller than those in FA, hence they did not dry out as quickly as the FA plants. By day seven, 9 FA plants and 3 ozone-fumigated plants had wilted. By day 9 these values rose to 10 FA and 8 O_3- plants. Taking account of initial plant size, there was no evidence of a significant effect of ozone on the date of wilting.

4. Discussion and Conclusions

This section briefly discusses the key findings of this research, drawing on the results from all the different experiments.

The most important conclusion is that the experiments provide clear support for the initial hypothesis that wetland plants are likely to be highly sensitive to ozone. In each of the three main experiments, the majority of the species were significantly negatively affected by ozone in terms of at least one parameter. In evaluating this finding, it is important to emphasise that the between-plant variability in wild plants is much greater than for crop species, making it more difficult to detect significant effects of ozone in standard study designs. Initial species selection for both the long-term and short-term studies did not involve any prior knowledge about relative sensitivities to ozone and was largely constrained by seed supply and germination success.

It is also important to consider the significance of the particular parameters which were influenced by ozone. Visible injury was found in most species in the long-term study but only in two species in the short-term study, presumably reflecting the lower ozone concentrations and shorter exposure durations. However, visible injury is not generally regarded as a good indicator of ecological effects (Davison & Barnes, 1998).

Of greater importance are the effects on growth and biomass. Five of the twelve species in the long-term experiment showed significant reductions in above- or below-ground biomass in the long-term experiment, while six of fourteen species showed significant reductions in total RGR in the short-term experiment, at a much lower ozone exposure although under artificial growth conditions. The magnitude of biomass reductions observed for the most affected species in the long-term study in field chambers was large (as high as 58% for *C. arvense* roots), given that the AOT40 values of 9000-15000 ppb.h are within the range reported for the UK.

The effects on growth are also relevant to assessment of the current critical level of ozone. The significant reductions in growth in the short-term experiments were at AOT40 values of between 3439 and 3605 ppb h., similar to the current critical level. In the exposure-response study, with selected sensitive species, two species had estimated threshold values for a 10% reduction in above-ground relative growth rate of about 3000 ppb.h. It is important to emphasise that this is a logarithmic growth rate; if plants were growing exponentially over a period of three weeks, the reduction in biomass due to ozone would be over 25%. This findings suggest that the critical level to prevent decreases in growth of sensitive wetland species may need to be even lower than 3000 ppb.h.

The threshold AOT40 values for adverse effects on root RGR were even lower on the most sensitive species – 1000-1500 ppb.h – and there was evidence of significant reductions in root/shoot ratios in both the long-term and short-term experiments. It habitats where soil water and soil nutrients were limited, these effects on roots could be critical to plant survival. However, in wetland

communities where water and nutrient supplies are high, the ecological significance of reduced root growth is less certain.

Stomatal conductance values in the short-term experiment were generally lower than in the open-top chamber experiment, probably reflecting the lower light intensities, and this may be a factor influencing differences in ozone response in the two experiments. Only *Typha latifolia* and *Phragmitis australis* were found to have conductance values in filtered air in the short-term experiment above 300 mmol m^{-2} s^{-1}. Values for other wetland species ranged from 107 in *Valeriana* to 273 mmol m^{-2} s^{-1} in *Epilobium*. Only two of the four woodland species were tested for gas exchange, but both showed much lower conductances (86 mmol m^{-2} s^{-1} in *Geum urbanum* and 64 mmol m^{-2} s^{-1} in *Viola*).

A significant positive relationship was found between growth rate reductions in ozone and stomatal conductance was found in the short-term experiment. The greater ozone flux into plants with higher stomatal conductances presumably explains their increased sensitivity. In the long-term experiment, a similar, but non-significant, positive association was found between stomatal conductance and reductions in root biomass, although not above-ground biomass.

It would be expected that faster growing species should take up higher ozone doses, and this in turn should relate to species sensitivity. In the present study no significant correlation was found between plant RGR and the % reduction in RGR when exposed to ozone, although *Stachys palustris, Eupatorium cannabinum* and *Epilobium hirsutum* were amongst the species with the highest growth rates and were species which showed the highest number of parameters affected by ozone. Similarly, it has been suggested that plants with large thin leaves should be more sensitive to ozone than species with compact leaves as they have a high surface area to volume ratio for gas uptake. *E. cannabinum, S. palustris* and *E. hirsutum* also showed the highest SLA, but the correlation between ozone effects on growth rate and SLA was not significant over the whole range of species.

The rare species showed very little effect of ozone, with only one species (*Verbascum lychnitis)* showing reductions in biomass. Four of the eight species (*Sedum rosea, Primula farinosa, Pulsatilla vulgaris* and *Lychnis viscaria*) were especially "slow starters". Although they germinated easily, their initial growth was slow relative to the other species tested. Wetland plants were found to have the highest mean RGR in FA (mean: 0.141; range 0.11-0.203), whilst the woodland and rare species had lower RGR's (woodland mean 0.11; range 0.098-0.138; rare species 0.108, range=0.061-0.141). No comparisons of stomatal conductance are possible, as gas exchange could not be determined on most rare species.

Species sensitivity showed no association with ecological strategy (C-S-R) or habitat preference (Ellenberg indices), although this may reflect the relatively narrow range within the species studied. Many of the wetland species are classified as competitors, or competitive ruderals, and an important outstanding question is how significant the reductions in growth rate which were found at relatively low exposures would be to the success of these species in competitive situations in the field

5 Community Sensitivity

A major challenge in evaluating the impacts of ozone on semi-natural communities in the U.K. is to identify factors associated with sensitivity to the pollutant. These include both the sensitivity of individual plants within the community and other climatic and edaphic factors associated with this community which might be related to ozone impacts. In assessing these factors, we have used both the National Vegetation Classification (NVC) and the Countryside Vegetation Survey (CVS). While the former includes all types of community, irrespective of their extent, the latter provides a basis for assessing if species are increasing or decreasing in frequency over time in the major plant communities.

