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CAPER 99

6-8th April
York

WEDNESDAY

CHAIR – NIGEL BELL

09.00	J Carroll, Manchester Metropolitan *Interactions between air pollutants and the leaf surfaces of roadside and urban vegetation.*
09.20	J. Binnie, ITE Edinburgh *The urban atmosphere and its impact on vegetation.*
09.40	G Taylor et al, Southampton University *The capture of $PM_{2.5}$ and PM_{10} by different tree species.*
10.00	Jim Nicholson, Edinburgh University *Modelling horizontal and vertical concentration profiles of ozone and nitrogen oxides within and downwind of high latitude urban areas.*
10.45	Coffee

CHAIR DAVID FOWLER

11.00	M. Ashmore, Bradford University *Applying the Critical Load Approach to Metal Deposition: does it make sense?*
11.20	J. Cotter-Howells, Aberdeen University *Changes in heavy metal speciation during acidification – implications for ecotoxicity.*
11.40	B. Alloway, Reading University *Atmospheric Deposition of Heavy Metals in rural areas of England and Wales.*
12.00	W. Nicholson et al, Newcastle University *Heavy metal and fluoride contamination of alluvial meadows and pastures in South Northumberland.*
12.30	Lunch

CHAIR – MALCOLM CRESSER

14.00	L. Cawley, Manchester Metropolitan University *Effects of elevated Nitrogen and imposed drought on the ecology of a lowland heath.*
14.20	M. Pilkington, Manchester Metropolitan University *Diagnostic indicators of nitrogen critical load exceedance on moorlands.*
14.40	C. Barker, Imperial College *Interactions between N deposition and heathland management.*
15.00	L. Jones et al, ITE Bangor *Grazing/atmospheric N interactions in upland acid grassland..*
15.20	E. Hewins, Bristol *A Greenhouse Experiment investigating the effects of management and atmospheric ammonia deposition on Cotswold grasslands.*
15.45	Coffee/tea

CHAIR – LUCY SHEPPARD

16.00	J. Heath, Lancaster University

Tree stomatal responses to atmospheric CO_2 enrichment and interactions with vapour pressure deficit.

16.20 T. Lyons, Newcastle University
Modelling O_3 detoxification by ascorbate in the leaf apoplast.

16.40 J. Maddison, Newcastle University
Examining the role of ascorbate mediating ozone resistance.

17.00 M. Coyle, ITE Edinburgh
High Resolution Maps of O_3 concentration and AOT40 for the UK.

17.20 N. Jarraud, Imperial College
Investigating the distribution of Tarspot of Sycamore along a transect based on gradients of O3 concentrations.

17.40 General discussion

THURSDAY

CHAIR – JEREMY BARNES

09.00 G. Mills, ITE Bangor
UNECE International Co-operative Programme on the effects of O_3 on vegetation – a brief overview.

09.20 P. Drogoudi, Imperial College
Effects of elevated O_3 and fruiting level of yield parameters, growth and photosynthesis in strawberry.

09.40 N.M. Abdel-Latif, Imperial College
Effects of O_3 on the growth and regrowth of an Egyptian variety of Jews Mallow (Corchus olitorus).

10.00 E. Clamp, Newcastle University
Effect of O_3 on lines of Brassica rapa which have evolved through natural selection.

10.45 Coffee

CHAIR – MIKE ASHMORE

11.00 C. Gillespie, Newcastle University
Potential effects of O_3 on commercially grown glasshouse crops.

11.20 C. Umponstira, Newcastle University
Ozone and water stress interaction.

11.40 S. Power, Imperial College
Responses of wetland communities to enhanced O_3 exposure.

12.00 News Items?

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PAPER ABSTRACTS

INTERACTIONS BETWEEN AIR POLLUTANTS AND THE LEAF SURFACES OF ROADSIDE AND URBAN VEGETATION

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Vegetation provides a number of acknowledged benefits in the urban environment. Trees and shrubs in particular can act as effective sinks for pollution, with the complex canopy surfaces forming effective traps for both gaseous and particulate pollutants. Urban and roadside vegetation screens may therefore represent an effective means of local pollution control. The effects of urban pollutant mixtures on roadside vegetation in general, and on the structural integrity and function of leaf surfaces in particular, are not well understood. Deposition of acidic pollutants is associated with erosion of the leaf cuticle in a number of species, and the deposition of fine particles has been shown to impair stomatal functioning. Both these effects would tend to increase overall pollutant uptake, and could in addition lead to an increased susceptibility to other environmental stresses. The aim of the investigations presented here has therefore been to obtain a clearer understanding both of the "stress profile" to which roadside plants are exposed, and the impact of roadside plantings on the spread of pollutants away from "point" sources such as motorways. Transects have been established across two conifer belts adjacent to the M6, and an area of deciduous trees close to the M60, with the intention of monitoring the spread of both particulate and gaseous pollutants away from the motorway and studying the interaction of these pollutants with leaf surfaces. Experimental material has also been established close to existing monitoring-stations at clean, suburban and polluted sites.

Results will be presented on the NO_2 dispersion profile across the M6 and M60 transects and the possible effects of pollutant gases and particulate loading on cuticular erosion, and needle transpiration rates.

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URBAN ATMOSPHERE AND ITS IMPACT ON VEGETATION

J.Binnie [1], P.C. Moonen [2], F.J.Hayes [2], J.N. Cape [1] and T.W. Ashenden [2].

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Urban environments often contain high levels of atmospheric pollutants such as NO, NO_2, CO, Volatile Organic Compounds (VOCs), as well as particulates. This is a result of the increasing volume of traffic on UK roads. The effect of these pollution mixtures on vegetation is potentially serious, not only through direct toxicity, but also indirectly as a possible increased susceptibility to other biotic and/or abiotic stress.
A short-term feasibility study was conducted to develop a realistic plant exposure system which would simulate urban kerb-side pollution concentrations and make preliminary assessments of the impact of exposure to this system on two plant species.
The system utilised by the study consisted of a diesel generator linked to two solardome glasshouses[1]. The atmospheric conditions created in the solardomes reflected the concentrations of pollutant gases at the kerbside in inner cities. VOCs and particulates were also generated to levels comparable with urban environments. This was confirmed by sampling air in the domes for analysis by thermal desorption GC-MS, in the case of VOCs, and a three-stage Dekati particle impactor, in addition to continuous NO_x measurement.

