CAPER 2005

COMMITTEE ON AIR POLLUTION EFFECTS RESEARCH

University of York
Environment Department
21st – 23rd March 2005

Programme

Monday 21st March

12:30	Registration at Environment Building, Building 21, Market Square, Heslington Campus
12:30	Lunch
13:30	Depart for field trip
19:00-20:00	Dinner
20:00	Assemble posters
20:30	Poster session (and wine!)

Accommodation in James College

Talks will be held in Langwith College Room L036 (next door to the Environment Building)

Meals will be in the Galleria Restaurant in the Roger Kirk Centre

Tuesday 22nd March

8:00		***Breakfast***
9:00		Welcome **Chairman - David Fowler:** Environment Department
9:05	Peter Brimblecombe (East Anglia)	The History of Sulfur and Nitrogen Deposition
10:05	Laura Shotbolt (Univ. York)	Use of Herbarium Moss Samples to Reconstruct Records of Historical Metal Deposition
10:30		***Coffee Break***
11:00	Emma Green (Imp. College)	The Effect of Nitrogen Deposition on Heathland Systems
11:20	Catherine Stanley (UCL)	Nitrogen (N)-Modelling in Mosses: from Metabolites to Membranes
11:40	Chris Field (MMU)	Nitrogen Bio-Monitoring in the Peak District Using the Moss *Hypnum jutlandicum*
12:00	Imogen Pearce (CEH Banchory)	Impacts of Nitrogen enrichment on Montane Heath in Wales and Potential for Recovery
12:30		***Lunch***
13:30		**Chairman – Simon Caporn**
13:30	Gareth Phoenix (Univ. Sheffield)	Temporal Dynamics of Nitrate, Ammonium and Phosphate Leaching from N Polluted Semi-Natural Grasslands
13:50	Matthew Jones (CEH Edinburgh)	Quantifying the Relationship Between Concentration Dependent Deposition Velocities and NH_3 Concentrations for Semi Natural Vegetation using a NH_3 Flux System
14:10	Sally Gadson (Imp. College)	State of the Health of Beech Trees in Epping Forest
14:30	Mike Ashmore (Univ. York)	Ecological Impacts of Air Pollution from Road Transport on Local Vegetation
14:55	Rebecca Keelan (Univ. York)	Impacts of Ozone on Woodland Ground Flora
15:30		***Tea***
16:00		**Chairwoman – Lucy Sheppard**
16:00	Colin Gillespie (SEPA)	Combining Science and Regulation for Improving Environmental Protection
16:20	Bridget Emmett (CEH Bangor)	The Defra-NERC Terrestrial Umbrella: Now and in the future
16:40	Carly Stevens (Imp. College)	What Should be the Way Forward? Assessing Methods to Investigate Pollutant Impacts.
18:00		***Dinner***
19:30		**Committee Meeting**

21:00		**Umbrella get together**

Wednesday 23rd March

8:00		***Breakfast***
9:00		**Chairman – Mike Ashmore**
9:00	Jeremy Barnes (NCL)	QTLs Governing the Impacts of Ozone on Wheat Yield
9:20	Chris Callaghan (NCL)	Probing the Effects of Ozone on Carbon Metabolism in Planta
9:40	Eleni Goumenaki (NCL)	Mechanisms Underlying the Impacts of Ozone on Photosynthetic Carbon Fixation
10:00	Agnieszka Kaminska (NCL)	Impacts of Ozone on Aspects of the Reproductive Biology of Oilseed Rape
10:30		***Coffee***
11:00	Patrick Büker (SEI)	Comparison of a Multiplicative and a Photosynthesis Based Algorithm for Modelling Stomatal Ozone Fluxes to Higher Plants
11:20	Ali Dodmani (NCL)	Ozone Exposure – Response Relationships for Commercially Grown Winter Wheat
11:40	Nikos (NCL)	Suppression of Fungal Pathogens by Ozone
12:00	David D'Haese (NCL)	The Role of Apoplastic Antioxidative Capacity in O_3 Tolerance and an O_3 Microarray Experiment
12:30		***Lunch & Departure***

THE HISTORY OF SULFUR AND NITROGEN DEPOSITION

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Sulfur and nitrogen have long been considered important elements because of their role in biochemistry, in addition to their links with pollution. This presentation will trace the history of sulphur and nitrogen deposition. Although we will examine the geochemical history and changes in emission and deposition over time, the lecture will also treat the intellectual history and the way growing knowledge has contributed to our study of the global cycles of these elements. The elements were related to biology and agriculture from classical times, although thinkers were also aware that they caused damage. The growth of science in the 17th century meant a great deal of thought was given to the presence of sulphur and nitrogen in the air. However, in the case of nitrogen, the links between the seemingly inert gas and agriculture and biology were not fully realised until the *Theory of Vitalism* had been abandoned in the early 19th century. Once nitrogen was understood to be an important element for plant nutrition there were many studies of rainwater chemistry worldwide. Growing urban pollution from coal-derived sulphur dioxide initiated a parallel interest in the sulphur chemistry of rain. These early analyses are a tantalising source of information about the changing chemistry of rainfall over the last 150 years, through a period of great concern with acid rain. However, in the 21st century interest may once again move into new areas with rising concerns over the long range transport of dust and forest fire smoke, especially in Asia.

USE OF HERBARIUM MOSS SAMPLES TO RECONSTRUCT RECORDS OF HISTORICAL METAL DEPOSITION

Laura Shotbolt[1], Mike ashmore[1], Patrick Büker[2]

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Current metal concentrations in soils and surface waters reflect the impact of historical atmospheric deposition, as well as current sources. This is particularly important in countries such as the U.K., which have a long history of metal mining and smelting. Historical deposition scenarios are also important in constructing meaningful dynamic models, which can predict the effects of changes in metal deposition on soil and surface water concentrations. We describe here the results of an exercise to reconstruct metal deposition histories for five contrasting catchments in different regions of the U.K., by analysis of herbarium moss samples.

Samples of four moss species (*Hylocomium splendens*, *Hypnum cupressiforme*, *Pleurozium schreberi* and *Rhytidiadelphus squarrosus*) were collected from four major British herbaria. A total of 143 samples were obtained, covering the period between 1850 and 2000 and originally collected from within 50km of each catchment Samples were analysed for 28 elements using ICP-MS. A small number of samples (4) were identified as contaminated, based on the results of visual appearance and principle components analysis of washings, and were discarded before data analysis.