5.1. Species sensitivity

5.1.1 Ellenberg Classification

The most detailed compilation to date of the responses of wild species to ozone is that of Franzaring et al. (1998), which lists 96 species which have been studied in at least one of six published studies (Bergmann et al., 1996, Pliejel et al., 1997, Grub et al., 1997, Mortensen et al., 1992, Reiling & Davion., 1992, and Ashmore et al. 1996). For the purposes of this analysis, the species in this compilation were simply classified as:-

Sensitive: growth reduction of 10% or more recorded in at least one study
Insensitive: no growth reduction of 10% or more recorded in any of the studies

Any attempt at more detail quantitative analysis could not be justified, as it would be impossible to realistically compare growth effects of different size from the different studies. Indeed, the fact that a range of studies with different growth and fumigation conditions are included in this compilation must limit the chances of finding statistically significant associations. It should also be noted that 42 of the 96 species fall into the Sensitive category, i.e. the chance of any studied species falling into the sensitive or insensitive category are not greatly different. However, those species which have been studied in more than one experiment may be more likely to show at least one growth reduction of 10% or more than those species only studied in one experiment.

A cross-tabulation was then conducted using the new Ellenberg classifications for UK vegetation (Hill et al., 2000), based on four axes – light, moisture, acidity and fertility. The results (Table 5.1) indicate no strong associations between sensitivity and the Ellenberg classifications. Indeed if there is any trend discernible it is for a higher proportion of species which are resistant to have a high light and moisture requirement – the opposite of what would be predicted based on the high sensitivity of wetland species found in the experimental component of the study. Nevertheless, the lack of any strong association between Ellenberg classifications and ozone sensitivity is, as was discussed in Section 4 above, consistent with our experimental data on wetland species and with other analyses of larger datasets (Franzaring et al., 1999).

Table 5.1: Summary of percentage of species in the compilation of Franzaring et al. (1998) which are classified as sensitive (S) and insensitive (R) to ozone in each Ellenberg class

Ellenberg class	LIGHT S	R	MOISTURE S	R	ACIDITY S	R	FERTILITY S	R
1	-	-	-	-	-	-	2.4	-
2	-	-	-	-	-	2.4	7.3	11.6
3	-	-	5.3	9.1	2.6	2.4	19.5	18.6
4	-	-	26.3	25.0	12.8	11.9	14.6	18.6
5	-	4.8	57.8	36.3	7.7	2.4	17.1	9.3
6	10.0	4.8	10.5	13.6	33.3	22.7	19.5	16.3
7	70.0	57.1	-	6.8	38.5	47.6	14.6	23.2
8	17.5	33.3	-	4.5	5.1	9.1	4.8	-
9	2.5	-	-	4.5	- / -		- / 2.3	

5.1.2. Countryside Vegetation System

The botanical data collected in the Countryside Survey has been used to develop a new classification of British vegetation – the Countryside Vegetation System (Bunce et al., 1999). This analysis identifies 37 distinct species group, for each of which Firbank et al. (2000) list a number of characteristic species; these 37 groups can be characterised by two prime axes: habitat and soil type. The numbers of species in the compilation of Franzaring et al. (1998) in each group were too small for any substantive analysis, and therefore the habitat groups were amalgamated. The results are summarised in Table 5.2. Given that 44% of the species in the compilation of Franzaring et al. (1998) were classed as sensitive, there are two very clear results from this analysis. Firstly, when combined, the crop/crop edge/grassland group has a smaller percentage of sensitive species (29%) than would be expected if they were a random selection from the compilation. Secondly, the percentage of sensitive species associated with woodland or woodland edge habitats was remarkably high (89%). This was the main reason that a small group of woodland species was included in the experimental programme.

Ideally, it would be have been of interest to group the species by soil type, rather than habitat type, as in Table 5.2. However, it was found that almost none of the species studied in the experiments used by Franzaring et al. (1998) was characteristic of soils described as wet, damp or saturated by Bunce et al.

(1999). This serves to emphasise that the ozone responses of wetland species have been poorly studied to date.

Table 5.2 Summary of numbers of species classed as sensitive or insensitive to ozone (Franzaring et al., 1998) in major species groupings within the Countryside Vegetation System (Bunce et al., 1999)

Habitat type	Number of species sensitive	Number of species Insensitive
Crop/crop edge	3	8
Wood/wood edge	16	2
Grassland	6	14
Water edge	0	2
Moorland/heath	0	3

5.1.3 Change in the Countryside Survey

The re-survey of fixed plots within the British Countryside Vegetation Survey (CVS) in 1978, 1990 and 1998 provides the opportunity to test if any of the observed changes might be consistent with effects of ozone. The published analysis of change to date has focussed on community level indices. Since to date no clear predictions of the overall direction of community change are available for ozone, in contrast, for example, to nitrogen deposition, there is little value in these community indices. However, Smart (pers.comm.) has recently developed a database of significant increases or decreases of frequency in individual species within the major vegetation categories of the CVS. A brief analysis of the database was conducted to test if there were any clear signals that ozone-sensitive species were tending to decrease in frequency.

Since the database records all significant changes in any major vegetation category it is possible for the same species to show significant increases in one category and significant decreases in another category. Taking this into account, there was no evidence of an association with ozone sensitivity in the compilation of Franzaring et al. (1998). Of the species which showed a significant decline in frequency in the CVS between 1978 and 1998, 52% were sensitive, while of the species which showed a significant increase in frequency, 46% were sensitive. A different type of comparison was made with the results from the experimental studies. In the CVS database, 20% of species showed a significant decrease in frequency; in the case of the species fumigated, 6 out of 25 (24%) showed a

significant frequency, again giving no clear signal of an ozone response in the CVS database.

Although these results show no evidence of national changes in distribution of individual species which might be associated with ozone, it is important to note that frequency is a fairly crude indicator of change and that there are many factors which have influenced change in vegetation composition over this period. Furthermore, a more rigorous test of the null hypothesis would be possible if plots in areas with different levels of ozone exposure were identified and compared.

5.2. Community Classification

While sensitivity of the component species is one major factor determining if changes in community composition are caused by ozone, it is by no means the only one. The distribution of the community in relation to ozone exposure patterns across the country will be important, as will soil moisture availability. A method was therefore developed, using these criteria to provide an empirical approach to identify communities where the risk of ozone impacts may be greatest.