The plants used for the vegetation study were *Viola wittrockiana* (pansy) and *Trifolium repens* (clover); these were exposed for a period of two months. Control plants were simultaneously grown in identical solardomes without exposure to pollutant mixtures.
Plants exposed to the simulated urban environment exhibited reduced stomatal conductance[2] and increased leaf wettability[3] in relation to the control plants. Both these responses have been generally thought to indicate pollution injury. However, a study of uncontrolled water loss from detached leaves produced inconclusive results.

The study produced results which indicate early signs of pollution damage to the plants, however, further long-term studies will be required to determine fully the impacts of urban pollution mixtures on vegetation.

[1]Rafarel, C.R. and Ashenden, T.W., 1991. A facility for the large-scale exposure of plants to gaseous atmospheric pollutants. *New Phytologist, 117*, 345-349.
[2]Darrall, N.M. 1989. The effect of air pollutants on physiological processes in plants. *Plant, Cell and Environment*, 12, 1-30.
[3]Cape, J.N., 1994. Evaluation of pollutant critical levels from leaf surface characteristics. In: Air Pollutants and the Leaf Cuticle. Eds. K.E.Percy, J.N.Cape, R. Jagels and C.J.Simpson. pp123-138. Springer-Verlag, Berlin.

THE CAPTURE OF $PM_{2.5}$ AND PM_{10} BY DIFFERENT TREE SPECIES

K.P.Beckett[1], Gail Taylor[2] and P.H.Freer-Smith[3]

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Earlier work has focused on source apportionment of particulates removed from the air by urban woodland (Freer-Smith *et al* 1997), and on the effects of tree structure on the amounts (weights) of anthropogentic and naturally occurring particulates taken up at different UK urban sites (Beckett *et al* 1999).

For the last two years Leyland cypress, Corsican pine, whitebeam, field maple and poplar have been grown in five replicated blocks at a polluted urban site (Withdean Park, Brighton) and a rural site some four miles north of Brighton (South Downs). Data from leaf washing indicate that there are significant differences in the amounts of $PM_{2.5}$ and PM_{10} deposited onto the foliage of the different species. As would be predicted, species with more complex stem morphology and smaller leaves (the cypress and pine) capture larger weights of both size category of particulates. Capture of PM_{10} is greater at the urban site while $PM_{2.5}$ is transported further from the city, giving deposition values which decline less than those of PM_{10} at the more rural site. Measurement of particulate concentrations in the air support this conclusion.

Windtunnel measurements have confirmed the species differences of particulate trapping efficiency (and deposition velocities) in controlled windspeeds and exposure concentrations. We are currently looking at the effects of windspeed on deposition. Species specific data which allow deposition velocities to be calculated for different windspeeds will allow particulate uptake to be modelled for specific planting designs giving planners the ability to maximise the beneficial effects of trees on urban air quality.

Freer-Smith P.H., Holloway S. & Goodman A. 1997 The uptake of particulates by an urban woodland: site description and particulate composition. **Environmental Pollution** 95 (1) 27-36

Beckett K.P., Freer-Smith P.H. & Taylor Gail. 1999 The capture of particulates by trees at five contrasting UK urban sites. Submitted for publication.

Modelling Horizontal and Vertical Concentration Profiles of Ozone and Nitrogen Oxides Within and Downwind of High-Latitude Urban Areas

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Urban ozone concentrations are determined by the balance between ozone destruction and photochemical production. At high latitudes, low levels of solar insolation and high horizontal advection speeds reduce photochemical production and the ozone concentration patterns are largely determined by the reaction of ozone with nitric oxide. A boundary-layer Lagrangian column model has been developed to simulate the three-dimensional structure in ozone and nitrogen oxides (NOx) concentrations in the boundary-layer within and downwind of urban areas. The short-timescale photochemical processes of ozone and NOx, as well as emissions and deposition to the ground are simulated.

The model is applied over a 100x100km domain, containing the city of Edinburgh (at latitude 56°N), to simulate the city-scale processes of pollutants and to allow comparison with monitoring stations within and downwind of the urban area. Results are presented, using averaged wind-flow frequencies and appropriate stability conditions to show the extent of the depletion of ozone within and downwind of the city. The long-term average spatial patterns in the surface ozone and NOx concentrations over the model domain are reproduced quantitatively. A series of monitoring sites that lie on a north-south transect through the city - from an urban, through a semi-rural, to a remote rural location - allows the comparison of modelled with observed data. The diurnal variations in ozone concentrations have also been modelled and compared with the monitoring site data.

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APPLYING THE CRITICAL LOAD APPROACH TO METAL DEPOSITION: DOES IT MAKE SENSE?

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The critical loads approach has been used to provide an effects basis for negotiations on control of emissions leading to soil and freshwater acidification. The approach requires the definition of a chemical threshold, in a medium such as soils, for an adverse biological response, and a model to calculate the level of atmospheric deposition above which that threshold will be exceeded at steady-state (the 'critical load'). More recently, there has been discussion on whether this concept can be applied to other types of atmospheric pollution. This paper reports on an interdisciplinary project, involving seven research groups, on the application of the critical load approach to metal deposition, focussing on the UK uplands. Issues such as defining the bioavailability of the metal, the need to consider several different contaminant pathways to biological targets, the timescales of change in response to deposition, and the significance of historical contamination, all mean that modifications of, or alternatives to, the critical load approach may be needed for potentially toxic metals.

CHANGES IN HEAVY METAL SPECIATION DURING ACIDIFICATION - IMPLICATIONS FOR ECOTOXICITY

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The speciation of metals (including heavy metals) in aqueous media (e.g. soil solution, river waters) is affected by several factors, notably pH and ionic strength. Therefore, whilst acidification of soils may cause increased concentrations of heavy metals in solution by virtue of desorption and increasing solubilisation of minerals, the aqueous speciation of heavy metals will also be affected. Although it has long been considered that aqueous speciation plays an important role in determining the organism (plants and animals) uptake of elements from solution, it is only relatively recently that rapid and precise measurements of toxicity and computer computations of metal speciation have been available. Together, these techniques are now allowing direct comparisons between heavy metal speciation and induced toxicity. We present data relating free metal ion activity to the response of the bioassay, *lux*-marked *E. coli* in an artificial soil solution. Highly significant correlations were obtained for Zn and Pb but weaker correlations were obtained for Cu. We conclude that free ion activity can be used as a predictor for ecotoxicity for Zn and Pb but that for Cu, either physiological effects are also important or other Cu complexes may be able to induce toxicity.