Analysis of trends in concentration of copper, lead, zinc, arsenic, cadmium, tin and antimony showed major contrasts between the five catchments. The lowest historical concentrations of most metals were found in a remote Scottish catchment and one in southern England, whereas the highest concentrations were found in the three northern England catchments which were heavily influenced by mining activity in the 19th century. Moss concentrations of Pb, As, Sb and Cd were converted to deposition rates using regression relationships determined by previous studies in the UK and Scandinavia; in addition, historical Pb deposition scenarios were constructed for each catchment from local peat and lake sediment archives and records of metal deposition, emissions and extraction. Despite the considerable scatter in the data from the herbaria samples, there was broad agreement in both absolute and relative terms with historical Pb deposition trends estimated from other sources, with the exception of one catchment, in the Lake District, where very localized mining activity may have influenced deposition rates.

The results demonstrate the feasibility of reconstructing past Pb, As, Sb and Cd deposition over a period of 150 years using herbaria samples The ability to reconstruct deposition histories for other metals is currently limited by the lack of data on the relationship between moss metal concentrations and rates of deposition.

THE EFFECT OF NITROGEN DEPOSITION ON HEATHLAND SYSTEMS

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Until recently little influence has been placed on the ecological consequences of perturbations of nutrient cycles by human activities. Initially, nitrogen (N) deposition was viewed without any sense of environmental concern due to the accepted nutrient properties of deposited N and its possible fertilising action in the terrestrial environment. However, there is strong evidence that the enhanced nitrogen deposition experienced in Europe has resulted in nutrient poor semi-natural ecosystems becoming less limited by nitrogen. Consequently, the prediction of the impact of N on the environment when it accumulates at levels above which ecosystems such as lowland heath have adapted is the focus of much scientific attention.

Within the UK, the area of heathland habitats has declined over the past 250 years, and now English Nature (2004) estimates lowland heath habitat covers just 41,000 ha., representing 20% of the European total. The need to protect these unstable habitats was recognised by the legislative bodies as early as the 1980s. However, protective legislation has not guaranteed the conservation of this habitat. Changes as a result of enhanced nitrogen availability and resulting stress factors are subtle and may only be noticed when whole ecosystems are affected. At present, it is unclear how this extra nitrogen is processed within the system, and even less is known on the effect of management on the response of heathlands to enhanced nitrogen deposition.

The main aims of the investigation were to determine the effects and fate of deposited nitrogen on a heathland system with a particular reference to the effects on nutrient cycling and the soil microbial community and to determine responses to nitrogen following different management treatments. This was achieved by applying new approaches to a long term manipulation study at Thursley Common, Surrey.

NITROGEN (N)-MODELLING IN MOSSES: FROM METABOLITES TO MEMBRANES

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Anthropogenic nitrogen (N) deposition from burning fossil fuels, applying fertilisers and growing legumes has resulted in an unnatural accumulation of N in atmospheric, terrestrial and marine environments. Recent research, such as the GANE (Global Nitrogen Enrichment) initiative, into N deposition effects has shown trends towards long-term environmental change.

Following on from research into the 'Foliar Uptake and Assimilation of Nitrogen in Bryophytes' (see poster), the rapid initial induction of nitrate reductase activity (NRA) was investigated further using inorganic-N and other established NRA inducers in both *Mnium hornum* and *Sphagnum fimbriatum*. From this work N-status models of both mosses were established over time.

Interestingly the moss *Mnium hornum* had a tremendous capacity to sustain itself whilst undergoing N-starved conditions. N-modelling focusing on *Mnium hornum* demonstrates that the NRA induction capacity of the moss is effected in accordance with its N-status and shows important relationships between the plants total % N, its tissue nitrate (NO_3^-) concentrations and other metabolites.

Further work using micro-electrodes and mV changes within *Mnium hornum* cells has investigated the NO_3^- assimilatory pathways across the cell membrane following inorganic-N and other NRA inducer treatments. These responses, along with continuing work on NRA and pH electrodes, should help further our understanding of these novel rapid responses in *Mnium hornum*.

The research has implications in the physiological partitioning of N within mosses and plant cell systems. It is also important with regards to monitoring environmental changes due to increased N deposition.

The protection of the environment, in conjunction with sustainable agriculture and industry, is important world-wide and this research will further our understanding of the implications of increased anthropogenic N on the environment.

NITROGEN BIO-MONITORING IN THE PEAK DISTRICT USING THE MOSS *HYPNUM JUTLANDICUM*

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More than any other National Park of Britain, the Peak District has received a large pollution load from the Industrial revolution to the present day. The majority of the moorlands in the region are in so-called 'unfavourable' condition and while the contribution of air pollution to the present condition of the moorlands remains uncertain it is likely to be significant. Nitrogen compounds are probably the most important air pollutants in terms of risk to semi-natural ecosystems and continued excessive N deposition to the Peak district moorlands limits their potential for achieving and sustaining 'favourable' condition which, in the SAC/SPA areas, compromises obligations under the European Habitats directive.

However, the actual extent of the N pollution problem in the Peak Park is uncertain for several reasons. Firstly, actual air pollution, rather than modeled data, is routinely monitored at only a few sites in the National Park; secondly, it is difficult to estimate the historical N pollution inputs and their residual effects. Thirdly, the cause and effect relationships remain poorly understood. Some of these difficulties may be overcome by the use of bio-monitors if they can provide detailed spatial mapping of the extent of N pollution and validation of its effects in areas where risk is perceived.

Several potential bio-monitoring tools, ranging from biochemical signals to changes in plant community composition, have been proposed. As part of a wider study of plant and soil bio-indicators in the Peak District for the *Moors for the Future* programme, this work reports on a detailed investigation of tissue Nitrogen concentration in *Hypnum jutlandicum* in the Peak District moorlands during summer 2004.

