

P2–PLANT CARBON–NITROGEN INTERACTIONS FROM RHIZOSPHERE TO PLANET

Organised by C.H. Foyer and C.G. Bowsher for the Plant Metabolism Group and Sponsored by the Journal of Experimental Biology

P2.1–The influence of carbon on nitrogen cycling in soil

Keith Goulding, Rothamsted Research

The enzymatic oxidation of soil organic matter (SOM) by a wide range of non-specific microorganisms (mineralization) yields not just energy but also the bulk of the microorganism's requirements for carbon (C) and nitrogen (N). The balance of N is obtained from the microbial assimilation of NH_4^+ and NO_3^- from soil (immobilization). But this assimilation of inorganic N is, in turn, closely linked to the presence of readily available carbonaceous material. Thus the C and N cycles in soil are clearly closely linked. There is a continual transfer of N within the soil from organic to inorganic forms and back again: mineralisation-immobilisation turnover (MIT). The net change determines the amount of inorganic N available for plant uptake and at risk of loss to the environment. It can be crudely related to the C/N ratio of the SOM, crop residues, root exudates, and organic amendments such as straw. Research using the stable isotopes of C, ^{13}C , and N, ^{15}N , has been invaluable for improving our understanding of factors controlling N cycling, testing hypotheses and parameterising and testing C/N cycling models. Another area of current interest is linking N (and other nutrient) cycling processes to microbial ecology. The paper will describe recent experimental research and modelling studies and outline plans for future work on C/N cycling in soils. Ref: Murphy et al. (2003) *Advances in Agronomy*, 79, 69–118.

P2.2–Signalling pathways in the regulation of root responses to nitrate

B.G. Forde, B. Abram, S. Filleur, Y. Gan and A. Rahman, Biological Sciences, Lancaster University

The NO_3^- ion is not only a valuable N source, it also acts as a signal to induce changes in gene expression, to alter the partitioning of growth between roots and shoots and to modify plant morphology (1). In the root, the developmental effects of NO_3^- are most pronounced on lateral root growth and these are of two kinds: 1) a positive effect of external NO_3^- on lateral root elongation and 2) a negative effect on lateral root development

when the plant accumulates high concentrations of NO_3^- . These antagonistic regulatory pathways allow root branching to be modulated according to the plant's demand for N. We are interested in understanding the local signalling pathway that enables the plant to respond to external NO_3^- , and the long-range signalling pathways that inform the root of changes in the shoot's N status.

References

1. Forde BG (2002) *Ann. Rev Plant Biol.* 53, 203–224

P2.3–The regulation of lateral root development – from environmental signals to hormone cross-talk

Hanma Zhang¹, Honglin Rong¹, Na-Kyeong Kim¹, Ive De-Smet¹, Laurent Signora^{1,2} and Christine H. Foyer²

¹Centre for Plant Sciences, School of Biology, University of Leeds, Leeds, LS2 9JT, UK

²Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, U.K.

Lateral root (LR) formation in higher plants displays considerable plasticity in response to many different (external and internal) regulatory signals. One such example is the influence of nitrate nutrition on LR development. In *Arabidopsis*, nitrate has two opposing effects on LRs: a localised stimulation on LR elongation and a systemic inhibition by high nitrate. Our current efforts focus on dissecting the underlying mechanism for the latter effect (the high nitrate-induced LR inhibition) and have led to a conclusion that ABA plays an important part in mediating the high nitrate signal and in LR regulation. Supporting evidence for the above conclusion includes: 1) The inhibitory effect of high NO_3^- is significantly reduced in three ABA insensitive mutants (*abi4-1*, *abi4-2* and *abi5-1*) and all the four ABA deficient mutants (*aba1-1*, *aba2-3*, *aba2-4* and *aba3-2*) examined; 2) exogenous ABA mimics the inhibitory effect of high nitrate on LRs. Detailed morphological analysis reveals that the ABA-induced LR inhibition is reversible and occurs immediately after the emergence at the LR primordium from the parent root. Comparison between LRs developed on the ABA^+ medium and those on the ABA^- medium suggests that there is a

