20th Anniversary

MEETING OF THE

COMMITTEE FOR AIR POLLUTION EFFECTS RESEARCH

[CAPER]

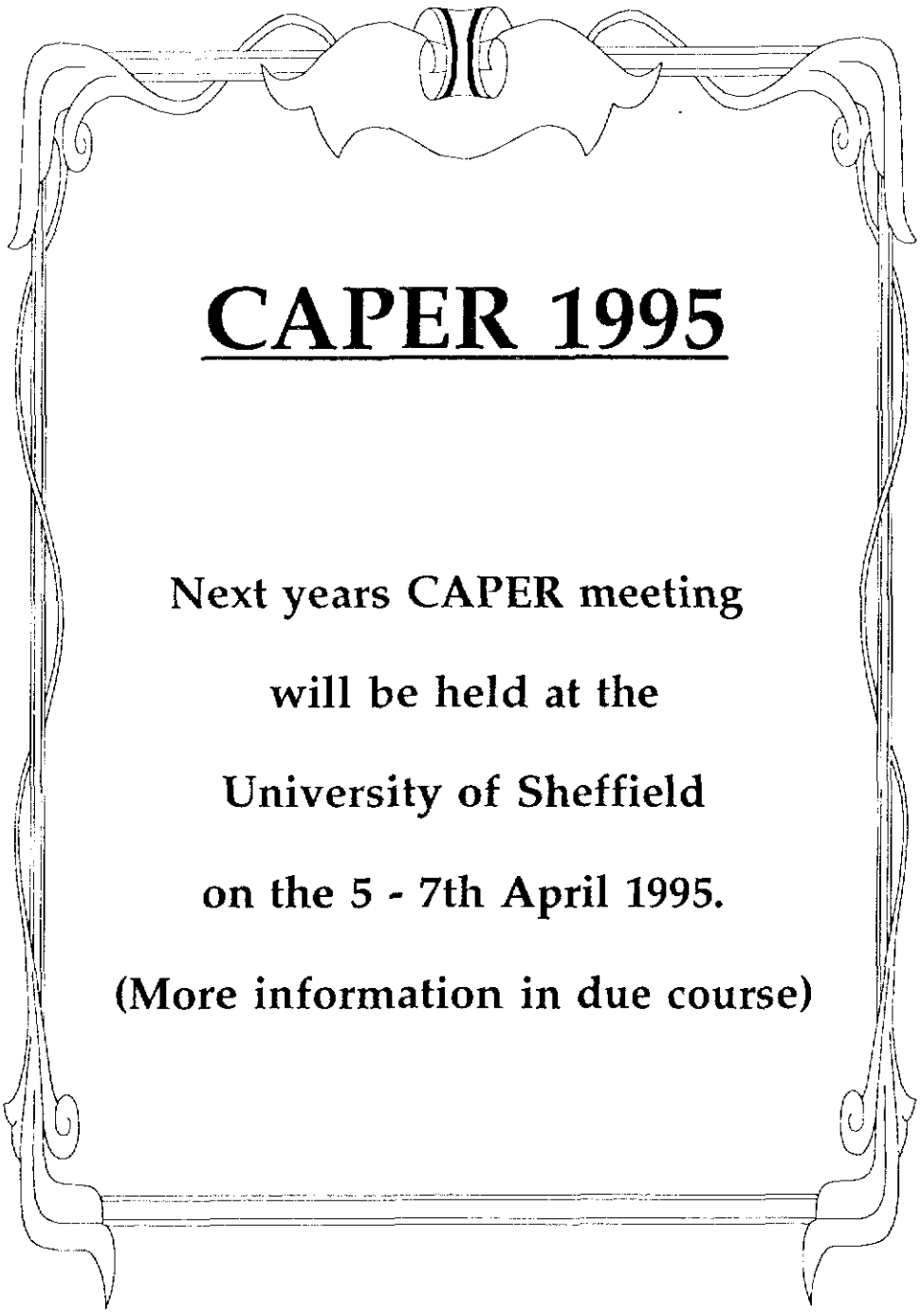
NEWCASTLE UNIVERSITY

20-22 APRIL 1994

ABSTRACTS

OF

PAPERS & POSTERS



CAPER 1995

Next years CAPER meeting

will be held at the

University of Sheffield

on the 5 - 7th April 1995.

(More information in due course)

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A KEYNOTE PRESENTATION

STRATOSPHERIC OZONE DEPLETION AND ENHANCED UV-B: EFFECTS ON VEGETATION

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This overview emphasized that, although there is a continuous column of O_3 from ground level to high up in the stratosphere, the troposphere and stratosphere are best regarded as distinct pools because there is very little up and down exchange and the chemical reactions in the atmosphere for the formation and destruction of O_3 in each are quite different. The nomenclature for describing the different types of UV was outlined (e.g. UV-B, 280-315 nm) emphasizing that very small changes in total radiant flux are brought about by depletion of stratospheric O_3 caused mainly by CFCs and N_2O. How these have brought about 'O_3 holes' at the South Pole depends on the cycle of O_3 destruction being interrupted in the Polar Winter by the freezing out of HNO_3 so that when Spring (and sunlight) returns monatomic chlorine and bromine scavenge the O_3 away. The different types of CFCs, their interim HCFC replacements, and the more desirable HFA substitutes were discussed in relation to the 1989 Montreal Protocol and 1992 Copenhagen amendment with emphasis that, on average, one chlorine atom destroys 40 O_3 molecules and bromine is 40 times more efficient than chlorine in destroying O_3. The final outcome is enhancement of penetrating UV-B to ground-level where for a 7% decrease in total atmospheric O_3 there would be approximately a 15% enhancement of UV-B flux.

The different means of exposing plants to enhanced UV-B were outlined using filters, controlled environment cabinets (CECs), and sun-tracking arrays (STAs) - all of which are in use at Lancaster and in the case of STAs also at cooperating sites at Wellersbourne (HRI) and Harper Hill (NETC). In the case of CECs, the total radiant flux must also be realistic and this involves high levels of lighting (and cooling) which in turn involves high energy costs. Time did not permit to outline in full the seasonal aspects of the STA experiments but the Lancaster initiative is following the Caldwell model which has high relative changes (i.e. increases) in UV-B in late Spring and early Autumn with lesser amounts in mid-Summer. This has several implications for developing and ripening crops. etc.

Results from the CECs indicate that there is considerable varietal response within crop species and that yield reductions are in general not related to visible symptoms. By and large, species with larger leaves are more sensitive than narrow-bladed plants. Certain pea species currently grown in the UK may have significant yield reductions for the predicted changes in UV-B over the next decade or so but cereals are not likely to be affected except for one barley cultivar, Forrester that is naturally very low in flavonoids.

One obvious visible change brought about by UV-B is an increase in leaf reflectivity, significantly so in the UV-B region of the spectrum. Experiments with tobacco have shown that enhanced UV-B reduces the quantity of wax on the adaxial surface of the most sensitive mutant, and results in marked changes in the chemical composition of the wax on the exposed leaf surface. Enhanced UV-B also decreases the quantity of straight-chain alkanes, increases the quantity of branched-chain alkanes and fatty acids, and results in shifts toward shorter straight-chain lengths. Furthermore, UV-B induced changes in wax composition are associated with increased wettability of tobacco leaf surfaces which has implications for rates of fungal infection and the composition of the phyllopane flora. Overall, the data are consistent with the view that UV-B radiation has a direct and fundamental effect on wax biosynthesis. We intend to pursue the full implications of these changes in the future.

1) EFFECT OF EPISODES OF SUMMER O_3 ON Δ^5 AND Δ^{12} FATTY ACID DESATURASES IN AUTUMNAL LIPIDS OF NORWAY SPRUCE (*PICEA ABIES* L. KARST.) DURING WINTER HARDENING.

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[2]Institute of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian, Scotland, U.K.