IMPACTS OF NITROGEN ENRICHMENT ON MONTANE HEATH IN WALES AND POTENTIAL FOR RECOVERY

Imogen Pearce[1], Andrea Britton[2], Barbara Jones[3]

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Atmospheric nitrogen (N) deposition is known to impact low-nutrient communities, causing loss of N sensitive species. Bryophytes are especially vulnerable to detrimental effects due to direct N toxicity and competition from the spread of more N tolerant vascular plants. Historical evidence suggests that sub-alpine moss heath, a unique community at the most southwest of its biogeographical range, has become degraded or lost over past decades, especially in northern England and Wales. Increasing atmospheric N pollution and sheep grazing have both been implicated in this habitat decline. Experimental studies in Scotland have shown that a raised N supply does indeed cause degradation of montane moss heath. There is therefore an urgent need to better understand the drivers of this habitat loss, and how targeted management can both conserve remaining heath and enable its recovery.

Work was carried out on the Carneddau summit ridge, Snowdonia, North Wales, to investigate current condition of the montane communities compared to less polluted montane heath in Scotland. Thirty-seven plots were laid out across the summit ridge, and vegetation cover, tissue chemistry and soil chemistry (including C,N,P, exchangeable cations and acidity) were sampled. Tissue N and P in the dominant N-sensitive moss, *Racomitrium,* and shoot growth were also measured. Soil chemistry suggested a build-up of nitrogen. *Racomitrium* tissue chemistry also indicated high pollution loading compared to montane heath in Scotland. Although tissue chemistry and physiology of the moss was comparable with that in Scottish *Racomitrium* heath that had been subjected to N additions of 10 kg N ha^{-1} yr^{-1} above the background rate (estimated as 18 kg N ha^{-1} yr^{-1}) for 6 years, it's growth rate was higher than would be predicted from the Scottish experimental results.

The most degraded areas along the ridge were characterised by low moss cover, large areas of bare soil and loss of the upper organic horizon, highest *Racomitrium* tissue N and lowest shoot growth. Degraded areas also had the greatest sheep presence, as measured by dung counts, and there was a close relationship between moss tissue N content and dung count. Localised heavy sheep grazing may therefore enhance habitat degradation. The implications of both high N pollution loading, and heavy grazing impacts, on recovery potential of moss heath habitat on the Carneddau are discussed.

TEMPORAL DYNAMICS OF NITRATE, AMMONIUM AND PHOSPHATE LEACHING FROM N POLLUTED SEMI-NATURAL GRASSLANDS

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The impacts of increased N deposition on inorganic N and P leaching from acidic and calcareous grasslands have been studied in detail. Intact, 15cm diameter, soil/turf cores were removed from experimental field plots receiving either ambient or long-term (8 yr) simulated enhanced N deposition (at rates of +3.5 or +14 g N m^{-2} yr^{-1} applied as NH_4NO_3 solution). These cores were placed in lysimeters allowing regular (up to twice weekly) collections of leachate to be made. A 12 month campaign of leachate sampling and analysis has provided a highly detailed data-set on leaching dynamics of nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^-) from these semi-natural acidic and calcareous grasslands under ambient and enhanced rates of N deposition.

In the calcareous grassland, NO_3^- leaching (under ambient N deposition) was up to an order of magnitude greater in January and February (peaking in early February) compared to leaching from May to December. Increased N deposition resulted in a similar temporal pattern of NO_3^- leaching (though at higher absolute rates), though the greater leaching in January and February seen under ambient conditions was extended through March and April. NH_4^+ leaching was also greatest in January and February, but in contrast to NO_3^-, greater rates of leaching were also observed between October and December. Increased N deposition had no impact on the temporal dynamics of NH_4^+ leaching and only minor impacts on absolute rates of leaching.

In the acidic grassland, greatest rates of NO_3^- leaching were observed between October and January and between April and August (despite low rainfall). Under the highest N deposition treatment (14 g N), NO_3^- leaching was comparable between different times of year, suggesting N saturation by this deposition rate occurs across all seasons. NH_4^+ leaching was greatest from October to January and declined throughout the rest of the year. N deposition had no effect on temporal dynamics of NH_4^+ leaching (though absolute rates were higher).

In both grasslands, greatest rates of PO_4^- leaching were observed during autumn and winter (October to February), with minimal leaching during March and April. A mid-July peak in PO_4^- leaching was also observed. In the calcareous grassland, reduced PO_4^- leaching under the 14 g N treatment was observed. In the acidic grassland, reduced PO_4^- leaching under the 14 g N treatment was also observed, though in contrast, the 3.5 g N treatment tended to increase PO_4^- leaching.

These fine-scale temporal data suggest that in most cases, while precipitation may be the primary driver of nutrient leaching on a day-to-day basis, biotic processes may be dominant drivers on a seasonal basis. Implications for N saturation and ground-water pollution are discussed.

QUANTIFYING THE RELATIONSHIP BETWEEN CONCENTRATION DEPENDENT DEPOSITION VELOCITIES AND NH_3 CONCENTRATIONS FOR SEMI NATURAL VEGETATION USING A NH_3 FLUX SYSTEM

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Keywords: Dry deposition, canopy resistance, *Calluna vulgaris*, Sphagnum sp.

Dry deposition modelling using inferential methods currently assumes that deposition velocity is independant of NH_3 concentration. Field measurements at a moorland site in south Scotland, suggested this was not the case; for low atmospheric ammonia concentrations, deposition velocity (Vd) decreased with increasing concentrations. A flux chamber was developed to investigate parameters influencing NH_3 deposition and to enable accurate quantification of Vd and canopy resistance (R_c). Results for a range of moorland semi-natural vegetation are reported here. Detailed study and testing of the chamber confirmed that chamber conditions, especially internal air mixing, were optimum for measuring NH_3 deposition.

The effects of interactions between NH_3 concentration and vegetation type on deposition rates were studied with *Calluna vulgaris* (L.) Hull., a combination of *Sphagnum capillifolium* (Ehrh.) Hedw. and *Sphagnum papillosum* Linb., and a mixed moorland canopy. Vd decreased (R_c increased) with increasing NH_3 concentration for the mixed canopy and the *C. vulgaris.* Increasing NH_3 concentrations did not affect Vd or R_c for the combined Sphagnum species

The linear increase between Rc and NH_3 concentrations for the mixed canopy and Calluna shows that conventional dry deposition modelling, especially close to NH_3 sources, systematically overestimates NH_3 deposition rates.