While the approach could be applied in principle to any community, adequate data on a sufficient proportion of the dominant species in the community is essential. It was not possible to apply the approach to wetland communities, because of the limited number of species studied. Therefore, the method was tested on grasslands, as there is the greatest amount of available data for such communities. The method uses the National Vegetation Classification (NVC) to classify the individual grassland communities, as the Countryside Vegetation System described above does not contain enough detail on the contribution of individual species. Following Rodwell et al. (1992), grassland communities may be grouped into mesotrophic, calcicolous and calcifugous/montane. This gives a total of 48 communities.

To classify the sensitivity, three criteria are combined: ozone exposure, moisture status and species composition. The methods used for each of these criteria are explained below.

1. Ozone exposure. At present, this is a simple visual comparison of the sample distributions in Rodwell et al. (1992) with the 5-year mean AOT40 distributions published by PORG (1998). One of three categories below is assigned, derived from the critical level for semi-natural vegetation:-

High: mean AOT 40 above 6 ppm.h (H)
Intermediate: mean AOT40 of 3-6 ppm.h (I)
Low: mean AOT40 below 3 ppm.h (L)

At this stage, classes are simply assigned based on the highest AOT40 value for any recorded sample location. In theory, with digitised NVC distributions, a mean and range of exposures could be calculated. However, it should be noted that

Rodwell et al. (1992) only record the location of their sample plots – the actual distribution of the community could be more extensive.

2. Soil moisture deficit

The description of climate and soil conditions associated with each NVC community provided by Rodwell et al. (1992) is used empirically to assigned the following categories:-

High: no significant soil moisture deficit in mid-summer (H)
Intermediate (I)
Low: significant soil moisture deficit in high ozone summers (L)

In theory, a more rigorous approach could be devised by using MORECS data for the appropriate land cover and soil type. However, since the distribution of particular communities is associated with specific types of drainage, aspect etc. even this would be a relatively simplistic approach.

3. Species sensitivity

In theory, if clear associations could be demonstrated between ecological characteristics and ozone sensitivity of individual species, it would be possible to assign a sensitivity category based on the broad characteristics of a community or an inferred sensitivity of component species. However, no such associations have been clearly demonstrated in this, or other, projects..

Therefore an empirical approach has been devised, based on the sensitivity of individual component vascular species. It is assumed that community response in grassland communities, where competition for light and other resources is high, will depend on the sensitivity of the dominant species of the community. Therefore only species which met both of the following empirical criteria, using the community descriptions of Rodwell et al. (1992), were included in this index:-

(a). Frequency of III (61-80%) or above in at least one sub-community
(b). Domin value of 6 (25-33% cover) or above in at least one sample

It should be noted that the use of these criteria will mean that a very different number of species will be selected for assessment in different NVC communities.

All these species were then assigned to one of three categories, based on the compilation of Franzaring et al. (1998). In addition to the sensitive and resistant categories described in Section 5.1, a third category exists:-

Unknown: not included in the compilation of Franzaring et al. (1998).

Tables 5.3 and 5.4 summarise the results from this method for mesotrophic and calcicolous grasslands. The method did not work successfully for calcifuge/montane grassland, because of the low numbers of species which dominate them, and the lack of experimental data for many of these. Even for mesotrophic and calcicolous grasslands, species numbers are in many cases low, and hence the method can only provide an indication of the main patterns of sensitivity.

An 'Ozone Index' was then calculated to summarise the data for each NVC community. To calculate the index, values of 3, 2, and 1 were assigned to categories high, intermediate and low for ozone exposure and moisture status. These values were multiplied together and then multiplied by the proportion of sensitive species to obtain the final index given in the final columns of Tables 5.3 and 5.4. This index is, however, based on a completely arbitrary weighting of the three categories.

The results in Table 5.3 and Table 5.4 should not be over-interpreted. There is a clear indication overall that mesotrophic grasslands are likely to more sensitive than calcicolous grasslands in the UK, but is important to note than the systems are not particularly well differentiated in terms of species sensitivity. Although the percentage of species which are known to be sensitive is higher in general in the mesotrophic grasslands, the percentage of species which are of unknown sensitivity is
also lower. A more useful method of using this approach is to identify NVC classes which are likely to be of high sensitivity to ozone – such as MH1, MG6, and MG7, all of which have over 50% of the dominant species listed as sensitive to ozone, are in areas of the country with relatively high ozone exposures, and in which ozone flux is less likely to be constrained by soil moisture deficit.

5.3 Conclusion

The analyses of species characteristics and species change presented in this section do not provide a strong basis for identifying either species or communities which are likely to be sensitive to ozone. A hypothetical classification system based, in terms of species sensitivity, on experimental data rather than predictive ecological characteristics, shows some promise as a possible basis for ecological risk assessment, but clearly needs more validation by field and experimental data. Nevertheless, development of such methods of assessment should continue because they provide a basis both for attempting to generalise from the limited body of experimental data and because they provide a basis for focussing limited experimental resources on key species and communities and on key hypotheses.

Table 5.3: Summary of community classification for ozone sensitivity for mesotrophic grasslands

NVC Class	Ozone Exposure	Drought Index	% species high	% species low	% species unknown	OZONE INDEX
MG1	H	I	61	23	15	3.7
MG2	I	H	26	10	63	1.6
MG3	I	H	40	40	20	2.4
MG4	H	H	20	80	-	1.8
MG5	H	I	41	29	29	2.4
MG6	H	I	57	29	14	3.4
MG7	H	H	55	22	22	4.9
MG8	H	H	50	17	33	4.5
MG9	H	I	50	-	50	3.0
MG10	H	H	33	-	67	3.0
MG11	H	I	50	10	40	3.0
MG12	H	H	44	12	44	4.0
MG13	H	H	25	-	75	2.2

Table 5.4: Summary of community classification for ozone sensitivity for calcicolous grasslands

NVC class	Ozone exposure	Drought index	% species high	% species low	% species unknown	OZONE INDEX
CG1	H	L	50	14	36	1.5
CG2	H	L	43	14	43	1.3
CG3	H	L	50	20	30	1.5
CG4	H	L	45	18	37	1.5
CG5	H	L	55	22	22	1.6
CG6	H	L	42	0	58	1.2
CG7	H	L	27	9	64	0.8
CG8	I	I	40	0	60	0.8
CG9	I	I	20	0	80	0.8
CG10	I	I	45	18	37	1.8
CG11	L	I	14	14	72	0.3
CG12	L	I	20	20	60	0.4
CG13	L	H	20	0	80	0.6
CG14	L	L	20	0	80	0.1

6. Project Outputs

The results of this work have been reported in poster form at the annual meetings of ICP Vegetation and of the NERC Committee on Air Pollution Effects Research (CAPER). A paper on the short-term experiments will be submitted shortly to Environmental Pollution.