Current year needles from 5 year-old Norway spruce trees, which had been exposed to either episodes of atmospheric O_3 or periodic mistings with simulated acid rainwater throughout 3 summer periods, were analyzed for changes in molar percentages and ratios of fatty acids isolated from different lipids at the time of maximum winter hardening. No significant changes due to acidic mistings were detected but significant decreases in the degree of unsaturation of both C_{16} and C_{18} fatty acids, the molar percentage of $\Delta^{5,9,12,15}$18:4, and the molar ratio of $\Delta^{5,9}$18:2 to $\Delta^{9,12}$18:2 in monogalactosyl diglyceride (MGDG) due to summer O_3 exposures were found. Molar percentages and ratios of fatty acids did not change much in other lipids but these changes in plastidic MGDG could be traced to a significant effect of summer O_3 on the Δ^5 -and Δ^{12} -desaturases acting upon phosphatidyl chorine (PC) in the endoplasmic reticulum. The replacement of the Δ^6 -subset of C_{18} fatty acids by an equivalent Δ^5 -series throughout was confirmed by gas chromatography and mass spectrometry. Molecular modelling also showed that the Δ^5 -forms,which resembled the Δ^9 -isomers , are very different in shape to the Δ^6 -series and this may account, in part, for the extremely low winter temperatures (<-40°C) from which Norway spruce needles may recover.

2) THE EFFECTS OF ACUTE OZONE EXPOSURE ON THE PHYSIOLOGY OF UPLAND BRYOPHYTES.

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Upland bryophytes are ecologically important, being significant components of upland vegetation. In the uplands the diurnal variation in ozone concentration, seen at lower elevations, is less pronounced and so bryophytes may be exposed to long periods of elevated ozone. Bryophytes differ from higher plants in that they lack stomatal control of gas flux. In addition their leaves are often only one cell thick.

A range of bryophyte species have been exposed to relatively high ozone concentrations, at low temperature, to determine their responses using measurements of photobleaching, photosynthesis and membrane leakiness.

With the fumigation conditions used, the bryophyte species so far studied are surprisingly resistant to damage by acute episodes of ozone, when exposed in a hydrated state. However, there are differential species responses. One *Sphagnum* species - *Sphagnum recurvum* was more sensitive than three other *Sphagnum* species investigated. There was a significant reduction in photosynthesis and a significant increase in membrane leakiness in *Sphagnum recurvum* following a single 6 hour fumigation with 150ppb ozone, at 5°C, and PFD of 500 mol m^{-2} s^{-1}. At a higher ozone concentration (200ppb) there was a significant reduction in photosynthesis and a significant increase in membrane leakiness of *Polytrichum commune*. This was following 4 consecutive days exposure.

A single exposure to a very high ozone concentration (800ppb) did not cause photobleaching. This is unlike the situation with SO^2.

3) DIFFERENTIAL STOMATAL RESPONSE TO O_3 IN EGYPTIAN CULTIVARS OF RADISH AND TURNIP

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The effect of O_3 80 nl l^{-1} on photosynthetic rates (A) and stomatal conductance (g_s) of Egyptian cultivars of radish (*Raphanus sativus* L. cv. Baladey) and turnip (*Brassica rapa* . cv. Sultani) were examined. Ozone caused decreases in A in both species, whilst g_s of radish increased and that of turnip decreased due to O_3. The effect of different concentrations of O_3 (50, 100 & 150 nl l^{-1}) on radish plants was examined in a further experiment and it was found that A decreased, while g_s increased in all concentrations of O_3.

Scanning Electron Microscopy (SEM) of leaf surfaces of fumigated leaves showed that the increase in g_s in radish resulted from the collapse and destruction of epidermal cells. Counts of the percentage of stomata which were fully opened demonstrated that O_3 caused an increase of 53% in the case of radish, and a decrease of 54% in the case of turnip. Moreover, in the second experiment the number of the fully opened stomata of radish was found to increase with the increasing O_3 concentration.

4) THE EFFECTS OF OZONE AND WATER DEFICIT ON TWO BROADLEAVED TREE SPECIES

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Common beech (*Fagus sylvatica*) and wild cherry (*Prunus avium*) saplings were exposed to episodic ozone and soil water deficit in solardomes at Lancaster University. Ozone increased the stomatal resistance of well watered beech. In water stressed plants however, ozone caused a reduction in stomatal resistance relative to the droughted control trees. No effect of ozone on pre-dawn xylem water potential could be detected, though there was a significant reduction due to soil drying.

In cherry, no such stomatal response to ozone was observed. Stomata of cherry closed due to soil drying and reopened when the plants were re-watered, regardless of the air quality treatment. Furthermore, ozone did not affect shoot height, final shoot biomass or basal stem diameter of cherry even after exposure during 2 growing seasons. However, final root biomass was found to be slightly less in ozone exposed plants. Water deficit led to significant reductions in all these variables.

In both species, spring bud-break was found to occur earlier in plants which were subjected to soil water deficit in the previous growing season. However, subsequent shoot growth was slower in these plants than controls. Previous exposure to ozone did not affect the timing or rate of bud-break. In beech trees to which no treatments were applied during 1992, total shoot growth in 1992 was less in previously ozone exposed plants, and was greatly reduced in previously water stressed plants. In well watered beech, this reduction due to ozone was the result of reduced internodal expansion. However, in plants which were unwatered in the 1991 growing season, ozone led to a reduction in the mean number of internodes per branch. It was proposed that declining crown densities observed in mature field-grown beech by other workers could be a result of this reduction in internodes (i.e. a reduction in nodes, and therefore in the number of leaves per branch).

Ozone was shown to promote earlier leaf abscission in cherry plants exposed to both artificial ozone regimes or to ambient air pollution (at Headley in Hampshire). This effect was seen in both systems in both 1992 and 1993.

It was concluded that both species were at risk from water deficit and ozone pollution. Beech and cherry

showed differing stomatal responses to ozone. In beech, ozone could reduce productivity by reducing CO2 uptake, by disrupting the stomatal response to soil drying and by reducing crown density through altered branch morphology. In cherry, ozone could have detrimental effects on tree health and yield by reducing root growth, thereby rendering plants more prone to water deficit, and by stimulating earlier leaf fall. This could result in a shortened growing season and may disrupt translocation of photoassimilates.

5) RESPONSE OF POPULATIONS OF *PLANTAGO MAJOR* TO FIELD FUMIGATION OF OZONE.

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Four populations of *Plantago major* were subjected to 10, five hour episodes of 30ppb above ambient ozone during 1992. Two of the populations had previously been classified as resistant and two as sensitive. Non-destructive measurements were carried out over 1992 and 1993. Resistant populations showed no significant effects over the two years.

Sensitive populations showed enhanced leaf growth but significant reductions in mature scape number, capsules per scape and seeds per capsule, giving up to a 39% reduction of seed number after the first year. The proportion of fumigated plants remaining wintergreen were also reduced. Although no ozone was added during the second year, the later start (not green in spring), decreased winter survival and persistent effects resulted in the fumigated plots producing less seed. Over the two years the fumigated plots produced up to 49% less seed than the control plots. We suggest this is strong evidence highlighting the degree of selection pressure ozone may be exerting on native vegetation.

6) CHANGES IN LEAF WETTABILITY CAUSED BY EXPOSURE TO ACID MIST AND OZONE

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A review of the mechanisms whereby air pollutants can influence leaf wettability concluded that ozone and sulphur dioxide were unlikely to react with leaf surface waxes but could influence wax biosynthesis, but that acidic mist had the potential to influence wax biosynthesis and change chemical composition of surface waxes in situ. Organic pollutants also were likely to change the physical structure of 'crystalline' structural waxes on conifers by promoting the change to an amorphous structure without chemical reaction or 'erosion', but few data were available.

Experiments with several spruce species at ITE Edinburgh over the past 8 years have shown: increased wettability (decreased contact angle) of red spruce needles with increasing acidity of applied mist; decreased wettability of Norway spruce needles in response to HNO_3 and NH_4NO_3 in the absence of SO_4^{2-} ions; no effect of ozone episodes of 140 ppb on the wettability of Norway spruce seedlings; increased wettability of Sitka spruce seedlings and grafted cuttings with artificial acid mist containing H^+, NH_4^+, NO_3^-, SO_4^{2-} ions. These experiments were conducted in open-top chambers from which ambient rainfall was excluded. A 4-year experiment with mature Sitka spruce trees in the field showed increased wettability in response to acid mist, with response proportional to dose. The inclusion of glass particles in the mist enhanced wettability of needle surfaces.