Atmospheric Deposition of Heavy Metals onto Agricultural Land in England and Wales

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A network of 35 sites for monitoring total atmospheric deposition of heavy metals was set up in England and Wales using Warren Spring Laboratory-type inverted Frisbees. Thirty four of the monitoring sites were at locations remote from major roads and industrial sites but for comparative purposes one was placed near to a known source of pollution, a lead-zinc smelter. The total deposits were on a monthly basis and filtered (0.45μm) and were analysed for 23 elements. The mean values and concentration ranges for the total deposition (gha^{-1}) of eight heavy metals over a period of 30 months were: As 2.9 (0.9-13.2), Cd 1.8 (0.9-3.6), Cr 8.7 (2.2-59.3), Cu 55.9 (25.7-378), Hg 2.0 (0.5-37.6), Ni 8.9 (3.4-40.6), Pb 42.4 (15.8-92.4) and Zn 220 (111-385).

The data for cadmium, lead and zinc showed the lowest coefficients of variation (31-34%) indicating relatively ubiquitous sources. However, copper, mercury and chromium showed highly variable and skewed data suggesting more localised sources. In contrast to remote rural sites, monitoring was also carried out 2.5 km downwind from a large lead-zinc smelter and industrial complex. Cadmium deposition was greater than the mean for the rural sites by a factor of 30, copper by 2, lead by 35 and zinc by 14.8. These data show that local atmospheric dispersion from a major source such as a smelter can give rise to highly elevated concentrations of potentially toxic elements in the air and soil which could have implications for the health of humans and livestock and crop quality.

HEAVY METAL AND FLUORIDE CONTAMINATION OF ALLUVIAL MEADOWS AND PASTURES IN SOUTH NORTHUMBERLAND

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The River East Allen flows through the North Pennine orefield in South Northumberland where mining for lead, zinc, and more recently fluorspar, has occurred in the past. At Sinderhope, (NY841514) rare plants associated with heavy metal contamination grow on riverside pastures. These metallophytes are threatened by river bank erosion and animal disturbance. In the adjacent meadow former land management practices have caused a decline in the native flora and depletion of the seed bank. The research project at this site is aimed at increasing plant species diversity. However, analysis of the soil has revealed high levels of heavy metals and fluoride, not only on the riverside pastures but also over alluvial meadows. This contamination has probably been the result of deposition of toxic sediments during past flood events but there may also have been some aerial deposition from former smelting activities. There is unexpected seasonal variation in the concentration of these elements in vegetation especially during the winter months. Different extraction methods have revealed a high proportion of non-labile fluoride in the vegetation. The fluoride content of plants is strongly correlated with the aluminium and the low pH environment of the meadow. Transects taken across the river, up and downstream of this site, have verified that other pastures and meadows along this valley have been similarly affected. These results may explain the decline in plant species diversity in this area, but also imply that there may be a potential health risk to domestic livestock and wild animals grazing on these riverside pastures and meadows.

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The Effects of Elevated Nitrogen and Imposed Drought on the Ecology of a Lowland Heath

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Elevated atmospheric nitrogen (N) deposition may result in increased drought injury suffered by *Calluna vulgaris* (L.) Hull. Experimental field trial plots were established at a lowland heath site in Cheshire and since March 1996 these plots have received NH_4NO_3, in aqueous solution, applied at 14 day intervals to provide a dose equivalent to: 0, 20, 60, 120 kg ha^{-1} yr^{-1}. During the summer of 1997, plots were split beneath rain shelters, one half receiving the average weekly summer rainfall for the site, the other half was droughted. Nitrogen applications continued throughout the six months of imposed drought.

Nitrogen application promoted heather growth, flower numbers and seed production. The adverse effects of drought were greatest on those plots treated with higher N applications. In those plots which had been droughted a shift in the onset of flowering was observed. In summer 1998 *Lochmaea suturalis* damage was observed, with initial surveys indicating an increased proportion of shoot damage in the high N plots. Annual vegetation surveys since 1996 have shown an increase in *Deschampsia flexuosa* (L.) Trin. in the high N treatments, particularly where gaps are appearing in the heather canopy.

Data relating to these aspects of this study will be presented.

T

DIAGNOSTIC INDICATORS OF NITROGEN CRITICAL LOAD EXCEEDENCE ON MOORLANDS

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The objective of this study is to find reliable indicators of excess nitrogen supply on *Calluna* dominated upland moor using the long-running field manipulation experiment at Ruabon, North Wales. These plots have experienced monthly application of ammonium nitrate sprayed on at rates of 0, 40, 80 and 120 kg N ha^{-1} yr^{-1} since 1989. Previous studies at this site have found significant effects of nitrogen on shoot extension, shoot nutrient content, frost hardiness, litter weight and litter nitrogen content of *Calluna vulgaris*[1], indicating strong N retention.
Our aims are to find evidence of N saturation by looking at monthly changes in the soil concentrations of major nutrients, base cations and aluminium in the rooting zone and as potential leachate. Simultaneous recording of environmental and climatic variation as well as changes in microbial and phosphatase activity and changes of nutrient content and demand within the plant will help to build a picture of nutrient cycling within the system. By combining these components we hope to find a comprehensive diagnostic indicator of nitrogen critical load exceedence.
In addition the study will investigate the effect of surplus phosphate, the potential for increased rates of mineralisation as a result of altered C/N ratios in the litter, the losses from denitrification and the ameliorating effect of burning on the N status of the system.

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T

THE INTERACTION BETWEEN NITROGEN DEPOSITION AND HEATHLAND MANAGEMENT

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Increased nitrogen deposition is believed to be responsible for the recent change in dominance at many of Europe's lowland heaths from the original heath community to one that is dominated by one or two grass species. Traditionally, management such as burning or grazing has been used to 'check' succession of heathlands, however this management has declined in recent years. In the light of enhanced nitrogen deposition, management of *Calluna* heathlands becomes an important area of research as management may represent an inexpensive, effective way of preventing unwanted changes occurring as a result of enhanced nitrogen deposition – effectively increasing the critical load for heathlands. Alternatively management may be actively damaging to heathlands, opening the *Calluna* canopy and allowing the invasion of grass species.

Whilst a lot of information exists about individual management practices, very little is known about the interaction between management and enhanced nitrogen deposition. Research has been carried out on a lowland heath site in Surrey investigating the effects of three management regimes and (simulated) accidental heath fires on the impact of additional nitrogen. Experiments were also carried out at on plots that had received nitrogen additions since 1989 and in which a significant store of nitrogen had accumulated in the soil. This allowed investigation of the effects of nitrogen stores on management outcome as well as the effects of current nitrogen additions of 30 kg N $ha^{-1}yr^{-1}$.