STATE OF THE HEALTH OF BEECH TREES IN EPPING FOREST

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Located in the north east of London, Epping Forest is a 2,500 ha open space which is downwind of London. The area contains beech forest, dry heath and wet heath, that are Special Area of Conservation habitats and two-thirds of the Forest is a SSSI. A preliminary study indicated that these habitats may be detrimentally affected by air quality; sources of pollutants come both from London and the local road network, including the M25.

This study is investigating the effects of air pollution on the habitats above. In 2004 the spotlight was on beech trees. In the summer, soil and leaf samples were collected from healthy and unhealthy trees for chemical analysis. In addition the health of the trees was assessed by scoring crown architecture and the foliage through a standard set of criteria. Beech trees in Burnham Beeches (Buckinghamshire) and the New Forest (Hampshire) were also assessed for crown health providing standard sites for comparison.

In addition, a pollution monitoring network was set up through the Forest to measure levels of NO, NO_2 and NH_3. Sites were located in beech woods and heathlands, in background and roadside positions, in order to obtain an overview of the pollution climate through the forest. The presentation will examine the results of the work detailed above.

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ECOLOGICAL IMPACTS OF AIR POLLUTION FROM ROAD TRANSPORT ON LOCAL VEGETATION

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Motor vehicles emit a cocktail of pollutants and are a major contributor to air pollution in many areas, both rural and urban, but realtively little is known regarding the ecological impacts of air pollution from roads on adjacent vegetation. Data are presented from studies conducted along transects away from roads at three sites adjacent to two major motorways in the UK: the M62 motorway in West Yorkshire, and the M40 in Buckinghamshire, comprising two woodland and one blanket bog site. Surveys of oak and beech tree health (at the woodland sites) and species composition (at the blanket bog site) were undertaken on transects away from the motorway. In addition, material of six bryophyte and three lichen species was transplanted from relatively 'clean-air' sites to different distances from the motorway at two sites.

The oak tree health survey found increased defoliation and insect damage near to the motorway, and the beech tree health survey also found poorer crown condition close to the motorway. No clear relationship was found between distance from the road and vegetation species diversity at the moorland site. However, the moss *Polytrichum commune* showed a significant decline in frequency with distance from the motorway. In a number of the transplanted bryophyte species, chlorophyll concentrations, membrane leakage and growth significantly increased with proximity to the motorway. Nitrogen concentration was determined for one species from each site and found to be significantly higher near the road in the woodland transplant. Few significant responses were seen in the lichen species tested, with the exception of increased visible damage or loss of electrolytes near to the motorway.

The 'edge effect' of the road was estimated to extend to approximately 100 m at these two sites, according to the measured parameters. This distance was consistent with the measured profile of NO_2, which dropped to background levels at about 100 m. The results suggest that the mixture of air pollutants from road transport can have significant ecological impacts within 100 m of major roads. It is likely that nitrogen oxides are the dominant pollutant within this mixture in terms of impacts on vegetation.

IMPACTS OF OZONE ON WOODLAND GROUND FLORA

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The impacts of ozone on crop yield and forest growth are well established, but those on the species composition of semi-natural vegetation are much less certain. A number of studies have examined the impacts of ozone on individual species, or on artificial mesocosms, but very few studies have examined the effects of ozone on real communities. Furthermore, the focus of these studies has been the growth and reproduction of established plants and communities, rather than the phase of seedling emergence and establishment. In communities such as ground flora of deciduous woodlands, species are adapted to short growth periods in the spring, and hence delays in establishment and development caused by ozone could have significant ecological consequences.

We studied the effects of exposure to 80ppb ozone on the emergence and development of woodland species from bare soil through to canopy closure. Mesocosms were established by collecting soil from Grass Wood in the Yorkshire Dales National Park, UK, and placing it in trays within fumigation chambers. Four of the chambers, each of which contained two or three replicate trays, received filtered air, while the other four chambers received a target concentration of 80ppb ozone for 8 hours per day. The light intensity reaching the plants was reduced in three stages: from weeks 1-4 there was no reduction, during weeks 5-8 photon flux densities were reduced by 50% and from weeks 9 onwards photon flux densities were reduced by 62%.

Several species showed characteristic symptoms of visible injury within a few days of emergence, followed by early leaf senescence. This appearance of visible injury then declined both in terms of the number of number of species affected and the extent of injury symptoms. However, when irradiance levels were reduced, there was an increase in the quantity of symptoms, with typical symptoms of ozone damage being found on species such as *Hypericum pulchrum, Glechoma hederacea, Lysimachia nummularia & Planatgo lanceolata*. Exposure to ozone caused a shift in the balance between grass and forb species, with grass cover decreasing relative to forb species, and ruderal species increasing in cover. The net effect was an increase in species diversity in the ozone-treated mesocosms, primarily because of the wider range of forb species. The impact of ozone on species composition changed with the decrease light penetration. The results demonstrate that ozone may have significant effects on woodland ground flora composition. The interactions between irradiance and ozone exposure found in this study suggest that the development of shade and changing ozone exposures over the spring and summer months may have complex impacts on woodland plant communities.

COMBINING SCIENCE AND REGULATION FOR IMPROVING ENVIRONMENTAL PROTECTION

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Atmospheric pollutants, sulphur dioxide (SO_2) and nitrogen (oxidized and reduced) are regarded as the main contributors to the acidification of sensitive ecosystems in Scotland. Emissions of sulphur have declined significantly since their peak in the 1970's, whereas emissions of nitrogen oxides (NO_x) have declined since the early 1990's. However, acidification and eutrophication still pose a major threat to the Scottish natural environment, with the potential acidification from nitrogen deposition now substantially exceeding that of sulphur, whilst the total nitrogen deposition, mainly responsible for eutrophication in both the UK and Scotland is dominated by reduced N (as NH_3 and NH_4^+).

Ammonia (NH_3) emissions are dominated by agricultural sources. However, source attribution of such emissions are uncertain because they depend on a number of complex factors that make interpretation of data difficult (e.g. decomposition rates, waste management, livestock, fertiliser use). In Scotland, cattle are the dominant source of ammonia production, with intensive agriculture (pig and poultry sector) dominating localised source attribution for NH_3 deposition on local sensitive receptor sites.