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Assessment of the Relative Sensitivity of Wetland Plant Species to Ozone.

Final Report

Contract EPG 1/3/121

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Assessment of the relative sensitivity of wetland plant species to ozone.

Final Report

Conclusions

! There is significant variation in the ozone response between populations of the common wetland species, *Epilobium hirsutum*.

! Ozone response is not associated with any particular regions of the UK so exceedance of the critical level would be expected to affect some populations in which ever part of the UK that it occurs.

! Early-season fumigation of a sensitive population leads to significant effects but they are transient.

! The effects of early-season exposure are modified by water stress. Ozone <u>reduces water use and the effects of water stress.</u>

! Intra-specific competition among dense patches of seedlings alters leaf morphology and makes them more susceptible to acute injury. Therefore <u>competition reduces the critical level for acute injury</u>.

! Late-season exposure has a detectable effect on the shoots but it has a much larger effect on stolon mass. <u>Stolons are the over-wintering organs so ozone reduces the potential for vegetative growth early in the following season. The long-term effects of this are unknown</u>. The critical level for this effect is around 5000 ppbh.

! Because of the seasonality of the work, an experiment is still underway on the effects of ozone and water stress on fecundity. It will be finished in October and a supplementary report will be issued soon after.

! The need to express critical levels in terms of flux rather than exposure is reinforced by the results of the ozone-water stress experiment. To do this measurements of stomatal conductance have been made over two years of OTC experiments. It is hoped that Ms Haley will be able to develop an ANN model to enable us to express the critical level in terms of flux.

! Finally, the work has shown that further work is needed on the interaction between ozone and water stress, and on the carry-over effects of ozone from one season to another.

Introduction

The overall aim was to assess the relative ozone sensitivity of wetland plant species to ozone and interactions with competition and water stress. Furthermore, the existing critical level for natural vegetation does not make any provision for geographical variation in ozone response between populations so the work included an examination of variation between populations of a model species.

The work was undertaken by Helen Haley, who is currently finishing a summer OTC experiment and writing it up for a PhD. The timing of the project in relation to the summer growing season meant that although this summer's fumigation experiments have been completed on time, more information will be available in the autumn when the over-wintering organs have been fully formed and harvested.

Several species were tested initially, including those being used by Professor Ashmore. This led to the selection of *Epilobium hirsutum* as our model species. It occurs mainly on wet soils but it is also tolerant of drier conditions so it grows in a wide range of situations. It is widespread in Europe and occurs in parts of N. America where it is naturalised. The research focussed on a comparison of two populations that differ in ozone response: Cowrigg (Scottish borders), which is sensitive, and Millers Dale (Derbyshire) which is more resistant.

This report briefly describes the more important results of the experiments, without giving much detail. It starts with a comparison of the relative sensitivity of 18 populations then OTC fumigation experiments in 2000 and 2001 are described.

Results

1. Variation in relative ozone resistance between populations of *E. hirsutum*.

Eighteen populations of *E. hirsutum* were screened for relative resistance to ozone using our standard procedure (Reiling & Davison, 1993). Table 1 shows that there was about a five times difference in effects of ozone on dry weight between populations. There was no geographical pattern to the sensitivity/resistance, ie there is no association between sensitivity/resistance and ozone climate. For example, two of the most sensitive populations were from Kent and Perthshire, while the two most resistant ones were from Tyne & Wear and Rutland. Therefore exceedance of the critical level would be expected to affect some populations in which ever part of the UK that it occurs.

2. The effects of ozone fumigation using OTCs, 2000.

In 2000 two experiments were conducted, a five-week 'early-season' fumigation to simulate a spring episode, and a later fumigation starting in August. In the early-season experiment (described in last year's report) ozone reduced plant performance but the effects were transient. In contrast, the late season fumigation had a lasting and potentially important effect.

Two populations of *Epilobium hirsutum,* Cowrigg and Miller's Dale, were fumigated over a period of eleven weeks from August to October 2000, in open top chambers. Four levels of ozone were employed, giving mean AOT 40s of 60, 4000, 10600 and 19000 ppb.h. at the end of the fumigation period. Plants were grown in pots, either individually or in stands of 4 where a centre plant was surrounded by a ring of three.

Figures 1-2 show the effects of ozone on a morphological character, leaf width. In plants grown without competition (Figure 1) there was no effect on Miller's Dale (=MD) but there was a significant reduction in Cowrigg (=C). However, in plants grown in competition, in the centre of a group of four, the effects of the ozone was much less pronounced (Figure 2).

At the end of the 2000 growing season the plants were harvested and the stolon weights calculated (Figures 3-4). Figure 3 shows that ozone had a very large effect on the stolon weight of both populations. However, the variability made it difficult to discern the critical level. Competition (Figure 4) almost halved stolon weight but there was still a significant effect of ozone, with a better defined critical level around 5000 ppbh. There are three important conclusions from this experiment:

!   Although the MD population was resistant in terms of morphological features, it still showed a reduction in stolon weight.

!   Competition had a greater effect on stolon weight than even the highest ozone exposure, and there was an indication of an ozone-competition interaction.

!   As the stolons are winter storage organs that provide the capital for the next year, ozone may have a carry-over effect into the next year.

3. Ozone and water stress interactions.

Research in the last 18 months focussed on ozone-water stress interactions. Laboratory experiments were used to test stomatal response and Merrill psychrometers for measuring water potentials.

Two experiments were set up in OTCs in spring 2000. The underlying philosophy was that the same weather that brings ozone episodes also tends to bring water stress. As the latter is very important during the seedling phase when roots are poorly developed, in the first experiment we simulated patches of dense, competing seedlings establishing in a gap during an ozone-water stress episode. The seedlings had three weeks of ozone then water was witheld from half of the 30cm pots. Wilting took 22 days then plants were re-watered. Half were harvested immediately after the drought and, at the time of writing, the rest are being kept until autumn to record the long-term effects on stolon production.