Comparison of reaction rates for ester hydrolysis with field data on cloud frequency and chemical composition at an upland forest site in South Scotland showed that significant chemical reaction of some compo-

nents of spruce surface waxes is possible over a year, and may account for changes in leaf surfaces observes as changes in wettability and in surface structure (using Scanning Electron Microscopy). Changes in leaf surface properties have the potential to affect cuticular transpiration, ion exchange and attack by insects and pathogenic fungi.

7) OZONE : HERBICIDE INTERACTIONS IN U.K. CROPS

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Ozone pollution episodes occur in late spring and early summer coinciding with the time farmers treat their spring sown crops with early post-emergence herbicides. This gives rise to the potential for interactions to occur between ozone and the herbicides. In this study 2-4 commonly grown cultivars of three spring sown crops (barley, oilseed rape and sugar beet) were exposed to 100 nl l^{-1} ozone for 7 h d^{-1} for 2 days followed 3 days later by a single treatment with field rate herbicide.

Ozone and diclofop-methyl (a fatty acid biosynthesis inhibitor) produced a less than additive interactive effect on the visible injury of the spring oilseed rape cultivar 'Galaxy'. 'Starlight', however showed an additive interaction. Results from fresh weight and leaf area data suggested additive to synergistic interactions in 'Galaxy'.

In the sugar beet cultivars 'Saxon' and 'Celt', ozone and phenmedipham (a PSII inhibitor via the D1 protein and ATPase uncoupler) produced a less than additive interaction for fresh weight. Injury deriving from phenmedipham was expressed as circular chlorotic areas 3-4 days after treatment. This was distinctly different from ozone injury, which appeared as millimetre long chlorotic flecks.

The effect of this interaction in 'Saxon' on the leakage of electrolytes from the tissue occurred after 4-8 days, coinciding with the main photosynthetic action of phenmedipham.

8) *APHIS FABAE* AND OZONE - CAN WE DETECT EFFECTS IN THE FIELD?

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Whilst the effects of SO_2 and NO_2 on aphids generally appear to be consistent with most fumigation experiments showing increased insect performance which is supported by field observations and experiments, the situation with O_3 is less clear. A wide range of different responses have been reported from O_3 fumigation experiments depending on the aphid/plant system investigated and on experimental conditions. In addition, supportive field evidence for the effects of O_3 is lacking due mainly to the regional occurrence of O_3 episodes and because the climatic conditions necessary for their formation are also favourable for the build up of aphid populations.

This paper presented results from a current NERC funded project on the effects of O_3 on different stages of the complex, host-alternating life cycle of the black bean aphid, *Aphis fabae*. When the primary host plant, spindle (*Euonymus europaeus*) was grown in OTCs receiving either O_3 (70ppb day / 30ppb night) or filtered air from May until September the MRGR of oviparae (egglaying stage) in October was increased by 40% on the O_3 fumigated trees in 1992 but no effect was found in 1993 (probably due to spider mite infestations in 1993). Subsequent experiments with the spring generation in the following year have shown no effects of fumigation.

The most consistent effects have been found on fat hen, *Chenopodium album*, which is the most important native secondary host in late summer. Fumigation studies conducted in OTCs have shown increases in MRGR of about 30% in 5 separate experiments and the size of the effect is equivalent to that which would be found with an increase in temperature of 4°C. Studies in closed chambers have shown that similar increases in MRGR can occur with this aphid on *Chenopodium* at much lower concentrations (threshold c40ppb O_3) and fumigation times.

The paper concluded with a discussion of how long term data sets on the populations of the aphid on crops and spindle together with O_3 monitoring data and meteorological records are being investigated in order to estimate the likely consequences of O3 on populations of *A. fabae* in the field.

9) INTERACTIVE EFFECTS OF ELEVATED CO_2 AND OZONE

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ELEVATED CO_2: AFRC WHEAT MINI-TOPIC

10) THE EFFECT OF CO2 CONCENTRATIONS AND TEMPERATURE ON TILLER PRODUCTION AND SURVIVAL IN WINTER WHEAT

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Work on winter wheat (cv. Mercia) has shown that the stimulation of grain yield by elevated CO_2 results from increased survival of tillers. We are examining how combinations of normal and elevated CO_2 and ambient and increased temperatures affect carbohydrate accumulation within the plant and whether this is correlated with tiller production and survival.

Experiments done last year compared the effects of normal and elevated CO_2 on plants sown in early November and grown in an open-sided glass roofed structure and transferred, at the beginning of April, to environment chambers with temperature controlled to follow the ambient. Plants were then exposed to 350 or 600 $cm^3\ m^{-3}$ CO_2 until harvest. Tiller number per plant was monitored weekly from the beginning of April through to the end of July. Plants exposed to elevated CO_2 had more tillers per plant than grown in normal, ambient CO_2. For example, plants grown in 600 $cm^3\ m^{-3}$ CO_2 for six weeks had 3.7 tillers per plant whilst those grown for the same time at 350 $cm^3\ m^{-3}$ CO_2 had 2.6 tillers per plant. Carbohydrate contents, in terms of glucose, fructose, sucrose and starch were analysed in each tiller class of three tiller plants. Two samples of tillers were taken, the first when the number of tillers per plant reached its maximum, the second during tiller dieback. In the first sample, carbohydrates in tillers 2 and 3 were most affected by 600 $cm^3\ m^{-3}$ CO_2 with glucose and fructose contents significantly ($p < 0.05$) decreased compared to normal CO_2. However, at the second harvest when tiller dieback was pronounced, the amounts of glucose and fructose were much decreased in plants growing in the lower CO_2 concentration to such an extent that sugar levels were similar for both treatments. We hypothesise that elevated CO_2 during the initial period of tiller growth leads to rapid increase in the number of tillers growing from tiller buds on each plant. However, the plant is not able to maintain this larger number of tillers, carbon assimilates became limiting and carbohydrate content decreases in the younger tillers, resulting in their senescence. However, the number of tillers per plant which survive is slightly greater in plants grown in elevated CO_2 resulting in more ears and grains and bigger yield of grain than in normal CO_2.

This year we are examining tiller formation and its relation to carbohydrate content, incorporating temperature as an additional experimental factor. Carbon dioxide concentrations have been set at 350 or 700 $cm^3 m^{-3}$ to amplify the effects. There are also two temperature regimes combined with the CO_2 concentrations. The ambient temperature tracks the outside air and the other is ambient temperature plus 4°C. An initial harvest made on three tiller plants growing at ambient +4°C (these plants are much more advanced than those grown at ambient temperatures) show that dry mass of both roots and shoots increase significantly ($p<0.01$) in 700 compared to 350 $cm^3 m^{-3}$ CO_2. The average dry mass for roots and shoots was, respectively, 1.1 and 2.5 g in elevated CO_2 and 0.8 and 2.2 g in normal CO_2. The increase in shoot dry mass in elevated CO_2 was evident in all tiller classes, but was most marked for tiller 3. Further harvests and analysis of tiller survival will be made and correlated with the carbohydrate content of the tillers and main stems.

11) EFFECTS OF ELEVATED CO_2 CONCENTRATION AND TEMPERATURE ON APEX AND CANOPY DEVELOPMENT IN WINTER WHEAT

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12) EFFECTS OF ELEVATED CO_2 ON GROWTH AND DEVELOPMENT OF WINTER WHEAT (*VAR.* HEREWARD) AT LOW TEMPERATURES.

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The project tested the hypothesis that contrary to what might be expected from limited published work, elevated CO_2 (700 μmol mol^{-1}) will affect carbon assimilation, growth, resource allocation, floral development and tiller survival of Winter Wheat during the Autumn period of low temperatures. A temperature controlled fumigation facility with 12 small chambers was designed and constructed for *c.* £14 K. Work showed conclusively that elevated CO_2 causes a significant increase in growth of Hereward Wheat at constant temperatures as low as 8°C. This is the first report of an effect of elevated CO_2 at low temperature. Elevated CO_2 increased tillering, mean relative growth rate and leaf area ratio but there was only an effect on specific leaf area at the highest temperature (17°C). The concentration of nitrogen in the leaves was not decreased with elevated CO_2, but the C/N ratio increased with lowering temperature. A dose response experiment indicated that even at a temperature as low as 8°C, there may be a very significant effect of CO_2 at concentrations as low as 450-500 μmol mol^{-1} CO_2. This observation has implications for the uplands and Northern areas in the near future so it needs to be confirmed in the field. Work was undertaken through funding by the AFRC under the Mini-Topic 'Rising CO_2 and The Wheat Crop'.