Plant growth responses to nitrogen treatments under contrasting management practices were recorded, as were effects on soil chemistry. Preliminary results show that the area of regeneration of *Calluna vulgaris* and length of regenerating shoots corresponded to a potential gradient of nutrient removal and disturbance. *Calluna* regeneration was strongest with the least nutrient removal and the least disturbance. Nitrogen additions had no significant effect on regeneration after any management investigated, but soil stores of nitrogen promoted denser regeneration of the *Calluna* canopy. These areas may be better able to resist invasion by grass species. These results may be due to the amount of disturbance caused by management at this stage in the investigation and not due to a gradient of nutrient removal. Findings of this investigation are expected to change with time as the nutrient removal by management takes affect, and regeneration is not dominated by the physical impacts of management allowing the more subtle effects of nitrogen additions to influence regeneration.

Other initial results are also presented, and future work discussed. This includes the introduction of grass seeds to the experimental area to allow investigation of the interacting effects of nitrogen and management on the outcome of competition between grasses and *Calluna*.

GRAZING/ATMOSPHERIC NITROGEN INTERACTIONS IN UPLAND ACID GRASSLAND

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The critical load of atmospheric nitrogen for upland acid grassland is set at 7-20 kg N $ha^{-1}y^{-1}$. However, it does not take into account the influence of management practices. This experiment uses monoliths from an acid grassland and aims to fine tune the critical load in relation to management by grazing. The monoliths experience a range of nitrogen deposition levels above and below the critical load and selective cutting regimes to simulate grazing by sheep.

Changes in vegetation composition in the first year show a trend towards reduced abundance of mosses at increasing levels of nitrogen. This was significant for *Racomitrium lanuginosum* which showed reduced cover even at low nitrogen levels. Cover of *Galium saxatile*, *Nardus stricta* and some moss species significantly increased under the heavier grazing treatments while *Vaccinium vitis idaea* significantly decreased. Time of bud break in *Vaccinium myrtilus* is advanced in the highest nitrogen treatment. In the first year, the management treatments have had a greater effect on the vegetation than the nitrogen treatments.

A GREENHOUSE EXPERIMENT INVESTIGATING THE EFFECTS OF MANAGEMENT AND ATMOSPHERIC AMMONIA DEPOSITION ON COTSWOLD GRASSLANDS

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The deposition of atmospheric nitrogen, particularly ammonia from agricultural sources, has been hypothesised to cause the dominance of the grass *Brachypodium pinnatum* in calcareous grasslands, with associated losses of species diversity and richness. However, management may also be important in determining the species composition of grasslands.

Brachypodium pinnatum and *Bromus erectus* are the two most dominant species in unimproved calcareous grasslands in the Cotswolds. A greenhouse experiment was designed to investigate the effects of cutting and three levels of nitrogen addition on the competition between these two species. A range of planting densities enabled the calculation of competition indices.

The experiment ran for a period of 15 months and results show that nitrogen addition increases the growth of both species, in monoculture and when grown in mixed species pots. Cutting stimulates biomass production tillering in *Brachypodium* but not *Bromus*. Neither nitrogen nor cutting had any significant effects on the outcome of competition between *Brachypodium* and *Bromus*. The results will be discussed in the light of findings from a field survey and other sources.

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TREE STOMATAL RESPONSES TO ATMOSPHERIC CO_2 ENRICHMENT AND INTERACTIONS WITH VAPOUR PRESSURE DEFICIT

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Atmospheric CO_2 enrichment is generally expected to enhance forest productivity, while at the same time improving water economy through reductions in the stomatal conductance to water vapour (g_s). This response has direct implications for tree water economy, drought tolerance and growth - especially given that climate change is likely to lead to increased frequency of drought and reduced soil moisture availability in many regions. Secondly, the effects of CO_2 enrichment on g_s will determine rates of evapotranspiration from forests, thereby affecting regional and global climate - both in terms of humidity/precipitation and in terms of direct feedbacks on surface temperature.

Although crops and herbaceous species have shown consistently large reductions in g_s in response to elevated CO_2 concentrations, the response of trees appears to be generally weaker and much more variable[1]. The increasing number of examples of weak stomatal responses to elevated [CO_2] may be due to the emergence of more data from longer term experiments using larger trees rooted directly in the soil[2], thus representing a more realistic scenario. Similarly, variability between different experiments using the same or different species might be mainly attributable to growth conditions[3] although detailed side-by-side species comparisons are lacking[3]. However, work carried out in Solardomes at Lancaster offers just such a comparison between three members of the Fagaceae: beech (*Fagus sylvatica*), chestnut (*Castanea sativa*) and oak (*Quercus robur*).

The magnitude of the stomatal response to CO_2 enrichment varied considerably between species, as well as according to other environmental parameters - in particular leaf-to-air vapour pressure deficit (LAVPD)[4] and soil moisture deficit[4, 5]. When LAVPD was low (~1 kPa) g_s was substantially lower at elevated compared to ambient [CO_2]. However, at high LAVPD (~2.5-3 kPa) the effect of [CO_2] on g_s was substantially weaker or disappeared altogether - sometimes g_s was actually higher at elevated [CO_2][4]. This has been shown consistently, both in response to natural variations in temperature and humidity (driven largely by solar radiation) and to imposed changes in LAVPD with a constant artificial light source. These effects may account for much of the variability reported between species and between experiments, since conditions during measurements so strongly affected the apparent response of g_s to [CO_2]. Applying the data to modelling showed that, in some species, future reductions in evapotranspiration may be far less than previously assumed[6]. CO_2 enrichment is also expected to protect against ozone pollution through reduced g_s - but this protective effect may also be less than often assumed, particularly as the lack of stomatal closure in elevated [CO_2] occurs at precisely those times of high LAVPD and/or drought that are most likely to coincide with ozone pollution episodes[6].