In the second experiment seedlings were grown either singly (no competition) or in groups, with one plant in the middle of a triangle of three others of the same species. The ozone and water treatments were the same as above but there have been no destructive harvests and the plants are being kept until the autumn to record fecundity (flowering, seed production, viability and stolon production).

Experiment 1.

Ozone reduced stomatal conductance, as shown in Table 2, as expected. An unexpected feature was that as the intense competition developed between the seedlings, the leaves became visibly paler and thinner than those in experiment 2. Furthermore, they developed visible injury in the highest ozone treatment whereas there was none in the plants grown in experiment 2. We considered that this sensitivity was probably due to reduced leaf thickness so specific leaf area was measured (Table 2). This supported the idea that intense competition had altered leaf morphology and probably increased susceptibility to ozone.

Table 2. Effects of ozone and competition on leaf conductance and morphology of *E. hirsutum*.

Stomatal conductance, 12.00h-14.00h	Experiment 1 Plans in intense competition from seed.	Experiment 2 Plants grown singly, no competition
NFA	363 ±13	372 ±14
NFA+75 ppb ozone	247 ±21	226 ±26
Specific leaf area mm^2 mg^{-1}		
NFA	44 ±5	31 ±5
NFA+75 ppb ozone	52 ±7	32 ±6

Figure 5 shows the rate of drying of the soil in two of the treatments, NFA (non-filtered air) and NFA+75 ppb ozone development of water. Soil water stayed around 40-50% by volume in the well watered plants but it fell to around 10% over 22 days. There was a significant difference in the rate of water depletion, it being slower in the NFA+75 treatment.

Figure 6 shows the development of leaf water potentials. There was some day to day variability and a tendency for a slight fall in Θ in the watered plants, but there was a significant difference in the rate of development of stress between the NFA and NFA+75 treatments. Ozone delayed the development of water stress. However, there was no effect on survivorship of the seedlings.

Plants were harvested immediately after the drought and the shoot dry weights are shown in Figure 7. The shoot dry weight of well watered plants declined in NFA+50 and NFA+75 compared with controls. Water stress reduced weight in all treatments except NFA+75ppb ozone. The effect of drought was related to the ozone treatment as can be seen in Figure 8. Clearly, ozone reduced the effect of water stress, probably by decreasing stomatal conductance and water use. This reinforces the need to gain a fuller understanding of ozone-water stress interactions and to express the critical level in terms of flux rather than exposure.

A second harvest, taken after three weeks recovery (Figure 9), showed that the effects of this early-season ozone exposure were transient, as in 2000.

At the time of writing, the experiment is being continued so the effects of the treatments on stolon production can be recorded in October. Figure 10 shows some of the latest data on the % of plants flowering. Water stress caused a significant increase in the % of plants flowering except in the highest ozone treatment, NFA+75 ppb.

Experiment 2.

The plants in the fecundity experiment developed water stress much more slowly than those in experiment 1. The rate of development of water stress was slower (Figure 11) than in experiment 1, presumably because the lower mass of plants had less drying effect on the soil.

At the end of the drought, non-destructive measurements showed that although there were effects of both ozone and water stress, there was no interaction (data not shown). Our interpretation is that this was because water stress did not progress as far as in the seedling competition-drought experiment.

The fecundity experiment has continued with records of flower, seed and stolon production. It will continue until about October, when a supplementary report will be issued.

Stomatal conductance and ozone flux.

As mentioned in the 2000 report, our aim is to express the critical level in terms of lfux as well as exposure so we have examined stomatal conductance in most of the experiments in order to model it. Work last year showed that conductance followed a similar diurnal trend most days but the maximum varied from about 300 to 600 mmol m^{-2} s^{-1} (water vapour). An example of diurnal curves are shown in Figure 12. There is no response to short-term (30 minute) changes in light or temperature. PAR, VPD and temperature are all so strongly correlated that conventional statistical techniques such as regression cannot be used to determine the influence of individual factors. Data collection has continued so Ms Haley is attempting to develop an ANN model that will estimate ozone flux.

<div align="center">

Reference

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Reiling, K. & Davison, A.W. (1993) Environmental Stress- Ozone. pp. 63-65. In: Methods in Comparative Ecology- A laboratory manual. Ed. by G.A.F. Hendry & J.P. Grime. Chapman & Hall, London.

Figure 1 Effects of ozone fumigation on leaf width of single (ie no competition) plants of *E. hirsutum*. Blue = MD, resistant; Red = C, sensitive population
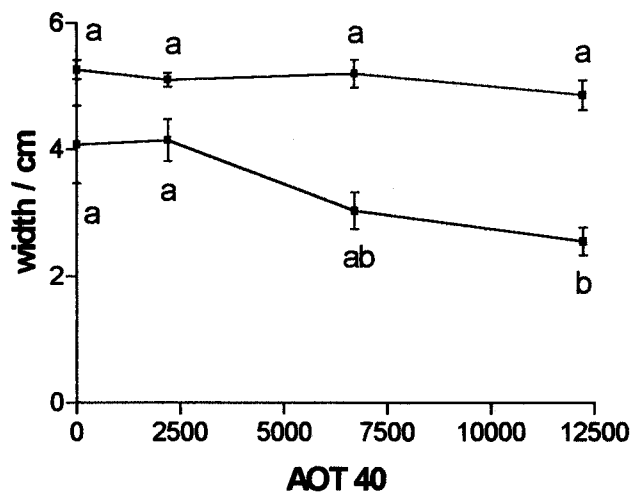