13) THE EFFECTS OF ELEVATED ATMOSPHERIC CARBON DIOXIDE CONCENTRATION ON THE GROWTH AND WATER USE OF SPRING WHEAT

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The effects of elevated atmospheric CO_2 concentration on the growth, yield and water use efficiency of spring wheat were investigated. This was accomplished by two field experiments in 1992 and 1993 seasons

using open top chambers (OTC) and modelling. Treatments included OTCs with elevated atmospheric CO_2(CO_2 Treatment), OTCs without CO_2 enrichment (Control) and the ambient plots (Ambient). There were two replicates in 1992 and three replicates in 1993. Continuous CO_2 enrichment started soon after plant emergence and lasted till final harvest. The CO_2 in the treatment chambers was manually controlled to aim at its concentration to be 350 ppm above the control chambers. Hourly and daily values of solar radiation, air temperature and humidity were monitored throughout the seasons.

The results showed that CO_2 increased both the grain yield and the total above ground dry matter of spring wheat by 21% and it had no effect on the harvest index. Detailed post-harvest analysis revealed that the increase (of 20%) in the number of grains per spikelet at high CO_2 concentration was responsible for almost all the increase in the grain yield, and that CO_2 did not appear to affect the number of spikelet per ear. The flag leaves grown in high CO_2 were longer, wider but thinner. Although CO_2 increased green leaf area index, it did not affect the water uptake by the wheat plants. This is consistent with the results of root measurement showing that the total try matter of roots in the soil profiles up to 1.2 meters deep was not affected by CO_2 enrichment. The whole canopy water use efficiency was increased by CO_2 enrichment by the same percentage as the increase in the total dry matter production. CO_2 did not influence the development and maturity of spring wheat plants.

14) ACCLIMATION OF PHOTOSYNTHESIS IN WHEAT TO GROWTH IN ELEVATED CO_2 AND VARIED NITROGEN SUPPLY

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Long term growth of plants at elevated concentrations of CO_2 commonly produces a decrease of photosynthetic capacity which partly offsets the gain resulting from decreased photorespiration. Such acclimation may arise if, through increased growth rate at elevated CO_2, the supply of nitrogen becomes limiting. This is because the primary carboxylase, ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco), normally accounts for a large proportion of leaf nitrogen. At elevated CO_2 Rubisco can operate more efficiently and so nitrogen may be redistributed away from this enzyme, leading to photosynthetic acclimation. This hypothesis is being investigated using *Triticum aestivum* (cv.Hereward) grown at 350 & 650 mol mol^{-1} CO_2 with 2 or 0.9 g l^{-1} nitrate supply.

Rates of net photosynthetic CO_2 uptake, measured at light saturation (A_{sat}) and at 350 mol mol^{-1} CO_2, show no significant differences between CO_2 treatments for either 2nd & 3rd or 4th & 6th leaves when plants are grown at the high rate of nitrogen supply. Conversely, at the low rate of nitrogen supply, A_{sat} is reduced in the high CO_2 treatment. This acclimation is such that, even when measured at 650 mol mol^{-1} CO_2, leaves from plants grown at 650 mol mol^{-1} CO_2 do not exceed the rates of leaves grown and measured at 350 mol mol^{-1} CO_2. To investigate the possible mechanism of this acclimation leaf gas exchange was used to calculate carboxylation capacity by examining the response of carbon dioxide assimilation to variation in intercellular CO_2 concentration. Maximum rates of carboxylation did not differ between CO_2 treatments for the plants grown with the high rate of nitrogen supply. However, carboxylation rates were significantly decreased in the high CO_2 grown plants at the 4th & 6th leaf stages when nitrogen was supplied at the lower rate, but no effect was found in the low CO_2 grown plants.

These results therefore concur with the hypothesis that acclimation of photosynthesis to growth in elevated CO_2 is most likely to occur if the supply of nitrogen is limiting and that the rate of carboxylation provides the likely mechanism. Conversely, under light limiting conditions no acclimation is apparent, nor is there any obvious theoretical means by which this could occur.

15) A MECHANISM FOR ACCLIMATION OF PHOTOSYNTHESIS IN HIGH CO_2

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Short-term photosynthetic responses to elevated CO_2 are often greater than long-term responses. Because of the central role played by C_3 plants in the global carbon cycle and global climate, it is essential to understand the mechanisms accounting for acclimation of photosynthesis and respiration to elevated CO_2. This will facilitate the construction of an interactive model taking into account these acclimations, which will enable more accurate predictions of the impact of elevating CO_2 on plants and *vice versa*. Our working hypothesis is that those plants which photosynthetically acclimate when grown in high CO_2 are progressively sink limited.

With cherry trees and tomato plants growth in high CO_2 leads to a loss of Rubisco protein which can account for the loss of photosynthetic capacity. In tomato leaves the abundance of transcripts for nuclear genes coding for the small subunit of Rubisco, as well as for chlorophyll a/b binding proteins and for Rubisco activase, was reduced in high CO_2. In contrast, transcript levels for chloroplast genes coding for the large subunit of Rubisco and for core proteins of photosystem I (A1-A2) and II (D1) were less affected. Abundance of mRNA from the nuclear gene for glycolate oxidase, involved in photorespiration, was not affected, whereas that from the nuclear gene for a subunit of ADP-glucose pyrophosphorylase was transiently increased after exposure to elevated CO_2.

The responses of the nuclear-encoded genes to high CO_2 were enhanced when leaves were detached so as to deprive them of any major sink. Also these responses were mimicked when sucrose or glucose was supplied to leaf tissue. Carbohydrate analyses of leaves grown in high CO_2 or supplied with sucrose revealed large increases in the amount of glucose and fructose.

It is proposed that acclimation to elevated CO_2 is the result of a feedback mechanism in which accumulated hexoses repress the transcriptional activity of nuclear-encoded photosynthetic genes and up-regulate nuclear-encoded genes involved in carbohydrate storage. The effects of pollutants such as O_3, SO_2 and NO_x on photosynthetic acclimation in elevated CO_2 are unknown.

16) CHANGES IN CELL AND CHLOROPLAST DEVELOPMENT IN THE YOUNG WHEAT LEAF (*TRITICUM AESTIVUM* CV. HEREWARD) GROWN IN ELEVATED CO_2.

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To detect early heterogeneites in cellular response to elevated CO_2, anatomical and molecular comparisons of young (7 day old) wheat leaf tissue grown in ambient (350il l^{-1}) and in elevated (650il l^{-1}) CO_2 levels were carried out. Perturbations to cell and chloroplast development or to the assembly of the photosynthetic apparatus at the earliest stages of leaf development caused by elevated CO_2 may be expected to have major knock-on effects on the carboxylation and net-assimilation rates of the mature plant.

The anatomical development of fully expanded seven day old first leaves from plants grown in ambient and elevated CO_2 were compared by image analysis. Elevated CO_2 was found to accelerate the cell and chloroplast expansion rate without disrupting the developmental sequence along the length of the leaf lamina. This developmental enhancement by elevated CO_2 was found to occur in cells at the very earliest stage of leaf growth in the base of the leaf. The enhancement to cell development was detected as precociously -developed chloroplast thylakoid membranes, as an acceleration in the rate of mitochondrial division and enhanced protein accumulation.

Mature chloroplasts grown in elevated CO_2 showed a marked *decrease* in starch accumulation compared to ambient grown chloroplasts. A developmental window in the expansion of the young leaf and consequent changes in source-sink demands may result in this atypical reduction in starch accumulation in tissue grown in elevated CO_2. In contrast, in older wheat leaf tissue (4 weeks) starch deposits are found to be

greater in elevated than in ambient-grown tissue.