SEPA has significant statutory responsibilities requiring the protection of Special Areas of Conservation and Special Protection Areas (under the Conservation (Natural Habitats, &c.) Regulations 1994), as well as Sites of Special Scientific Interest and biodiversity more generally under the Nature Conservation (Scotland) Act 2004. The successful implementation of these duties requires that regulatory activities be supported by the best available scientific understanding on ecosystem impacts associated with airborne pollutants. Regulatory staff require ecological scientific input during the assessment and licensing of applications, and the subsequent monitoring and review stages of the regulatory cycle. The paper will highlight the appropriate use of current scientific information and potential application through the regulatory role of SEPA.

It is also recognised that air pollution does not respect the geographical boundaries of organisational responsibility. In order to tackle air pollution impacts on protected sites in an integrated and holistic manner a major revision is taking place in the way air pollution is assessed and regulated in the UK. The paper will provide more details of the steps taken so far, the tools being developed and the work in progress to achieve collaboration and maximise protection of our natural heritage.

THE DEFRA-NERC TERRESTRIAL UMBRELLA: NOW AND IN THE FUTURE

Bridget Emmett

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The Defra-NERC Terrestrial Umbrella has been funding research into the impacts of acidic and eutrophying air pollution in terrestrial systems for many years. The focus of the work has changed following the policy needs and scientific questions but has always included a commitment to long term manipulation experiments, process studies and modelling. An outline of the current work will be given together with suggestions on gaps and future needs to be followed by a discussion session.

WHAT SHOULD BE THE WAY FORWARD? ASSESSING METHODS TO INVESTIGATE POLLUTANT IMPACTS.

C. J. Stevens
Imperial College.

Making observations across a UK-wide gradient of nitrogen deposition was very challenging. We were attempting to determine the impact of nitrogen deposition amongst a great deal of noise from other variables. Despite this the study gave very interesting results. We showed a clear decline in the species richness of UK acid grasslands correlated with increasing nitrogen deposition. The same could be said about other large scale observational studies including the countryside survey, which showed changes in the Ellenberg N (nutrient status) score with increasing nitrogen deposition, but the number of variables that could be potentially influencing the species present over such a large area is considerable.

The value of such observational studies has been questioned as there is no way the effects can be statistically identified as causal (rather than just a correlation) and there are difficulties separating the impacts of the variable in question from other potentially confounding variables. Indeed this may not be possible at all. I will briefly examine the difficulties of such large scale studies and discuss the variables that should be measured in the field given limited time, financial resources and the differing scales at which they vary and are measured at. I will also discuss whether observational studies are the best way to detect the effect of pollutants on grasslands or should we in fact continue to invest our resources predominantly in experimental manipulations.

Whether we chose to work on experimental studies, large scale gradients or modelling, scale is a very important issue. Are the results of experiments or observations carried out at a small scale relevant to the wider landscape and what is the best scale to work at to address policy questions? Large scale long term manipulation studies would perhaps be the ideal, however, they are not feasible and only yield results after a long time period which raises the question what is the compromise we should be coming to?

QTLs GOVERNING OZONE IMPACTS ON WHEAT YIELD

†Colin Gillespie[1], †Agnieszka Kaminska[1,2], Ali Dodmani, Paul Bilsborrow[3], Steve Quarrie[1,4], Jeremy Barnes[1]

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Despite 30 years' few efforts have been directed toward the identification of genetic traits governing the impacts of ozone on vegetation. This poster reports findings from a well-replicated study performed in open top chambers on a population of 96 recombinant inbred wheat lines, for which there is a comprehensive genetic map, in a bid to identify QTLs (quantitative trait loci) governing ozone "tolerance".

In toto, >7000 plants (96 inbred lines plus parents) resulting from a cross between Chinese Spring and SQ1 spring wheat were exposed in 16 open top chambers (OTCs) to four levels of ozone (NFA, NFA+25 ppb, NFA+50 ppb, NFA+75 ppb) during the 2003 growing season. Impacts of ozone on yield and yield components were determined and QTLs mapped and ranked.

Parental lines displayed considerable variation in their response to ozone, and there was considerable genetic variation between lines raised in 'clean air' as well as in the response of the lines to challenge by ozone. In sensitive lines, ozone exposure reduced yield by up to 50%, with no effect on others. Data revealed much coincidence with yield and component QTLs identified in previous independent experiments conducted over the past decade on the same mapping population. Data also exhibited a reassuring coincidence in yield and yield component QTLs across ozone treatments. Chromosomes 5A and 5D were identified as containing QTLs specifically governing the impact of ozone on wheat yield

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EFFECTS OF OZONE ON CARBON METABOLISM *IN PLANTA*.

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The principal objective of the work presented was to test the hypothesis that ozone tolerance is determined by the ability of plants to mobilize carbohydrates for repair purposes. Fifteen partially-mapped ecotypes of *Arabidopsis thaliana* were screened for ozone resistance/sensitivity. Plants were fumigated with charcoal/Purafil® - filtered air (CFA) or CFA + ozone (15 nmol mol^{-1} overnight rising for 8h per day to 250 ppb) then harvested and total seed weight per plant, the weight of 100 seeds, specific leaf area (SLA), and rosette fresh/dry weight to determine resistance / sensitivity. Three genotypes were selected for detailed analysis based on their contrasting responses to O_3; Nok-3 (resistant), WS (sensitive) and the stress susceptible mutant (LT2404). Measurements using a leaf disc oxygen electrode revealed a substantial decline in photosynthetic capacity in WS and LT2404 – a finding consistent with the observed decline in foliar carbohydrate content in WS, but not Lt2404. The 'resistant' genotype, on the other hand, displayed no change in photosynthetic capacity when exposed to the same O_3 environment. High Performance Liquid Chromatography (HPLC) profiling of non-structural soluble carbohydrates revealed a significant decline (30-70%) in foliar starch and soluble content in Nok-3 and WS. In contrast, there was little change in the starch content of O_3-treated foliage of LT2404. Native-PAGE for starch degrading enzymes revealed shifts in the activity of specific amylase isoforms as well as in chloroplastic starch phosphorylase activity in the 'susceptible' genotypes, that were not apparent in the 'resistant' strain, Nok-3. This is consistent with reports by Zeeman *et al*, (2004) that chloroplastic starch phosphorylase could play a role in stress resistance.