Figure 2 Effects of ozone fumigation on leaf width of plants of *E. hirsutum* grown in competition with three others of the same species. Blue = MD, resistant; Red = C, sensitive population.
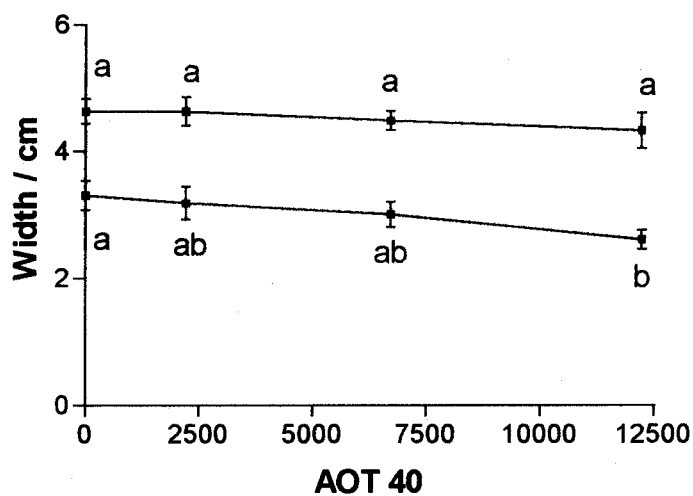
Figure 3 Effects of ozone fumigation on stolon weight (g) single (ie no competition) plants of *E. hirsutum*. Blue = MD, resistant; Red = C, sensitive population.
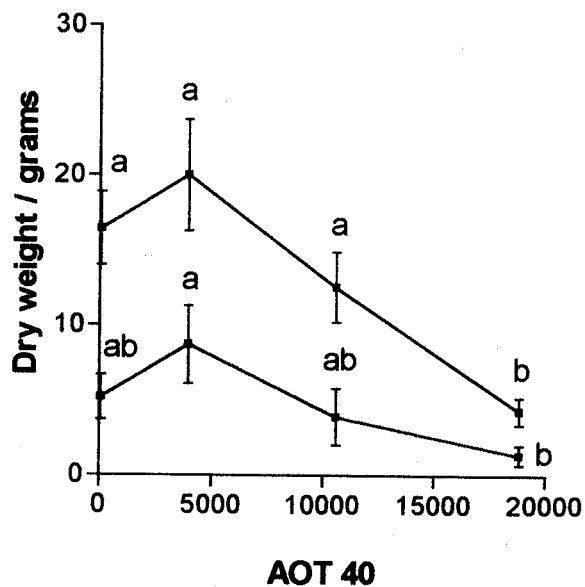
Figure 4 Effects of ozone fumigation on stolon weight of plants of *E. hirsutum* grown in competition with three others of the same species. Blue = MD, resistant; Red = C, sensitive population
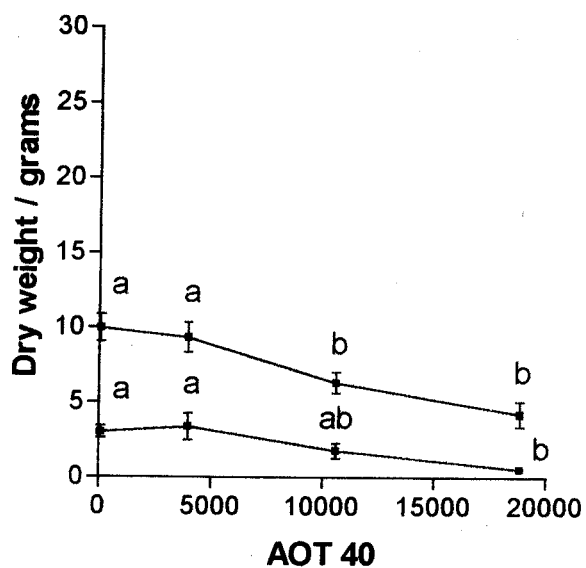
Figure 5 Rate of reduction in volumetric soil water content in 30 cm pots containing seedlings of *E. hirsutum* and subjected to ozone and water stress.Blue squares= NF watered; Green triangles= NF+75 ppb watered; Red squares= NF water stress; Black triangles= NF+75 ppb water stressed.
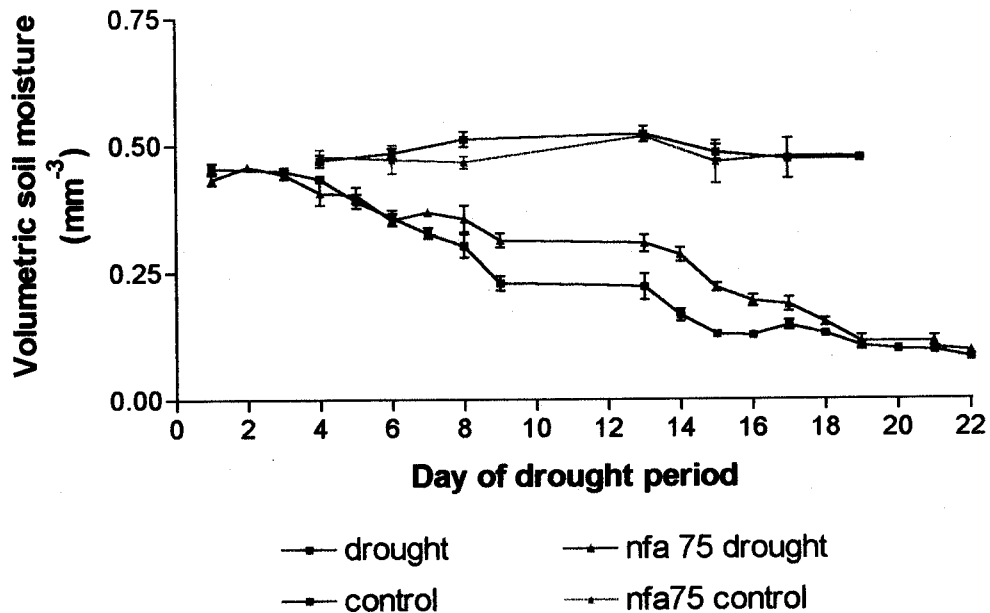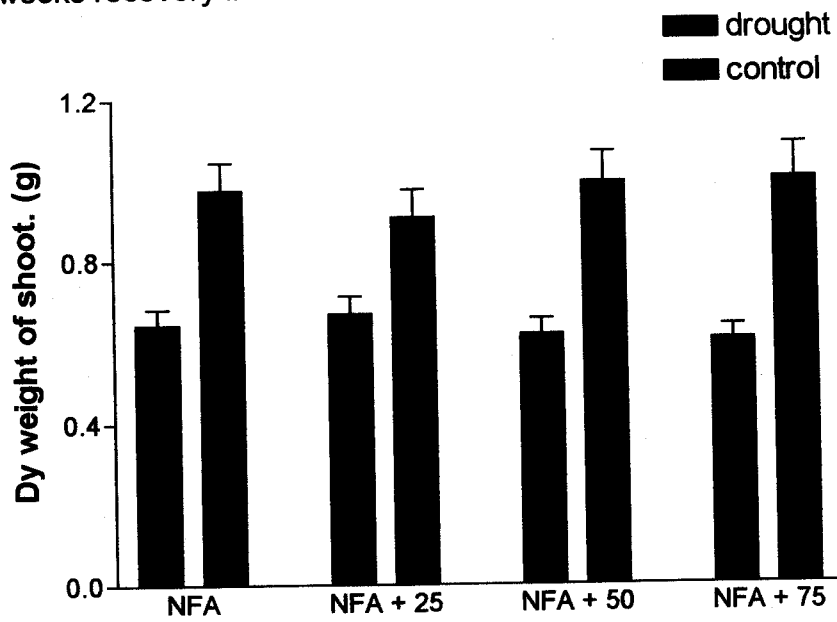