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2. Eamus D. (1996) Responses of field grown trees to CO_2 enrichment. *Commonwealth Forestry Review* **75**, 39-47.
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4. Heath J. (1998) Stomata of trees growing in CO_2-enriched air show reduced sensitivity to vapour pressure deficit and drought. *Plant, Cell and Environment* **21**, 1077-1088.
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Modeling ozone detoxification by ascorbate in the leaf apoplast

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This paper presents a computer-based model to simulate ozone detoxification by ascorbate in the leaf apoplast. The model describes ozone transfer from the external atmosphere through the various resistances imposed by a plant leaf (laminar boundary layer, stomatal aperture, gas/aqueous interface at the cell wall, plasmalemma), and its direct reaction with ascorbate in the cell wall (the apoplast). In addition, the distribution of ascorbate between subcellular compartments is estimated, based upon the permeability of the neutral species, ascorbic acid, across membranes, and the pH of the various compartments. Model simulations indicate that 'realistic' cellular concentrations of ascorbate can potentially detoxify a substantial portion of the incoming ozone, under environmentally-relevant conditions. Additional leaf characteristics are highlighted as important factors controlling flux of ozone to the plasmalemma; stomatal conductance, mesophyll cell wall thickness, chloroplast volume, apoplast pH and leaf temperature. The results are discussed in relation to factors determining intra- and interspecific variations in ozone sensitivity.

EXAMINING THE ROLE OF ASCORBATE IN MEDIATING OZONE RESISTANCE

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In this study, genotypes of *Raphanus sativus* have been employed as a model to determine the role of apoplastic ascorbate in mediating resistance to ozone.

Nine genotypes of *R. sativus* were screened for sensitivity to ozone (75 nmol mol^{-1} 7 h d^{-1} 3 $weeks^{-1}$) in terms of effects on relative growth rate, and R% was calculated from $(R_{O3}/R_{CF}) \times 100$. Effects on ascorbate concentrations in the apoplast and symplast of the leaf were also determined. Research demonstrated a positive correlation across genotypes between apoplastic ascorbate concentration in ozone treated plants and R%.

R. sativus cv Cherry Belle was then selected in order to investigate whether shifts in ascorbate concentration were consistent with a role in ozone detoxification. Apoplastic and symplastic ascorbate concentrations were determined during acute ozone exposure (120 nmol mol^{-1} 6 $hours^{-1}$) and at different stages of leaf development. Ascorbate concentrations in the apoplast were found to be significantly reduced, over the period of leaf development, following acute ozone exposure.

Ascorbate levels in *R. sativus* cv Cherry Belle were then manipulated by feeding plants (in nutrient media) for 24 hours with L-gactono-1,4-lactone (GAL), to determine if an increase in ascorbate is reflected by an upwards shift in ozone resistance. Feeding 50mM GAL to plant roots provided a two-fold increase in ascorbate concentrations in leaves: this was found to significantly protect photosynthesis against acute ozone exposure and reduce the appearance of visible damage. These results are consistent with a role for ascorbate in mediating ozone resistance in plants.

High Resolution Maps of Ozone Concentration and AOT40 for the UK

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ITE Edinburgh

Measurements from the UK national air quality monitoring network are used to calculate maps of annual mean and AOT40 for crops, semi-natural vegetation and forests in the UK. The techniques used rely on our understanding of the diurnal cycle in ozone concentration and how it is influenced by meteorology and topography in rural regions. Generally ozone concentrations exhibit a large diurnal cycle with peak levels in the afternoon and a minimum during the evening and overnight. In the afternoon when the boundary layer is well developed and turbulent, ozone concentrations are very similar across wide geographical areas. In the evening and over-night the boundary layer close to the ground cools and becomes stable, leading to reduced concentrations as ozone is lost to dry deposition at the surface. The degree of nocturnal depletion depends on the stability of the boundary layer as in a turbulent atmosphere losses will be replaced by the mixing down of ozone rich air from above. These processes are very sensitive to local topographic effects and therefore lead to large spatial variability in ozone concentration during periods of strong stability.

In the UK wind speed and hence turbulence increases with altitude and so altitude can be used to indicate the range in the diurnal cycle of ozone at any location. To calculate maps of ozone, concentrations during the afternoon are interpolated from rural sites then modified to account for the level of nocturnal depletion in each grid square using a 1 km x 1km altitude map of the UK. Although the resulting maps are quite uncertain they give a broad picture of ozone in the UK, incorporating our current understanding of the processes which influence surface ozone concentrations and give and indication of the regions most at risk of damage .

The high resolution ozone concentration fields can also be used to quantify deposition fluxes. These fluxes may be partitioned into stomatal and external fluxes. The paper shows results of these modelling approaches and comparisons with field measurements.

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INVESTIGATING THE DISTRIBUTION OF TARSPOT OF SYCAMORE ALONG A TRANSECT BASED ON GRADIENTS OF OZONE CONCENTRATIONS.

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Rhytisma acerinum, which causes tarspot of sycamore, has been claimed to be sensitive to air pollution[1]. Moreover, a study in south-east Scotland[2] confirmed that tarspot is abundant in the rural areas but absent from Edinburgh city centre. However, it was unclear whether this was because the source of inoculum (i.e. infected leaf litter) was removed in city parks or because of the higher pollution levels within central Edinburgh. The aim of the present study was to establish a transect in the London area in order to make field observations relating to the distribution of the disease along a gradient of air pollution. In parallel with this, a transect was established in Edinburgh and ozone fumigations were carried out in open-top chambers.
There was a significant decrease in Tar-Spot-Index (TSI) as a function of distance from central London both in 1997 and 1998 and from central Edinburgh. There was also a positive correlation between ozone levels and TSI in the field. This is in contradiction with fumigation data, which suggests the contrary. Further fumigations are planned in 1999 to elucidate this mystery. The most likely explanation so far is that in the field, ozone is but one of many factors affecting the distribution of *Rhytisma.* Another explanation for the relationship between TSI and distance from London might be that one is dealing with a 'memory effect' (i.e. The tarspot was once regulated by a steep pollution gradient, such as SO_2, which has since disappeared, but recolonisation has not yet occurred).

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THE UNECE INTERNATIONAL COOPERATIVE PROGRAMME ON THE EFFECTS OF OZONE ON VEGETATION : A BRIEF OVERVIEW.

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The UN/ECE ICP-NWPC[1] is coordinated from ITE-Bangor. The main objective of the programme is to provide information for the UN/ECE Working Group on Effects (WGE) on the effects of ambient ozone pollution and other pollutants on crops and non-woody plants in Europe and North America. Experiments are conducted in about fifteen countries each year. The data are used to validate existing critical levels, and to develop new critical levels for these types of vegetation. In 1998, as in the previous three years, ozone-sensitive and -resistant clones of white clover (*Trifolium repens* cv Regal, selected by Al Heagle, USA) were grown at 35 sites according to a standard protocol issued by the Coordination Centre (CC). Biomass, pollutant, and climatic data were collated at ITE and jointly analysed by the Data Modelling Centre at Nottingham Trent University and the CC.