In the mature tissue protein accumulation as defined by indirect immunolabelling using fluorescently labelled polyclonal antibodies to thylakoid proteins and to Rubisco showed no significant increase per unit plastid area in elevated CO_2 compared to ambient grown tissue. However, a dramatic effect on the development of the photosynthetic apparatus in elevated-grown tissue was revealed when tissue sections were probed for the 33 kD protein of the extrinsic oxygen evolving complex and for cytochrome *f*. 10-20% of mesophyll cells in a single leaf section contained chloroplasts that showed a extremely weak reaction to these *immuno*-probes (c. 75% less than in chloroplasts in adjacent mesophyll cells). The effect of this heterogeneity on the characteristics of the photosynthetic tissue in the long-term development of the photosynthetic apparatus remains to be determined.

17) THE 'GREENHOUSE EFFECT' ALTERS WHEAT LIPID METABOLISM AND STORAGE

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Previous studies have shown that increased CO_2 can alter plant metabolism. Therefore, we have studied the possible effect of elevated atmospheric CO_2 on lipid metabolism in wheat. Two systems have been used: (a) mature grains, where lipid composition has been determined, and (b) developing leaves, which have helped us evaluate the effects on the quantity and quality of lipid biosynthesis.

Although the lipid content of wheat seeds is low, lipids play an important role in the stability, nutritional quality and baking properties of flours, but there have been no previous studies which analyse the effect of elevated CO_2 levels on lipid metabolism. We have shown that both temperature and CO_2 levels affect directly both the quantity and quality of wheat grain lipids. Such lipid changes are significant for the baking industry because grain lipid composition influences flour quality. Both non-starch and starch lipids were affected by these environmental growth changes. Moreover, we have also observed that, during development of the grain, there are also concomitant changes in lipid composition.

The influence of environmental changes on lipid metabolism in expanding wheat leaves have also been investigated. During chronic temperature experiments with radiolabelled acetate, our initial data suggest that temperature has significant qualitative and quantitative effects. In addition, elevated CO_2 levels have been shown to affect phosphatidylcholine metabolism, in particular. These data are of relevance to studies examining the influence of elevated CO_2 on leaf growth and morphology.

NITROGEN EFFECTS

18) PHOTOSYNTHETIC AND DOSE RESPONSE COMPARISONS OF PLANTS TO ATMOSPHERIC AMMONIA.

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Photosynthetic activities of a wide range of plants were investigated in response to both gaseous and wet applied ammonia. IRGA was used to determine net photosynthesis (NP) for pre-treated material. A leaf disc oxygen electrode was used to determine NP in real time. Ammonia was applied directly to the electrode leaf chamber, so any changes in NP could be recorded as they were happening.

Both wet and dry applied ammonia caused an initial stimulation in NP. Increasing ammonia concentrations and repeat applications of ammonia caused a decline in the stimulation effect, with a subsequent

decline in NP below control levels. Ammonia concentrations between 0.5 - 12 mM NH_4 were used as reported in upland cloud water in the UK.

The stimulation effect was more evident in nitrophilous plants such as alder and poplar which are regarded as being less susceptible to acidifying atmospheric pollutants. In comparison, less nitrophilous plants such as heather and pine were found to be more detrimentally affected by lower concentrations of ammonia, over shorter periods of time.

Experiments with *Phaseolus vulgaris* cv tendergreen showed an increase in stomatal conductance with ammonia application. This effect may suggest a direct or indirect effect on guard cell regulation in the presence of ammonia. Drought sensitivity may also be increased as a function of this.

19) TERRESTRIAL INPUTS OF OXIDIZED AND REDUCED NITROGEN IN THE UK AND THEIR UNCERTAINTIES

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Maps of wet deposited nitrogen as NO3- and NH4+ are currently obtained from a combination of a low density network of precipitation chemistry monitors to define the concentration field and high density precipitation amount collectors from the UK Met Office. The resulting maps are corrected for orographic effects (the seeder-feeder) effect and provide 20km x 20km estimates of input. These values can be improved considerably at sites where direct monitoring data are available OR where detailed modelling of the airflow/wet deposition processes have been studied (e.g. Great Dun Fell or Ben More Assynt). Where the detailed information is not available, and especially in complex terrain, the 20 x 20 km grid square estimate may not be appropriate to define inputs at the 1km x 1km grid square level. In extreme cases inputs at particular sites may differ from the 20km x 20km value by a factor of 2.

For dry deposition of fixed nitrogen as NO2 and NH3, similar problems arise. In the case of NO2, the current estimates of deposition have been calculated using a concentration field for NO2 with a process based model of dry deposition which has been validated with field measurements over grassland. For other vegetation, the rates of deposition in the field are uncertain. For NH3 the problems are rather different, as at present we do not know whether arable cropland is a net source or a net sink for NH3. For semi-natural vegetation the current estimates of NH3 deposition rely on the assumption that the average canopy resistance is 10 s m-1, and this is uncertain on an annual timescale within ± 5 s m-1. Together with the uncertainty in NH3 concentration field this leads to a factor of 2 uncertainties in NH3 dry deposition for many areas.

The sum of wet and dry deposition inputs of nitrogen for the UK provides a good guide to the national inputs and relative importance of oxidized and reduced nitrogen inputs (and export). For site specific information in the absence of local monitoring or any attempt to provide high resolution in total nitrogen, the current knowledge of processes and concentration does not justify spatial resolution at the 1km x 1km or even 5km x 5km scale at this stage.

20) EVIDENCE FOR ATMOSPHERIC N DEPOSITION IN ORGANIC SOILS AND FOR SOME BIOLOGICAL AND CHEMICAL CONSEQUENCES

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Litter horizons of Scottish forest soils have been shown, by re-analysis after a 39-year interval, to have accumulated atmospheric N deposition at rates of up to 50 kg N ha^{-1} yr^{-1}. Some peats in Grampian have

become N-saturated, and nitrate inputs in rain pass through into rivers even in summer months when uptake is high. A simulation experiment with large intact peat microcosms from along a pollution gradient shows that nitrate uptake and organic N leaching are effective proton sinks in peat ecosystems. Ammonium leaching is also important in this context. Over a wide N deposition range, nitrate, ammonium and organic N leaching increase with N input. At very high deposition rates, N accumulates in undecomposed plant remains, and N leaching in all forms falls.

The high N deposition resulted in loss of *Calluna* at three of the 9 sites over 18 months, at one site there was clear evidence in the plant remains in the peat for *Calluna* loss in the field, before the start of the experiment. The *Calluna* loss may be a result of an observed sharp decline in mycorrhizal infection with increasing N deposition.

Evidence for N accumulation also comes from a fall in C:N ratio from 40 to 20 along the pollution gradient, the lowest value being found at the most polluted site.

21) LONG TERM EFFECTS OF AMMONIUM SULPHATE ON A LOWLAND DRY HEATH - AN UPDATE OF RESULTS

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In recent decades, large areas of lowland heath have been disappearing across much of northern Europe. Atmospheric nitrogen deposition has increased substantially over the same period, largely as a result of intensified livestock production and increased vehicle emissions. Research has shown that elevated foliar nitrogen concentrations of heather (*Calluna vulgaris* L.) are associated with an increased sensitivity to biotic and abiotic stresses which may in turn lead to a breakdown of the canopy, allowing invasion by grass species. A critical load for nitrogen of 15-20 kg ha-1 yr-1 has been suggested for the conversion to heathland to grassland.

Since 1989, we have been applying low levels of ammonium sulphate to a heathland site within the Thursley Common NNR in Surrey. The experiment involves four treatments: control (artificial rain only), low (7.7 kg N ha-1 yr-1), high (15.4 kg N ha-1 yr-1) and alternating (either control or high, in alternate years). The background deposition at the site is in the region of 19 kg N ha-1 yr-1, such that total nitrogen depositions are only slightly in excess of the proposed critical loads.