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Figure 6 Rate of change in leaf water potentials seedlings of *E. hirsutum* and subjected to ozone and water stress. Blue squares= NF watered; Green triangles= NF+75 ppb watered; Red squares= NF water stress; Black triangles= NF+75 ppb water stressed.
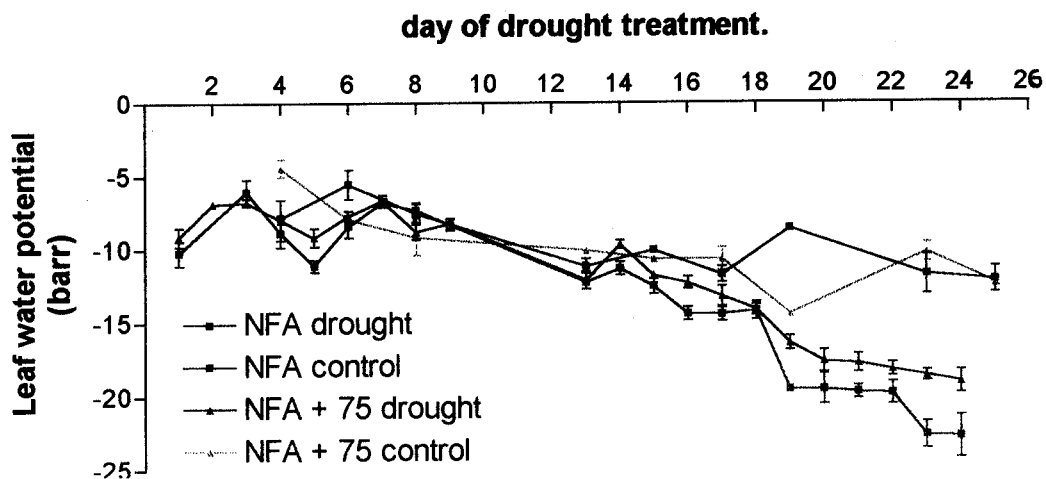
Figure 7 Dry weight (g per plant) of *E. hirsutum* immediately after three weeks with ozone followed by 24 days of ozone and water stress..
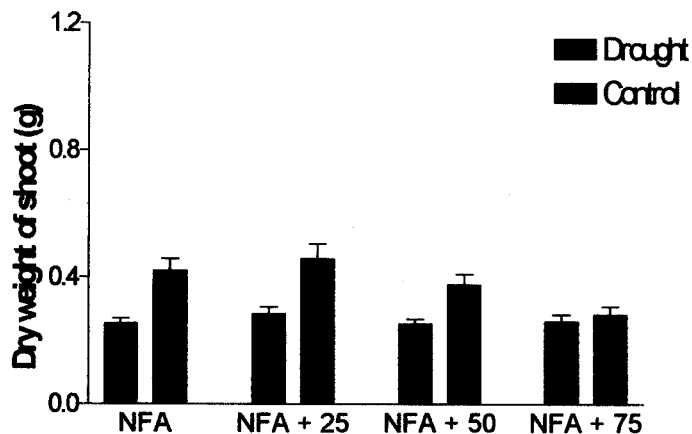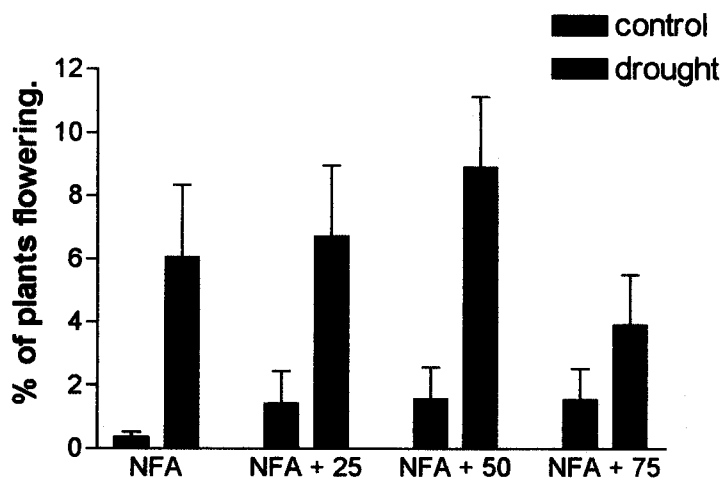