Ambient ozone concentrations were lower in central and northern Europe in 1998 than in 1997, but were higher at most southern sites. The current critical level of a three-month AOT40 of 3000 ppb.h was exceeded at 60% of the sites in 1998 compared to 84% of the sites in 1997. Due to the relatively cool wet summer last year, the critical level was not exceeded at the two UK sites (Bangor and Ascot). The ratio of the biomass of the sensitive to that of the resistant clover clone fell to as low as 0.56 at the site at Italy-Isola Serafina (near Milan) where the highest three-month AOT40 of 32,785 ppb.h was recorded.

The data from the clover clone experiment has been used to develop models of the effect of level II factors on the biomass response to ozone. Temperature, VPD, and NOx have all been identified as important influencing factors. Several different indices are currently being investigated for each of these parameters after initial models using the daytime means, and the means when the ozone concentration exceeded 40 ppb were only moderately successful (r^2 for the training and test data were 0.64 and 0.45 respectively). Even so, the ANN model performed better than a model produced by multiple linear regression using the same inputs, or the best performing model selected by stepwise linear regression. An equation extracted from an ANN has been used by the CCE to produce a map of predicted clover biomass ratio in Europe. The unique feature of this work is that the models are being developed from data from experiments in which plants were exposed to ambient ozone episodes in a range of climatic conditions, without the modifying influence of any chamber system on ozone flux.

[1] United Nations Economic Commission for Europe International Cooperative Programme on Effects of Air Pollution and Other Stresses on Non-Wood Plants and Crops

EFFECTS OF ELEVATED OZONE AND FRUITING LEVEL ON YIELD PARAMETERS, GROWTH AND PHOTOSYNTHESIS IN STRAWBERRY.

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Fragaria x ananassa Duch. cv. "Cambridge Favorite" fruiting and deblossomed plants were exposed to 92 ppb ozone or filtered air in open-top chambers for 69 days. Flower and fruit production, relative growth rate (RGR) of leaf area, leaf gas exchange, chlorophyll fluorescence kinetics and plant biomass were investigated. Ozone caused a reduction in fruit set or initial fruit growth, followed by a decrease in the number of fruits per inflorescence and subsequently individual fruit weight; however, total fruit yield over the fumigation period was not significantly affected by ozone. Ozone accelerated leaf senescence and had a greater negative effect on the rate of photosynthesis in older than younger leaves. The area above the fluorescence curve between F_m and F_o was the only parameter responding significantly to ozone, while the F_v/F_m ratio was not altered. The effects of ozone on photosynthesis and chlorophyll fluorescence were not affected by the presence of fruits. The RGR of leaf area was the first parameter to respond to O_3 fumigation, resulting in a significant decrease in leaf blade biomass. Root and crown biomass did not significantly decrease in ozone compared with filtered air. There was little evidence that the presence of fruit altered plant response to ozone.

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EFFECTS OF OZONE ON THE GROWTH AND REGROWTH OFAN EGYPTIAN VARIETY OF JEW'S MALLOW (*CORCHORUS OLITORUS*)

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Measurements of ozone concentrations in Egypt have indicated that this pollutant is present in concentrations high enough to have adverse impacts on vegetation. This paper reports the responses to ozone of one of widely grown and the most important summer leafy vegetable crop in Egypt, jew's mallow (*Corchorus olitorus* cv. Balady). This crop is characterised by multiple growth cycles over the growing season, and thus effects of O_3 on the capacity for regrowth are of particular interest.

Plants were exposed in closed chambers to ozone concentrations in the range 50-100 ppb. Fumigation with O_3 caused large reductions in leaf, stem and root biomass, in addition to foliar necrosis and accelerated leaf senescence during the first growth cycle. As a result of the these effects of O_3 in the first growth cycle, the regrowth of cut plants was inhibited during the regrowth cycle, even in the absence of O_3. There was also evidence that the effect of ozone was grater when plants were exposed from germination, rather than from seedling establishment. These observations suggest that *Corchorus olitorus* cv. Balady is susceptible to the concentrations of O_3 which have been recorded in Egypt during the last decade.

THE EFFECT OF OZONE ON LINES OF *BRASSICA RAPA* WHICH HAVE EVOLVED THROUGH NATURAL SELECTION

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This paper reports a comparison of the effects of ozone exposure to three lines of *Brassica rapa*:

Tremayne, Plowman and Richards (in press[1]) investigated the effects of microclimate on germination time and time to flowering in three annual species. In the case of *Brassica rapa*, after three generations differences in aspect led to evolution of lines with different phenological responses with regard to time to germination ($p = 0.0001$), and also time from germination to flowering ($p = 0.0001$). This raises the question of co-tolerance. Did selection for a dry sunny aspect alter response to other factors such as ozone, as it has been suggested that all stresses exhibit common mechanisms for resistance (Reiling and Davison 1992[2]).

Seed was taken from the two extreme lines of the afore mentioned study. The first line was taken from the north facing slope which was artifically shaded from direct sunlight and uncovered so it received direct rainfall (NFS). The second line was taken from the south facing slope which was unshaded but covered, receiving no direct rainfall (SFD). These two lines were compared together with seed from Generation 0 (third line) which received no selection pressures. All three lines were given a chronic ozone dose of 70 nl l^{-1} for 7h d^{-1} over their entire life cycle and the response was measured in terms of growth and other parameters considered to contribute to ozone resistance such as, chlorophyll content, ethylene emissions, ascorbate levels, and stable isotope discrimination. Significant results were obtained which will be presented in this paper.

[1]M.A.Tremayne, A.B.Plowman, A.J. Richards (in press) Changes in time to germination and time to flowering after microclimatic manipulation in three species of annual weed.

[2]K.Reiling & A.W.Davison (1992) Spatial variation in ozone resistance of British populations of Plantago major L., New Phytologist,122, 699-708.

IMPACTS OF OZONE ON COMMERCIALLY-GROWN GLASSHOUSE CROPS: A RISK ASSESSMENT

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Ambient levels of ozone (O_3) have been shown to be high enough to induce visible injury and reduce the productivity of several field-grown crops. However, little is known about the impact of O_3 on crops grown under protected cultivation (ie. glasshouses, polytunnels etc.). This paper reports on monitoring data, gathered over successive summers, which show that potentially phytotoxic concentrations of O_3 can be attained during the summer months inside commercial glasshouses on the south coast of England - when external O_3 concentrations are at their highest and growers ventilate their glasshouses to prevent over-heating.