These dats imply that ozone tolerance may be determined by the ability to sustain high rates of photosynthesis and carbohydrate mobilisation. Downstream investigations will investigate the impacts of ozone on starch excess and starch deficient mutants of *Arabidopsis* in a bid to determine if ozone tolerance is dictated by the amount of starch that can be mobilised under oxidative conditions.

MECHANISMS UNDERLYING THE IMPACTS OF OZONE ON CARBON ASSIMILATION

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Lactuca sativa L. cvs. Paris Island and Grenada were exposed in controlled environment chambers to either charcoal/Purafil®-filtered air (CFA) or CFA plus ozone (75 nmol mol^{-1} or 100 nmol mol^{-1} between 8:00 and 16:00 h). 'Sensitivity' to ozone was gauged in terms of visible foliar injury and effects on relative growth, biomass, stomatal conductance and the capacity for carbon assimilation.

Plants exposed to ozone exhibited reduced growth rates and decreased biomass accumulation after 24 and 40 d. Both genotypes were sensitive to ozone in terms of effects on shoot dry weight; environmentally-relevant ozone exposure resulting in a 25% reduction shoot weight. In contrast to generally-observed patterns of change in dry matter partitioning driven by ozone, no significant change in root:shoot investment was observed in the present study.

Diagnostic gas exchange measurements revealed an O_3-induced decline in the light- and CO_2-saturated rate of CO_2 assimilation (A_{max}). This was accompanied by a parallel reduction in Rubisco activity (V_{cmax}), and the maximum capacity for RuBP regeneration (J_{max}). No changes were observed in the relative stomatal limitation (RSL) of CO_2 assimilation in ozone-treated plants. Furthermore, no significant change was found in the quantum yield of CO_2 assimilation, suggesting effects on the capacity for CO_2 assimilation were attributable to shifts in carboxylation efficiency rather than maximal PSII photochemical efficiency or PSII-mediated electron flow.

Current studies are focusing on the molecular basis for the observed decrease in carboxylation efficiency in the ozone-treated plants.

Keywords: ozone, lettuce, photosynthesis, carboxylation efficiency, CO_2 assimilation, dry weight

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IMPACTS OF OZONE ON ASPECTS OF PLANT REPRODUCTIVE BIOLOGY

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Impacts of ozone on plant reproductive processes, and interactions with insect pollinators, are poorly understood. In the present study, rc-*Brassica napus* L. mutants were employed as a vehicle to probe such interactions.

Environmentally-relevant concentrations of ozone were shown to depress pollen germination and pollen germ tube development *in vitro*. Moreover, exposure to ozone depressed nectar production and HPLC profiling of nectar composition revealed a marked increase in nectar glucose and fructose content. Impacts on nectar quantity and quality were reflected in marked changes in feeding preference and insect development (i.e. butterfly longevity and fecundity).

The findings of this study will be presented in a bid to highlight their significance in a wider ecological context.

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COMPARISON OF A MULTIPLICATIVE AND A PHOTOSYNTHESIS-BASED ALGORITHM FOR MODELLING STOMATAL OZONE FLUXES TO HIGHER PLANTS

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A purely empirical (i.e. multiplicative) approach to model stomatal ozone fluxes to plants was compared with a more mechanistic approach, which directly links stomatal conductance to photosynthesis. Both types of algorithms have been tested with several European datasets (e.g. grapevine, wheat, Scots pine) to investigate the advantages and disadvantages of both approaches within the context of modelling ozone deposition at both site-specific and regional scales.

OZONE EXPOSURE-RESPONSE RELATIONSHIPS FOR WINTER WHEAT

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Ozone-exposure response relationships have been defined for spring wheat, but not winter wheat – by far the most commercially important crop in a UK agricultural context.

During the 2004 growing season, sixteen (partially mapped) commercially-grown British and Polish winter wheat cultivars were grown in 12 open-top chambers at Newcastle University's Close House Field Station. These cultivars span commercial uses for bread-making, biscuit-making and animal feed. Plants were grown at planting densities equivalent to those in the field and exposed in triplicate chambers to four targeted ozone treatments: non-filtered air (NFA), NFA+25 ppb, NFA+50 ppb and NFA+75 ppb ozone.

Exposure-response relationships were established for effects of ozone on yield and there was considerable variation between genotypes in their response to ozone. At the highest ozone concentrations, yield reductions ranged from 5% in 'Beaver' to 30% in 'Rialto'. The effects of ozone on yield were largely due to a decrease in 1000 grain weight, seed number/ear and number of grains/plant.

Strong relationships were shown between the AOT40 and the decline in yield, but better relationships were established employing stomatal flux calculated using the big-leaf approach of Emberson *et al*. (1999).

IMPACTS OF ATMOSPHERIC OZONE-ENRICHMENT ON PLANT-PATHOGEN INTERACTIONS

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Ozone is considered the most economically important air pollutant worldwide. Experimental studies reveal contrasting effects of ozone on foliar pathogens. The present studies focus on the impacts of the gas on fungal infection of fruit. Organically-grown tomato fruit (*Lycopersicon esculentum L.*) were exposed to ozone concentrations ranging between 5 (controls) and 200 ppb, then inoculated with specific pathogens. Ozone exposure suppressed lesion development/spore production, spore germination (with clear dose*response relationships evident). *In vitro* studies performed on fungi raised on Potato Dextrose Agar (PDA) revealed no direct effects of ozone on fungal development *per se*, implying that suppression of pathogen development was due in a large part to impacts of ozone on fruit-pathogen interactions, rather than fungi *per se*. 'Vaccination' was accompanied by shifts in the expression of several defence-related genes (e.g. *Aco1* and *Aos1* (signalling genes), *Chi 3a, Chi 9b, Gluc ac* and *Gluc bs* (PR genes*)* and *Hpl* (oxidative metabolism)), plus beneficial changes in quality-related attributes and organoleptic characteristics. Work is currently focussing on the mechanisms underlying the impacts of ozone on pathogen development and the role played by the identified shifts in gene expression in modulating the response.

This work was supported partly by National Foundation of Greece (IKY). The findings are covered by a European PCT application. Enquiries relating to the immediate commercial exploitation of these findings should be directed to BioFresh Ltd (http://www.bio-fresh.co.uk/).