Significant increases in shoot growth, flower production and litter production of heather have been observed in the past four years. Plots receiving the high nitrogen treatment (15.4 kg N ha-1 yr-1) currently produce approximately twice the amount of shoot growth and litter as the control plots, and the magnitude of the growth response has increased in the past two years. The proportion of flowering shoots has shown a similar increase with nitrogen additions, but one which is reversible when nitrogen additions are suspended. There has been an overall trend towards increasing shoot nitrogen content in all plots receiving additional nitrogen, although this was only significant in 1990 and 1992.

To date the effects of low nitrogen additions appear to have been largely beneficial and there is no evidence of a breakdown in the heather canopy. It is hoped that the experiment can be continued for a few more years to see if the plants continue to respond positively to nitrogen additions in the region of the critical load, whether they accumulate high concentrations of nitrogen in the shoots or whether they reach a new, stable, equilibrium.

22) ARE HIGH LEVELS OF NITROGEN DEPOSITION AFFECTING CALCAREOUS GRASSLANDS IN THE UK? EVIDENCE FROM FIELD OBSERVATIONS AND A CONTROLLED SPRAY EXPERIMENT.

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At the 1992 Critical Loads for Nitrogen Workshop, species-rich calcareous grasslands were identified as being vulnerable to increases in atmospheric nitrogen deposition. This was based largely on observations in the Netherlands which show that the rhizomatous grass *Brachypodium pinnatum* has expanded at the expense of other species, resulting in a decrease in floristic diversity. It has been suggested that nitrogen enrichment is driving this change since these semi-natural ecosystems are adapted to low nitrogen (and phosphorus) availability. A survey of chalk grasslands in the UK (Pitcairn et al. 1991) showed some increase in *Brachypodium* during this century, but it was difficult to separate the influences of management (decline in grazing) and N deposition.

Field Observations

The species composition at Parsonage Down, a chalk grassland in Wiltshire was recorded in 1970 as part of a study of Celtic field systems. These quadrats were relocated in 1990 to measure the change in species composition over the 20 year period. Current levels of N deposition here are estimated at 16-20 kb N ha^{-1} yr^{-1} and are likely to have increased since 1970. The site is unique in that it has been managed traditionally by sheep and cattle grazing for the last 53 years - hence any changes in composition can reasonably be attributed to increases in N disposition.

The spray experiment

A controlled spray experiment looked at the effect of applying nitrogen in simulated rain to artificial chalk grasslands for 2 years. The aim was to verify the observations at Parsonage Down and quantify a critical load. The experiment investigated the effect of different forms (ammonium and nitrate) and doses (0-90 kg N ha^{-1} yr^{-1}) of N and grazing on species composition.

Results and Conclusions

Field observations at Parsonage Down showed that there had been no loss of forb species, nor significant reduction in their numbers between 1970 and 1990. These results were supported by observations at other sites near Peterborough. In the spray experiment, even inputs of N as high at 90 kg N ha^{-1} yr^{-1} did not increase the growth or N assimilation of *Brachypodium*, although changes in species composition over longer timescales can not be ruled out. Total module dry weight increased with N dose but the contribution of *Brachypodium* actually decreased during the experiment. Some forbs responded positively to N inputs while others were not affected; there was no evidence that the Ellenberg value was a good indicator of competitive ability under N deposition.

This work shows that floristic diversity in chalk grasslands can be maintained even under fairly high nitrogen deposition if it is managed appropriately. Grazing is likely to be the most effective strategy. Critical loads of 14-25 kg N ha^{-1} yr^{-1} proposed at the Lokeburg conference appear too low, and need to incorporate a modifying factor for management practice.

23) IMPACTS OF POLLUTION ON THE ECOLOGY OF SNOWBED VEGETATION

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Snowbed vegetation in Scotland is very much on the edge of its European range, and is dominated by a distinctive bryophyte flora. Snow is a very efficient scavenger of atmospheric pollutants and, due to its melt dynamics these pollutants are released to the underlying vegetation in a highly concentrated 'flush'. Sensitivity of the bryophytes to pollutants in subnivean conditions and at various stages after exposure from under the snow has been investigated. Results demonstrate that the bryophytes do assimilate pollutants from the snowmelt, even in conditions of very low metabolic activity under the snow, and can be physiologically damaged by them. National and historical surveys of the pollutant contents of snowbed bryophytes demonstrate the possible extent of the pollutant threat to these sensitive communities, and the significant increase in this through this century.

24) EFFECTS OF INCREASED NITROGEN DEPOSITION (NH_4NO_3 AND $NaNO_3$) IN A SPRUCE STAND IN UPLAND WALES

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Work carried out in the last 5 years at ITE Bangor has focused on the impacts of atmospheric nitrogen deposition on spruce stands in the uplands of Wales and the consequences for streamwater quality.

Results from a survey of 25 catchments in the uplands of Wales have indicated that mature stands of spruce are receiving pollutant-derived nitrogen in excess of tree and microbial requirements as a consequence of the intensification of agriculture and industrialisation in recent decades. This is resulting in elevated nitrogen leaching which is accompanied by increased aluminium concentrations in stream and soilwater. A manipulation experiment in N.Wales in which nitrogen has been applied weekly as ammonium and/or nitrate at 35 or 75kgN ha^{-1} yr^{-1} to a mature spruce stand has confirmed that these systems are indeed nitrate saturated with increased nitrate inputs resulting in equivalent elevated nitrate leaching losses. However after 2.5 years of ammonium application there has been no increase in nitrogen leaching probably due to ammonium fixation in clay lattices and the absence of any stimulation of the soil nitrification rate. Changes in net nitrogen mineralisation and nitrification and litter decomposition have been slower than predicted from large, single-dose fertiliser experiments with no response observed after 2.5 years of application in all treatments. ^{15}N is being applied to enable the fate of nitrate and ammonium to be followed more closely with the results being used to test a new ^{13}C and ^{15}N forest growth model developed by the University of Wageningen, NIICE (van Dam, In Press).

The first indications that the trees may be responding to the water and nitrogen applications have been recorded. Litterfall increased with nitrate inputs in the third winter. The response by the roots (<5mm) is more complex as there is a combination of an increase in root biomass with water applications and a decrease as nitrate concentrations increase. Various other measurements have also indicated that water availability is an important limitation on tree growth at this site. Work is now focused on the changes in canopy structure and dynamics, the long term fate of ammonium and the impacts of nitrogen additions on N_2O, CH_4 and CO_2 fluxes from the soil. Similar nitrogen addition or exclusion experiments are being conducted across a pollution gradient as part of the EC-funded NITREX consortium.

25) NO3 LOADING IN FRESH WATERS; THE USE OF STABLE ISOTOPES OF N TO ASSESS THE IMPACT ON LITTORELLA UNIFLORA

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POSTERS

OZONE

26) THE IMPACT OF OZONE ON THE REPRODUCTIVE BIOLOGY OF PLANTS

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This work is investigating the direct impact of ozone on the reproductive biology of two plant species with contrasting reproductive habits. The species being used are *Brassica campestris*, an indeterminate species and *Plantago major*, a more determinate species. If ozone has a direct effect on the reproductive structures of these plants, the ability of individual plants to compensate for such damage may differ between habits, with the determinate species being more adversely effected. For this study, two exposure chambers have been specifically designed and constructed such that each permits the reproductive structures of up to twelve plants to be exposed simultaneously to ozone. One chamber acts as a control and the other allows the introduction of ozone. Currently investigations are under way to determine if the reproductive structures of both species are sensitive to concentrations of ozone. Future work will include investigations of the site of ozone action, the ability of each species to compensate for damage and the inheritance of sensitivity to ozone over a number of generations.

This project is funded by NERC.