Controlled environment studies on several commercially-grown herbs have indicated that the detrimental effects of environmentally-relevant O_3 concentrations may be mitigated by the common practice of elevating CO_2 concentrations in commercial glasshouses. Interestingly, however, in-depth investigations on commercially-grown tomato cultivars do not indicate the same amelioration of O_3 damage by CO_2-enrichment. Data are presented which investigate the mechanisms underlying the differential responses of commercially-grown herbs and tomatoes to the combination of elevated O_3 and elevated CO_2.

Ozone and water stress interaction

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In Britain, ozone episodes occur during warm, settled weather so plants may be exposed to the combination of ozone and water stress. As the first episodes often occur in spring and early summer, seedlings may be particularly prone to this combination of stresses. *Plantago major* was used to study the interaction of ozone and water stress. *P. major* seedlings were treated with ozone and charcoal filtered air (CF), and both treatments were water stressed for 7 days. Three days into the water stress the results showed that the water potential in the ozone stressed plants had declined slower than in the CF plants. (-1.37 MPa CF, -0.91 MPa ozone). After 7 days these had decreased to -3.70 MPa and -4.78 MPa respectively. Furthermore, the fumigated ozone plants had a pressure potential loss point (water potential = osmotic potential) greater than CF plants, 5 and 3.5 days respectively. This suggests the stomatal conductance decreased and the cell walls retained their rigidity in comparison with the CF plants. There was an increase in the biomass of the ozone fumigated plants over the first 3 days of the water stress, but the was no change in the CF plant biomass. After the full 7 days the ozone plants still had a greater biomass in comparison with the CF plants. This suggests that the ozone may play an important role in the protection of plants against water stress damage in dry seasons.

RESPONSES OF WETLAND COMMUNITIES TO ENHANCED OZONE EXPOSURE

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Critical levels of ozone for semi-natural vegetation (3,000 ppb.h > 40ppb) are based on experiments involving relatively few species, principally from grassland communities However, low stomatal conductances during periods of summer drought may restrict the ozone flux to grassland species at precisely the time when ambient concentrations are highest. In contrast, plants growing in wetland environments (e.g. fens or wet meadows), with an unrestricted water supply, would be expected to maintain higher stomatal conductances throughout the growth season, and hence might be expected to be more sensitive to ozone than species from drier grasslands.

Summer long fumigations of species characteristic of tall herb fens/fen meadows were carried out in open top chambers:- 5 species in 1997 (*Symphytum officinale, Valeriana officinalis, Lythrum salicaria, Iris pseudacorus, Mentha aquatica*) and a further 7 species in 1998 *(Rumex acetosa, Vicia cracca, Lathyrus pratensis, Cirsium arvense, Lychnis flos-cuculi, Lotus uliginosus, Filipendula ulmaria*). Cumulative AOT40 exposures ranged from 9,200 ppb.h to 14,200 ppb.h. All 12 species were affected by ozone in at least one of the measured parameters; of these, 9 species experienced significant negative effects i.e. a reduction in ozone. Two thirds of the species used in these experiments were visibly injured - a substantially higher proportion than in our earlier experiments involving species from grassland communities. Whilst several wetland species experienced significant reductions in above-ground weight, impacts on root weight and root:shoot ratios were more consistent and generally of a greater magnitude.

Significant reductions in photosynthetic rate in response to ozone were seen for 5 of the species, although the timing of these effects in relation to ozone exposure differed considerably between species. The mean stomatal conductance of the fen / fen meadow species used in this experiment was approximately 50% higher than that of grassland species used in earlier screening experiments. However, whilst there was a negative relationship between stomatal conductance of wetland plants and live weight reductions, data variability was high and only a small amount of the variance in plant response was explained by stomatal conductance.

POSTER ABSTRACTS

CAUSES OF WINTER INJURY IN NITROGEN-ENRICHED *CALLUNA VULGARIS*

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A heather moorland in North Wales has been fertilized regularly since 1989 with ammonium nitrate solutions at the rates of 0, 4, 8, 12, 20 g N m^{-2} y^{-1}. In April 1993 and every late winter since that time we have observed injury to the shoots of *Calluna* which resembles a condition known as 'winter browning'. Affected shoots appear pale brown or bleached and eventually drop off. The scale of this damage has repeatedly been greater in the highest Nitrogen treated plants. The cause of this injury is not entirely clear but in other locations has been attributed to low temperature dessication. This presentation presents observations and laboratory experiments which have been used to understand the physiology of this condition and to discover why this injury is greatest in nitrogen enriched plants.

MINERAL NITROGEN UTILISATION BY ECTOMYCORRHIZAL FUNGI WITH RESPECT TO ATMOSPHERIC DEPOSITION.

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Increases in pollutant mineral N deposition in the form of nitrogen oxides (NO_x) and ammonium (NH_3) is taking place across Europe and North America resulting in an increase in the ratio of mineral to organic N and affecting the ratio of available nitrate (NO_3^-) to ammonium (NH_4^+) in soils. The objective of this study was to examine the effect of varying NO_3^- and NH_4^+ ratio on N utilisation in the ectomycorrhizal species, *Tylospora fibrillosa* and *Paxillus involutus,* in pure culture. *T.fibrillosa* cultures were isolated from ectomycorrhizal root tips which were subjected to different of chronic levels of mineral N exposure as part of an EC project (NITREX). Ectomycorrhizal cultures were assessed for their ability to use NO_3^- by means of a nitrate reductase (NR) assay before being grown in liquid culture with five different mineral N sources:- NO_3-N only, NH_4-N only, and NO_3-N with NH_4-N in different ratios. N utilisation was assessed by quantifying residual NO_3-N and NH_4-N in the culture media and by determining the N content and biomass of the fungal mycelia over sequential harvests. Both species were found to use exhibit NR although there were found to be intraspecific differences in *T.fibrillosa* in its ability to utilise NO_3-N. A preference for NH_4-N was generally observed. The implication of the results for the community dynamics of ectomycorrhizal systems will be discussed.