Figure 8 % reduction in shoot weight due to drought treatment observed at end of the ozone/drought treatment
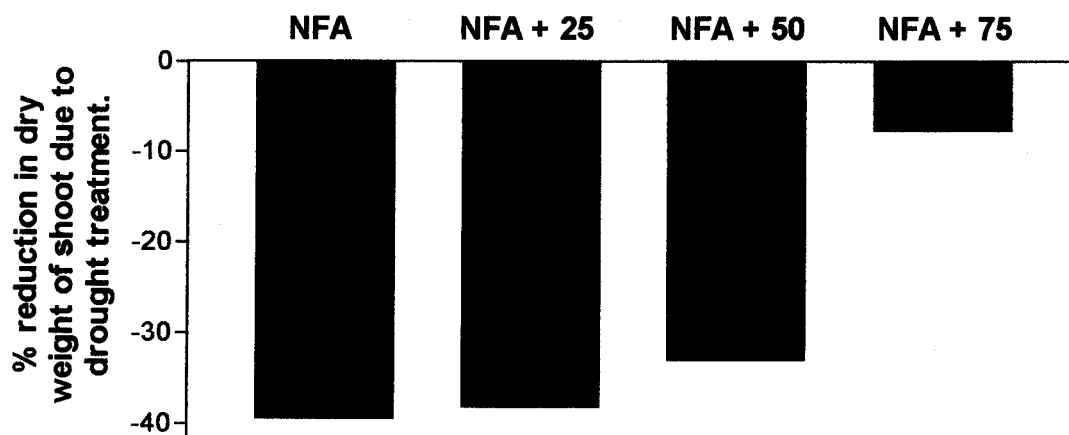
Figure 9 Dry weight (g per plant) of E. hirsutum plants after 3 weeks recovery in ambient air without water stress.


Figure 10. The effects of ozone and water stress on the % of *E. hirsutum* plants flowering in early September 2001.

Figure 11 Rate of reduction in volumetric soil water content in 30 cm pots containing plants four individual plants of *E. hirsutum* and subjected to ozone and water stress. Blue squares= NF watered; Green triangles= NF+75 ppb watered; Red squares= NF water stress; Black triangles= NF+75 ppb water stressed.

— NFA control   — NFA + 75 control
— NFA drought   — NFA + 75 drought
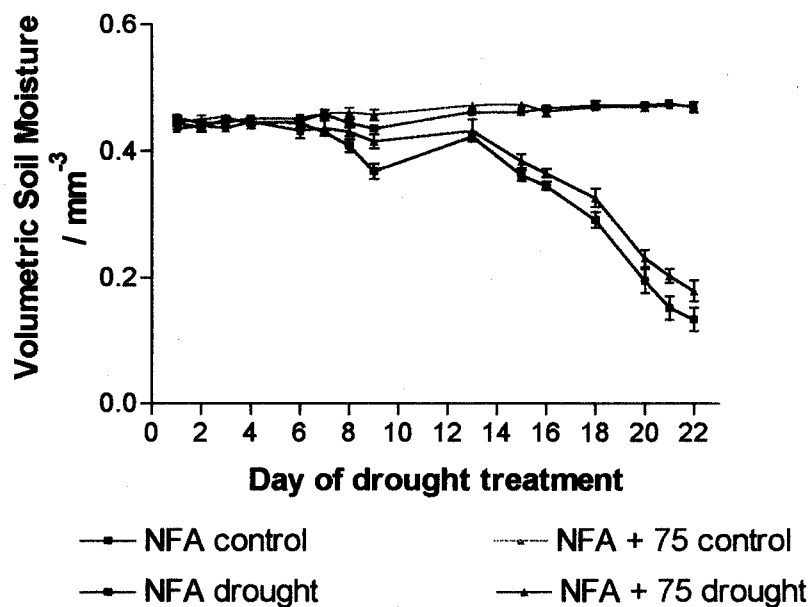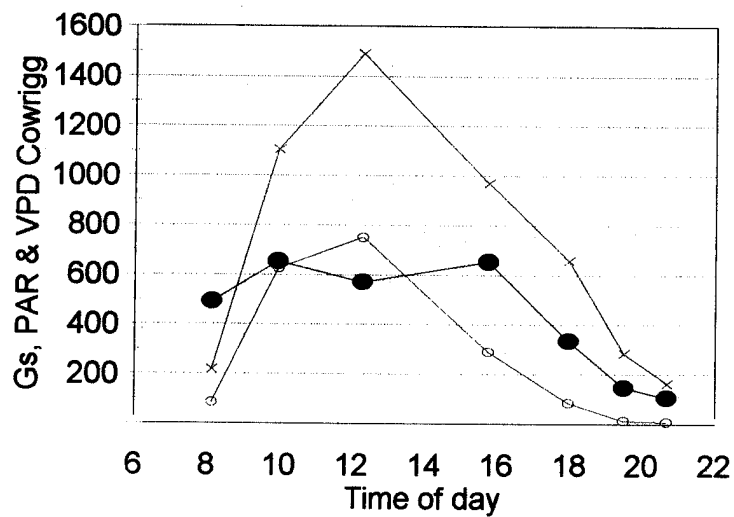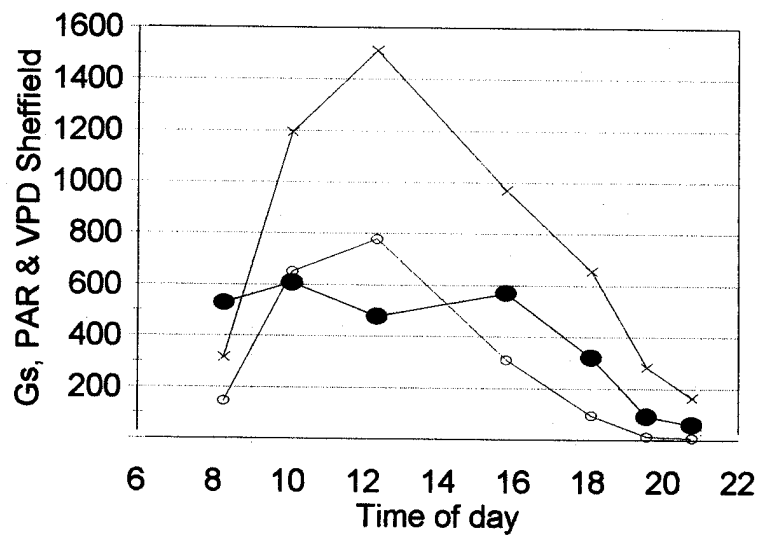
Figure 12. Examples of diurnal stomatal conductance (black, solid circles) in the top stem leaves of two populations of *Epilobium hirsutum*, vpd (red, crosses) and PAR (blue, open circles). The diurnal pattern stays much the same day to day but the maximum varies between about 300 and 600 mmol m^{-2} s^{-1}. It is unaffected by the instantaneous or previous light or vpd.