27) AN *IN VIVO* ANALYSIS OF OZONE INHIBITION OF PHOTOSYNTHESIS FOLLOWING SHORT- AND LONG-TERM EXPOSURE IN THREE CONTRASTING SPECIES

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The effects on leaf gas exchange of short-term O_3 fumigation (4-16 h, 200 & 400 nmol mol^{-1}) on mature leaves of *Triticum aestivum*, *Pisum sativum* and *Quercus robur* and on leaves that had developed in O_3 during long-term fumigation (80 nmol mol^{-1} 7 h d^{-1} for 3 weeks on *T. aestivum & P. sativum* and for 5 months on *Q.robur*) were examined. On all occasions when the light saturated rate of CO_2 uptake was reduced, this could be accounted for by a decreased carboxylation capacity of the enzyme ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco). Photosynthetic parameters associated with thylakoid integrity (regeneration of ribulose bisphosphate, quantum yield and F_v/F_m) were unaffected, except after the most severe treatment, by which time substantial damage was apparent. These results are supported by biochemical analyses of *T. aestivum* which shown no selective loss of thylakoid membrane proteins, but a preferential loss of the small and large subunits of Rubisco. Furthermore, the gas exchange analyses indicated that the accompanying decreases in stomatal conductance were not responsible for inhibiting CO_2 uptake but were in fact a secondary effect brought about in response to the decreased rates of photosynthesis. In conclusion, the primary cause of inhibition of photosynthesis *in vivo* following both short- and long-term exposure to O_3 in all three species investigated is a decline in the maximum rate of carboxylation.

28) EFFECTS OF AIR POLLUTANTS ON THE SEARCHING BEHAVIOUR OF INSECT PARASITOIDS.

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To assess the impact of air pollutants on the population dynamics of herbivores, the effects of pollutants on their natural enemies including predators, parasites, and pathogens must also be evaluated. This study looks at the effects of air pollutants (ozone (O_3), sulphur dioxide (SO_2), and nitrogen dioxide (NO_2)) on the searching behaviour of insect parasitoids.

A set of 2 trophic level experiments comprised closed chamber fumigations of O_3, SO_2, and NO_2 with aggregated distributions of host larvae (*Drosophila subobscura*) and its braconid parasitoid (*Asobara tabida*).

Analysis of chamber results showed that the proportion of hosts parasitised and the searching efficiency of the parasitoids were both significantly reduced with O_3 fumigation, but not with NO_2 or SO_2 fumigations. O_3 fumigation reduced % parasitism by approximately 10%.

When exposed to pollutants, the parasitoids appear equally able to avoid patches with no hosts. However the proportions of total patch times associated with patches of different host densities, in the O_3 and NO_2 treatments, suggests a reduced ability to discriminate between different host densities. There was no significant increase in the levels of superparasitism.

These results suggest the potential for air pollutants, particularly ozone, to negatively influence the searching behaviour of parasitoids. Due to the widespread occurrence of ozone in rural, and hence agricultural, areas such effects could prove a very significant factor in the efficiency of natural enemy control of many pest species.

29) THE INFLUENCE OF MICROCLIMATE ON CROP SENSITIVITY TO OZONE: A NEURAL NETWORKS APPROACH.

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Clover (*Trifolium subteranum*) plants were exposed to a range of ozone concentrations in a closed chamber facility. During exposure, light, temperature and relative humidity were measured. Microclimate had a strong influence on the amount of foliar injury and the critical level required for injury development.

The data set from the fumigations was modelled using neural networks, the principles of which were outlined. Results from the testing of the neural network model showed accurate prediction and that neural networks were a useful tool in clarifying the interactions between microclimate and ozone injury.

30) THE IMPACT OF OZONE ON THE WATER RELATIONS OF 2-YEAR OLD ASH TREES

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Two year old potted saplings of ash (Fraxinus excelsior) were intermittently treated with three different air qualities (150 ppb ozone, ambient air, charcoal filtered air). The plants were subjected to water stress in cycles of two to three weeks. Stomatal conductivity, extension growth and stem diameter increment at the base were assessed regularly. After the harvest in
September 1993, sections from the stem base were used to determine ringwidth, cell number per ringwidth and cell size of the latest annual ring.

On days after fumigation treatments the polluted plants showed decreased stomatal conductivity. Water stress intensified this effect. After a two week period, where seven fumigations had been imposed, the ozone treated plants showed increased extension growth, which coincided with reduced radial growth at the stem base. Thus indicating an altered pattern of biomass allocation. The analysis of the annual ring proved the decreased ringwidth of the fumigated plants, which was due to lower cell numbers per annual ring.

These results suggest, that water absorption from the soil and transport and storage of the water may be less efficient for polluted plants and dry summers may affect them more severely than unpolluted plants.

31) THE EFFECT OF OZONE FUMIGATION OVER-WINTER ON THE GROWTH AND PHYSIOLOGY OF *CALLUNA VULGARIS*.

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Ozone is a major phytotoxic air pollutant, which has been shown to reduce crop yields, and to damage other vegetation. Unlike many other pollutants, levels are continuing to rise. During ozone episodes upland sites can be exposed to high concentrations over prolonged periods. The diurnal variation of the ozone concentration at higher altitudes is less variable than that for lowland sites. Upland vegetation including *Calluna vulgaris*, may therefore be vulnerable to prolonged exposure to ozone. There is also evidence that elevated concentrations of ozone occur outside the growing season, and so many overwintering evergreens/perennials may be subjected to the combined stresses of ozone pollution, freezing and winter desiccation. It is important therefore, to assess if exposure to ozone during winter affects the plant's growth and survival.

Calluna vulgaris cuttings were raised from a stock plant taken from N.Wales, and grown in a typical heathland peat soil. The plants were fumigated in open-top chambers at Bangor I.T.E , with ambient charcoal filtered air, or 70ppb ozone, for 8 hours per day, 5 days a week. Fumigation started on 01:11:93 and the plants received a dose above 40ppb of 14,640 ppb hrs at the first harvest; and a dose above 40ppb of 18,480 at the 2nd harvest.

Exposures to ozone of 70ppb during winter increased the rate of electrolyte leakage from the plant's cells following controlled frosting; therefore the C. vulgaris was less frost resistant. The root dry weight was decreased, whilst the shoot dry weight was unaffected by the ozone exposure. The allometric root/shoot ratio indicated that the ozone had decreased the partitioning of dry weight to the roots. The chlorophyll content tended to decrease; whilst anthocyanin pigmentation increased for plants that had been fumigated with the ozone. The decrease of the chlorophyll content might have some serious implications for photosynthesis during the warmer spring weather. Further work is required to clarify the effects of ozone and low temperature upon net photosynthesis; this may relate to the alterations in frost tolerance.

32) DOES BENOMYL PROTECT PLANTS AGAINST OZONE?

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NITROGEN

33) AN ASSESSMENT OF THE NEED TO INCORPORATE LEACHED ORGANIC N AND S AND NITRIFICATION INTO CRITICAL LOAD VALUES FOR PEAT SOILS

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To quantify critical loads for organic soils it is necessary to estimate the proportions of the deposited N and S species involved in acidification, and leached straight out of the plant/soil system. In an 18-month experiment, peat turfs from 9 sites along a pollution gradient were subjected twice weekly to simulated acid rain similar in composition to that received in the field. Leachate volume was determined weekly. Bulked leachate samples were analysed for inorganic and total organic N and S species.

When nitrate inputs are low to moderate, nitrate is largely retained in the soil/plant system. At higher

nitrate inputs, uptake becomes relatively less important, and more of the nitrate input is leached. Except for the two most polluted sites, ammonium leached exceeded ammonium deposition.

To a first approximation, leached organic N increases linearly with N input. Soluble organic S showed a similar trend. At higher N inputs, there was a different trend, with organic N accumulation.

The N flux results can be used to model N outputs from total N inputs, and these results should be incorporated into critical loads calculations.

Organic S and TOC in the leachate were highly correlated. The best way of dealing with organic S leaching in critical load calculations is still under consideration.

34) CRITICAL LOADS OF NITROGEN FOR HEATHER MOORLAND

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CO_2 / CLIMATE CHANGE

35) HERBICIDE RESISTANCE IN AN ELEVATED CO_2 ENVIRONMENT

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Few experiments have investigated the herbicide-response of weeds in a future elevated atmospheric CO_2 environment ($[CO_2]_a$). Despite this there is potential for herbicide / $[CO_2]_a$ interactions to occur because photosynthesis is a primary site of action of many herbicides. In addition the growth and development of herbicide-resistant weeds may be altered in elevated $[CO_2]_a$. Resistance to an increasing number of chemicals has been found in many weed populations due to repeated use of the same herbicides.
Increased herbicide dose reduced total dry weight and leaf area of susceptible biotypes of blackgrass (*Alopecurus myosuroides*) and fat hen (*Chenopodium album*) but had no effect on resistant biotypes.
Future work will examine the effect of elevated $[CO_2]_a$ on the growth and development of susceptible and resistant biotypes of fat hen and blackgrass and quantify any interaction between herbicides and elevated $[CO_2]_a$.