Keywords: *ozone-enriched atmospheres; plant-pathogen interactions; tomato fruit; defence-related gene expression*

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THE ROLE OF APOPLASTIC ANTIOXIDATIVE CAPACITY IN O_3 TOLERANCE AND AN O_3 MICROARRAY EXPERIMENT

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The proposed protective role of apoplastic antioxidants against the adverse effects of O_3 led us to investigate the levels of ascorbate (ASC), glutathione derivatives (GSH) and total antioxidative capacity in the apoplast of two white clover clones (*Trifolium repens L.*) exhibiting differential sensitivity to O_3. Against all expectations, three independent experiments proved that apoplastic ASC was significantly more abundant in the O_3-sensitive clone (NC-S) than in the O_3-tolerant clone (NC-R). During a five day exposure to 71.1 ± 1.6 $nL \cdot L^{-1}$ O_3, 7 hours per day, apoplastic ASC even increased in NC-S. Similar results were found for the pool of undetermined antioxidants. Our data show that not all cases of differential O_3 tolerance originate from differences in apoplastic antioxidant levels. Based on scanning electron micrographs of sectioned leaves and stomatal conductance measurements, it was shown that neither stomatal conductance (g_s) nor the intercellular space conductance (g_i) contributed to the differential O_3 tolerance of the selected clover clones. In our quest to determine other factors causing the differential O_3 sensitivity in NC-S and NC-R, we also investigated the turnover rate of total ASC and GSH-derivatives by performing pulse-chase experiments using radioactively labelled precursors ^{14}C-mannose and ^{14}C-glutamate. In contrast to the absolute concentrations of these antioxidants, their turnover rates could much better explain the differences in O_3 tolerance. These findings led us to concludee that the sensitivity of NC-S may be determined by disturbed communication between apoplast and symplast ASC pools.

In a bid to further probe the reasons underlying the differential O3 sensitivty of the selected clover clones, microarray analyses were performed on *Arabidopsis* plants exposed to 150 $nL \cdot L^{-1}$ O_3, 8 hours per day over two days. The expression of about 21 500 genes was assessed using Agilent Arabidopsis 2 Oligo Microarrays, with expression profile reliability confirmed by quantitative real-time PCR of nine selected genes. Among the transcripts affected by O_3 were many genes implicated in the reorganisation and stiffening of the cell wall. Furthermore, O_3 induced genes involved in cell death and senescence, starch hydrolysis, antimicrobial activity, anthocyanin diglucosides, protein folding capacity, GSH-*S*-transferase activity and possibly also GSH synthesis. Unexpectedly, genes encoding for jasmonate and ethylene signalling were down-regulated, possibly because of the reported transient nature of their expression level following the onset of oxidative stress. Other pathways down-regulated under O_3 included lipid breakdown and the synthesis of alkyl cinnamates, phenylpyruvate and coumarate. Many O_3-induced shifts in gene expression were noted for the first time e.g. heat shock proteins, abscisic acid-responsive genes, xyloglucan xyloglucosyl transferases, the dirigent protein, gibberellin oxidases, □-dioxygenase, nitrilase thioredoxins and glutaredoxins.

Posters

SUPPRESSION OF GREY MOULD (BOTRYTIS CINEREA) ON TOMATO FRUIT BY OZONE

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The purpose of this study was to examine the potential of atmospheric ozone-enrichment for the preservation of fresh produce. Organically-grown tomato fruit (*Lycopersicon esculentum, L*, cv. *Mareta*) were inoculated with grey mould (*Botrytis cinerea*) and exposed to ozone concentrations ranging between 0.005 (controls) and 5.0 ppm. Ozone-enrichment resulted in a marked reduction in lesion development, with higher concentrations/duration of exposure resulting in greater suppression of lesion development (i.e. clear dose*response relationship). Impacts of ozone on lesion development were mirrored by a marked decline in spore production in treated fruit. *In vitro* studies performed on fungi raised on Potato Dextrose Agar (PDA) for six days at 13^{o}C and 95% relative humidity (RH) revealed no significant effects of ozone on fungal development *per se*. Interestingly, however, fruit exposed to even the lowest level of ozone (0.05 ppm) were shown to display suppressed expression of several defence-related genes (e.g. *Aco 1* and *Aos* (signalling genes), *Chi 3a*, *Chi 9b*, *Gluc ac* and *Gluc bs* (PR genes) and *Hpl* (oxidative metabolism)). Work is currently focussing on the mechanisms underlying the impacts of ozone on plant-pathogen interaction and the role played by the identified shifts in gene expression in modulating the response.

This work was supported partly by National Foundation of Greece (IKY). The findings are covered by a European PCT application.

Keywords: tomato; ozone-enriched atmospheres; *Botrytis cinerea*; defence-related gene expression

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ESTIMATION OF OZONE-INDUCED YIELD LOSSES IN SPANISH CEREAL CROPS USING OZONE EXPOSURE INDICES AND CUMULATIVE UPTAKE APPROACHES

De la Torre, D., González Fernández, I., González, A., Gimeno, B. S.

New ozone critical levels for agricultural crops have been defined (UNECE, 2004) based on foliar uptake of the pollutant rather than relying on exposure-based indices. Several approaches have been considered for the calculation of stomatal ozone flux and thus, the determination of ozone uptake.

This poster reports a study in which ozone deposition fluxes for a commercially-grown durum (*Triticum durum* L.) wheat crop were determined and compared with calculated ozone stomatal fluxes (F_{st}) using different models (Baldocchi *et al*., 1987, Erisman *et al*., 1994, Coyle *et al*., 2000) including the multiplicative approach adopted by Emberson *et al*., (2000). The experimental site was located near Madrid, Spain.

Estimates of ozone-induced yield losses based on the accumulated fluxes of ozone (AF_{st}), calculated using an approach similar to that adopted by Pleijel *et al*., (2003) and Danielsson *et al*., (2003), are compared with exposure-response data derived using the AOT40 approach proposed by Fuhrer *et al*. (1997). Relative yield losses are compared for several varieties across two irrigation treatments (representative of Northem European and Mediterranean conditions, respectively).

EFFECTS OF 2 YEARS EXPOSURE TO AMMONIA ON THE M19 VEGETATION AT WHIM MOSS

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At Whim Moss, NVC M19, dominated by *Calluna vulgaris, Erica tetralix, Eriophorium vaginatum, Sphagnum capillifolium* and *Cladonia portentosa* . The release of NH_3, simulating emissions from a 100,000 bird poultry unit, has caused visible damage to a variety of vegetation types. This poster illustrates the types of injury found on the sensitive species growing along the release transect.