SAP FLOW IN BEECH SEEDLINGS EXPOSED TO OZONE

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Beech (*Fagus sylvatica*) are sensitive to drought, and it has been suggested that exposure to high ozone concentrations may exacerbate damage in hot, dry summers[1]. Even without drought stress, beech may be sensitive to high ozone concentrations.
In an open-top chamber experiment, one-year-old beech saplings were exposed for three field seasons (1995-1997) to (a) charcoal filtered air (control) and (b) episodes of ozone at 100 ppb. Ozone was generated by electrical discharge in oxygen and maintained at 100 ppb by a computer controlled active feedback loop.
Annual exposures close to the Critical Level AOT40 for forest trees of 10 ppm.h were provided over the growing season[2] in the first and third years. In the second year, ozone exposure was equivalent to an AOT40 of > 20 ppm.h.
In the final year, sap flow gauges (Dynagage, Dynamax Inc.) were fitted on 4 ozone-treated and 4 control saplings.

Sap flow was strongly correlated with sunlight intensity in all trees, but the relationship 1) differed for different trees, and 2) changed through the season. Early and late in the season there was no difference between ozone-treated and control trees, but for a period of 3 weeks, in relatively cool, cloudy weather, the ozone-treated trees showed greater sap-flow than controls, and a loss of the relationship with sunlight intensity, particularly at dawn and dusk.

This effect did not appear to be an instantaneous response to ozone exposure, as ozone exposure episodes were only applied in warm, sunny weather, and may therefore represent the cumulative effect of exposure. Loss of stomatal control in mid-summer may increase drought stress in the field.

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2. Kärenlampi, L. & Skärby, L. (Eds.) 1996. *Critical Levels for Ozone in Europe:Testing and Finalizing the Concepts.* UN-ECE Workshop Report, Kuopio, Finland.

Abstract

Title: ...... **ANALYSING EXCESS INVESTMENT IN RUBISCO CONTENT OF WHEAT LEAVES AT ELEVATED CO_2**

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Text:

Light-saturated photosynthetic rate (A) is determined by the amounts of active photosynthetic components in the leaf and is closely correlated with leaf N content. As CO_2 partial pressure (pC) increases, Rubisco becomes more efficient compared with RuBP-regeneration and other processes. For optimal A per unit leaf N (N-use efficiency), a decrease in Rubisco relative to other components is therefore required at high pC[1]. N-use efficiency at high pC has been increased in rice lines manipulated to decrease Rubisco content[2]. We have demonstrated in wheat that most of the excess Rubisco at high pC persists even when plants are grown at high pC and with low N supply[3]. Here, we (1) show the relationship between photosynthetic components and photosynthetic capacities, estimate excess Rubisco content as a function of pC for wheat leaves grown under different pC and N supply (2) use models to identify the implications of our findings for genetic manipulation to improve N-use efficiency in wheat and sensitivity to assumptions (3) report status of our new project which aims to generate transgenic wheat lines with decreased Rubisco content.

1. B.E. Medlyn 1996, Australian Journal of Plant Physiology, 23, 593-603
2. A. Makino et al. 1997, Plant Physiology, 114, 483-491
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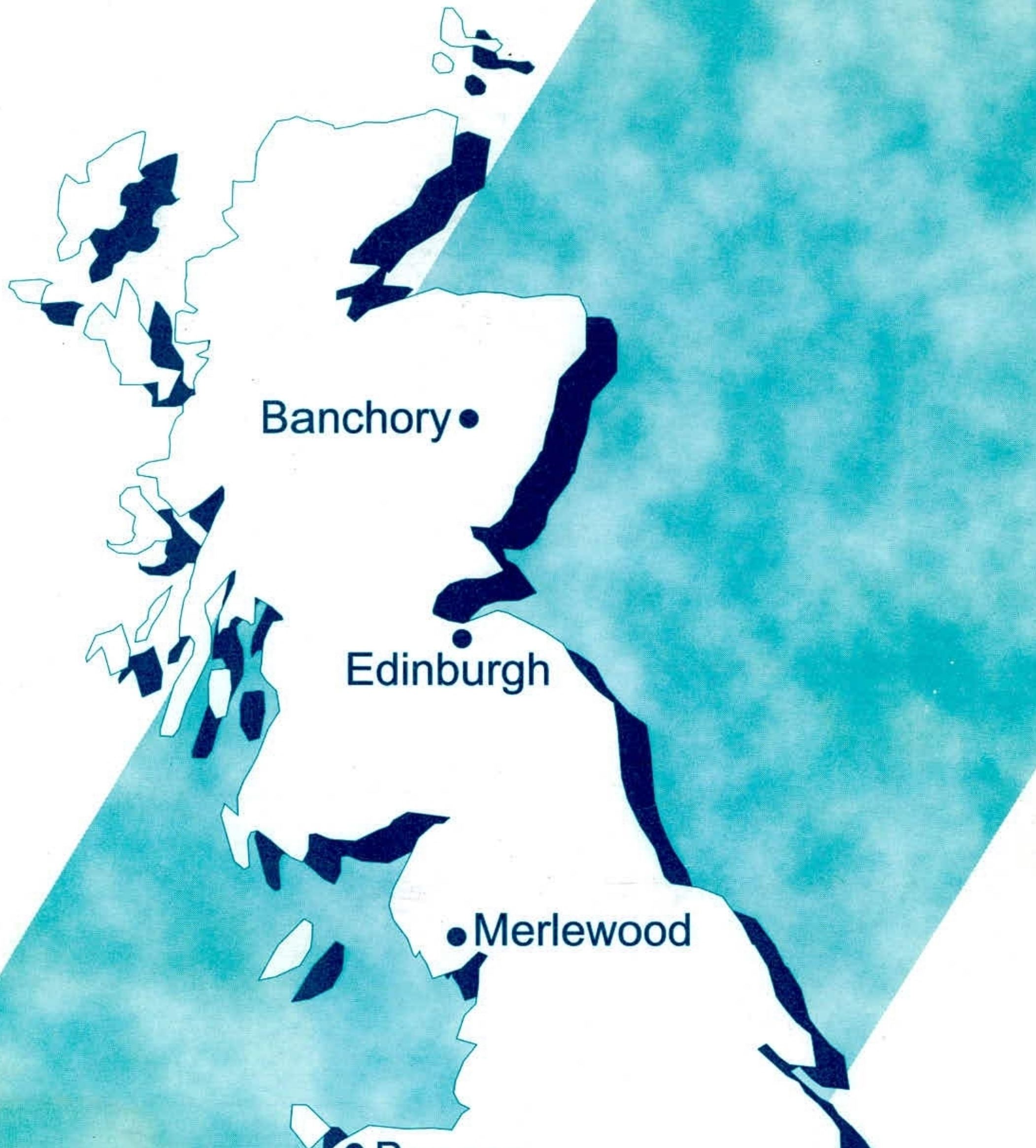

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