36) INFLUENCE OF ELEVATED CO_2 AND NUTRIENT SUPPLY ON GROWTH AND NUTRIENT UPTAKE OF *AGROSTIS CAPILLARIS*.

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A prominent upland grass species *Agrostis capillaris* was grown for 6 months at 3 levels of N, P and K and at 2 concentrations of CO_2 (ambient air and ambient air plus 250 ppm CO_2), in 'Solardome' growth chambers. The effects of elevated CO_2 on plant growth, morphology, nutrient requirements, uptake rates and tissue concentrations were investigated in relation to the plants nutritional status. A root bioassay technique was employed to look closely at the nutrient status of the plant. The results from this method correlated well with the more conventional foliar analysis results.
A. capillaris showed a positive growth response to elevated CO_2, even at low nutrient supplies. Plant shoot weight was enhanced due to increases in both plant leaf number and tiller number, and an overall increase

in average leaf number per tiller. Conversely root growth showed no significant trends with CO_2 concentration. There were no interactions between the growth response to elevated CO_2 and nutrient supply, even at the lowest N and P supplies, which were limiting to plant growth.
An imbalance between C-fixation and nutrient acquisition was found, with uptake of N, P and K not increasing in line with plant growth at elevated CO^2, even at above adequate nutrient supplies. *A. capillaris* still maintained shoot concentrations of N and P similar to those in ambient air, probably due to internal redistribution of these elements, both of which are important to the maintenance of photosynthetic rates, while in contrast shoot %K declined. The bioassay results indicated no change in the plant's requirements for N or P but a large increase in the plant's demands for K, which was not simply due to increased plant size and was postulated to be related to osmoregulatory changes within the plant.
The multitude of nutrient interactions between N, P and K evident throughout the study, highlighted the importance of not only looking at absolute concentrations of elements but also at the balance between them in nutritional studies.

37) EFFECTS OF CLIMATE CHANGES ON GROWTH AND STOMATAL BEHAVIOUR OF *BELLIS PERENNIS* L.

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Epidermal strips of *Bellis perennis* L. were incubated in the light, in 10 cm^3 of 10 mol m^{-3} MES + 50 mol m^{-3} KCl, adjusted to pH 6.15. Strips were exposed to CO_2 concentrations of 0, 325 or 770 ppm and ABA concentrations from 0-10^{-1} mol m^{-3} at eight temperatures from 5-40°C.
In the absence of ABA and CO_2, increasing temperatures stimulated stomatal opening. At low temperatures (10°C), ABA stimulated stomatal opening; as temperature increased, inhibition of opening due to ABA was restored, with stomatal sensitivity increasing with increasing temperature. Stomatal closure in response to CO_2 was temperature dependent, with increasing temperatures promoting greater stomatal sensitivity to CO_2. A three-way interaction occurred between temperature, CO_2 and ABA, showing that the greatest sensitivity to CO_2 occurred between 20-25°C, with no stomatal closure due to CO_2 enhancement at 5-10°C and 35-40°C. The higher the ABA concentration, the lower was the optimal temperature for stomatal closure due to CO_2.
The complex responses of stomata to temperature, CO_2 and ABA suggest that all environmental variables must be considered before making any assessment of plant growth responses to elevated atmospheric CO_2.

38) DOSE-RESPONSE RELATIONSHIPS OF CO_2 EFFECTS ON GROWTH AND PHYSIOLOGY OF WHITE CLOVER (*TRIFOLIUM REPENS* L.)

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The objectives of the present study were to look for the dose-response relationship of the CO_2 effects on the growth of white clover and on the water consumption of the canopy. Additionally, the long-term effects of CO_2 enrichment on chlorophyll and protein concentration and thus forage quality were determined.
White clover plants (*Trifolium repens* cv. Karina) were sown in pots containing 10 l of a natural unfertilized soil and grown under different CO_2 levels (377, 463, 543, 620 ppm) in open-top chambers during the vegetation period of 1993. Fertilizer was added at the beginning (N, P, K, Mg) and after each harvest (P, K, Mg). Plants were clipped to a height of 5 cm in about monthly interval and the cuttings were used for analysis (biomass, leaf number, chlorophyll content, nitrogen content). The daily water consumption of the pots was measured gravimetrically just before sampling.

The CO_2 enrichment resulted in an increase in the biomass at all harvests. This was due to a stimulation in the height growth rate and in the leaf appearance rate. There was a saturation curve between the CO_2 concentration and the forage biomass. In the lower CO_2 concentration range (380-540 ppm) forage biomass increased 0.2-0.3% per ppm CO_2. Chlorophyll content per leaf area and water consumption of the canopy was hardly affected by the CO_2 treatment. Thus, water use efficiency was increased. During the early phase of clover development, when the plants depended on the soil nitrogen, protein concentration of the forage decreased under high CO_2. However, when the root nodules had evolved, this phenomenon disappeared.

39) USE OF STABLE ISOTOPES ($S_{13}O$, $S_{13}C$) TO INTERPRET WATER AND CARBON DIOXIDE FLUXES FROM VEGETATION

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40) THE EFFECT OF HIGH CO_2 ON THE BIOPHYSICAL CO_2 CONCENTRATION MECHANISM OF TWO GROUPS OF LICHENS

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41) EFFECTS OF CLIMATE CHANGE ON THE BOUNDARY BETWEEN BRACKEN AND HEATHER.

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Species are at their natural limit of survival where boundaries between plant communities are controlled directly by climatic, rather than anthropogenic factors. Changes in climate here would be expected to result in differential responses in abundance of the interacting species and an eventual shift in the boundary. A field site was established at Upper Teesdale to investigate this, with small plots on and around the boundary between heather (*Calluna vulgaris* (L.) Hull) and bracken (*Pteridium aquilinum* (L.) Kuhn.). Polythene tents were erected to artificially increase the air temperature on half of the plots. A second treatment consisted of additional nitrogen inputs to the plots, to simulate both increased atmospheric deposition rates, and increased decomposition rates expected under higher temperatures.

Despite the fact that this project has only been run for one growing season, some interesting results have already been shown. Measurements of temperatures in and outside the tents in September 1993 showed a good correlation between the solar radiation and temperature increases, and increases of air temperature of up to about 5 {SYMBOL 176 \f "Symbol"}C can be expected on a clear summer day, which represents a realistic increase according to current predictions. The data also showed consistent reductions in air temperature at night, which is probably caused by a reduced mixing of air layers inside the tents. Bracken responded quite strongly to the addition of the tents, and it is assumed that the increase in temperature is the main cause. Heather density did not respond, but this may be attributed to the fact that the treatments only started after the beginning of the growing season. A considerable proportion of the bracken fronds emerged later during the growing season. As far as height increment growth is concerned, bracken responded very favourably to increased temperatures, but no results are available yet on the

response of the heather extension growth. It seems likely, however, that bracken will increase its dominance over heather in the boundary plots, considering the difference in stature, and will also invade areas now completely dominated by heather, something which has already started. The nutrient treatment has not shown any definite results yet, but this was not really expected during the first growing season anyway.

42) A FIELD EXPERIMENT TO ASSESS RESPONSE OF HYBRID POPLARS TO AMBIENT OZONE BY MEANS OF EDU STEM INJECTION.

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Cuttings of an ozone-sensitive hybrid poplar clone (*Populus deltoides x maximowiczii* 'Eridano') were grown from spring 1991 to autumn 1992 at a site in the Po plain of northern Italy. Plants were injected repeatedly in both summers with water or a solution of ethylenediurea (EDU). Treatment with EDU reduced ozone-induced visible injury, especially in the second summer when it was almost eliminated. Growth and final biomass (aboveground) were not affected by EDU treatment. Injection with EDU is straightforward and may provide a means to study large trees which are otherwise difficult to deal with but the results should be interpreted cautiously.