The lichen, *Cladonia portentosa* was first to succumb and after nearly 3 years has all but disappeared from the 60m transect, a few healthy clumps remaining beyond 50m. The damage has moved, occurring further and further from the source transect as the N deposition accumulates. The initial damage was in response to an input of >100 kg N ha^{-1} spread over 4 months. However, the speed at which the damage was observed closer to the source implies that the gas concentration and not just the cumulative N deposition contributed to the damage. The annual N deposition where damage is being observed 3 years on is ~ 40 kg N ha^{-1}. Interestingly, in the wet treatments damage was observed in the 64 kg N ha^{-1} plots.

Damage has also been seen on many Sphagnum clumps and the pleurocarpous mosses. Judging from the visible appearance *Sphagnum capillifolium* appeared to be more NH_3-N tolerant in the desiccated state. In January 2005, when samples were removed for analysis the *S. capillifolium* capitulum broke away easily from the stems of all samples along the first 40 m of the transect. Inputs at these positions exceeded 50 kg N ha^{-1}, calculated from NH_3 concentrations measured 0.1m above the vegetation, taking in to account canopy height, influential meteorological variables at the time of release and a concentration dependent R_c. Interestingly, damage was not apparent in the 'red' *S. capillifolium* suggesting the beta carotenes may offer protection against NH_3-N toxicity. By contrast *Hypnum jutlandium* appeared to be relatively NH_3-N tolerant. *Polytrichum commune* an acrocarpous moss was highly NH_3-N sensitive but capable of recovery possibly suggesting a response to concentration rather than accumulated dose.

Amongst the higher plants damage has been restricted to Calluna while *Erica tetralix* appears to be thriving. Some suggestions as to why this is the case will be offered for comment.

OZONE-ENRICHED ATMOSPHERES ENHANCE TOMATO FRUIT QUALITY

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Organically-grown tomato fruit (*Lycopersicon esculentum L.*, cv. *Carousel*) were exposed to ozone concentrations ranging between 0.005 (controls) and 1.0 ppm at 13 ^{o}C and 95% RH. Quality-related attributes and organoleptic characteristics were examined during and following exposure. Ozone-enrichment increased soluble sugar content (glucose, fructose) content and depressed organic acid content (citric, malic) and the effects persisted following transfer to 'clean air'. Moreover fruit firmness was maintained in ozone-treated fruit in comparison with the count-parts maintained in 'clean air'. Fruit (pulp and seed) antioxidant status and Vitamin C content was constant during low levels of ozone exposure and declined at higher levels of ozone exposure whereas no effects revealed during shelf-life storage. No effects on rates of fruit respiration, transpiration and ethylene emissions were observed during or following ozone exposures. Taste panels and processing trials indicate significant benefits from ozone exposure.

This work was supported by grants from the Royal Society and The National Foundation of Greece (IKY). Enquiries relating to the commercial exploitation of these findings should be directed to BioFresh Ltd (http://www.bio-fresh.co.uk/).

Keywords: fresh produce; ozone-enriched atmospheres; shelf-life; quality-related characteristics

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IMPACT OF ATMOSPHERIC OZONE-ENRICHMENT ON MICROBIAL SPOILAGE OF FRESH PRODUCE

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Tomatoes, strawberries, table grapes and plums were infected with grey mould (*Botrytis cinerea*), then fruit incubated in 'clean air' or an ozone-enriched atmosphere (concentrations ranging from 0.05 to 1.0 ppm). *In vivo* and *in vitro* experimentation revealed lesion development, spore production and spore germination to be markedly reduced in fruit maintained in an ozone-enriched atmosphere. Higher concentrations/duration of exposure resulted in greater impacts on lesion development/spore production (i.e. clear dose*response relationships were evident), with considerable benefits resulting from exposure to low levels of ozone (i.e. below the 0.2 ppm threshold set for the protection of human health). *In vitro*, effects on spore germination depended on concentration and duration of exposure ($P < 0.01$), whereas studies performed on fungi raised on Potato Dextrose Agar (PDA) for 6-7 days at 13ºC and 95% relative humidity (RH) revealed no direct effects of ozone on fungal development *per se*, implying that suppression of pathogen development was due in a large part to the impacts of ozone on fruit-pathogen interactions. Work is currently focussing on the mechanisms underlying the impacts of ozone on disease development.

This work was supported by grants from the Royal Society, The National Foundation of Greece (IKY) and the EU Marie Curie Programme. Enquiries relating to the commercial exploitation of these findings should be directed to BioFresh Ltd (http://www.bio-fresh.co.uk/).

Keywords: fresh produce; ozone-enriched atmospheres; spoilage; *Botrytis cinerea*

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FOLIAR UPTAKE AND ASSIMILATION OF NITROGEN IN BRYOPHYTES

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The aim of this research is to assess how mosses take up nitrogen (N), assimilate this N and sense N status, using biochemical profiling and ion-selective micro-electrodes by measuring changes in metabolite pools.

Mosses are being used as they are non-vascular plants which lack roots and take up nutrients though their external leaf structure. They lack the complex tissues found in higher plants and associated with this the problem of the translocation of metabolites. Mosses acquire N from the atmosphere as nitrate (NO_3^-) or ammonium (NH_4^+) in rainfall or in run-off from groundwater. Once taken up by the cells these ions are metabolised in the cytoplasm.

Two species of moss from contrasting habitats – the bog moss, *Sphagnum fimbriatum*, and the woodland floor moss, *Mnium hornum*, are being used. The rate of NO_3^- uptake and use has been measured by the in vivo nitrate reductase (NR) activity, allied with the tissue NO_3^- concentrations. NR is a substrate inducible enzyme and its induction is therefore indicative of uptake and assimilation of NO_3^-; it is an established indicator for measuring the N status of the plant.

Biochemically both species of moss show a rapid initial induction of NR with NO_3^- treatment, but each has a contrasting NO_3^- storage capacity. This project is bringing together the biochemistry and localised, real-time changes in the tissue concentrations of NO_3^- using the micro-electrodes to explain why these mosses behave so differently.