novel ABA-sensitive developmental checkpoint at the activation step of the LR meristem. This novel checkpoint is auxin-independent, as the inhibition can not be rescued by either an exogenous auxin application or enhanced auxin synthesis. The ABA-induced LR inhibition requires 10-fold less ABA than the ABA-induced inhibition on seed germination and is only slightly reduced in characterised *abi* mutants, suggesting that it may involve novel ABA signalling mechanisms. Interestingly, our analysis of LR regulation by nitrate and ABA has also uncovered novel cross-talks between nitrate, ABA and auxin in *Arabidopsis* roots. Data on such cross-talks will be presented at the meeting.

P2.4—Adaptative responses of root development to nitrogen availability and plant growth promoting rhizobacteria

B. Touraine, UMR 1063 Symbioses Méditerranéennes et Tropicales, Université Montpellier 2

Due to their sessile lifestyle, it is crucial for plants to adjust nitrate uptake capacity to nitrogen demand despite the variability in space and time of nitrate availability in soil. Although nitrogen homeostasis can be maintained via the adjustment of nitrate transporters expression and activity when nitrate supply changes rapidly, this process is unlikely to provide an efficient adaptative response in the long term. Rather, plant adaptation to nitrate resource changes seems to rely on sid7883312 the extent to which roots explore their surroundings. This involves dual control pathways of root branching by nitrate, a localized response to external nitrate and a systemic control by internal nitrogen status (1). Key genes involved in the former has been identified (1,2) but the latter remains largely obscure. In addition, roots are normally surrounded by a complex environment, called the rhizosphere, that contains large populations of microorganisms and several of these microbes are likely to affect both root development and nitrate availability or/and nitrate uptake. The two aspects, namely the regulation of root development by nitrogen status and the responses to soil microorganisms, will be illustrated by studies of the responses of root development to nitrate availability and to a plant growth promoting rhizobacteria (PGPR) strain isolated from rape rhizosphere. Approaches designed to identify genes involved in both responses will be presented.

(1) Zhang H, Jennings AJ, Barlow PW, Forde BG 1999 Proc Natl Acad Sci USA 96, 6529–6534

(2) Zhang H, Forde BG 1998 Science 279, 407–409

P2.5—What can mitochondrial electron transport mutants tell us about carbon–nitrogen interactions in leaves?

G Noctor¹, C Dutilleul¹, R De Paepe¹, CH Foyer²

¹Institut de Biotechnologie de Plantes, Université Paris XI, 91405 Orsay, France

²Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

Mitochondria play several roles during photosynthetic leaf metabolism, including glycine oxidation and respiration of other substrates that may be important in producing organic acids for nitrogen assimilation. Both processes require sinks for NAD(P)H. To analyse the importance of mitochondrial electron transport in photosynthetic carbon and nitrogen metabolism, we exploited a *Nicotiana sylvestris* mutant (CMSII) lacking functional mitochondrial complex I. Although dark respiratory rates are not decreased in CMSII leaves, and although the mutant has a photosynthetic capacity at least as high as the WT, CO₂ uptake is significantly decreased in physiological conditions [1]. Moreover, metabolite measurements indicate that loss of complex I function modulates processes involved in nitrogen assimilation: contents of ammonia, amino acids, malate and citrate are increased in CMSII while starch and 2-oxoglutarate are lower than in WT leaves. As in other species [2], leaf contents of most minor amino acids are co-ordinated in the WT, but this relationship breaks down in CMSII because of a marked shift towards accumulation of amino acids with low C/N. It is concluded that complex I functions as an electron sink important both in optimizing net CO₂ fixation under photorespiratory conditions and in co-ordinating the rate of leaf nitrogen assimilation with organic acid production.

[1] C Dutilleul, S Driscoll, G Cornic, R De Paepe, CH Foyer, G Noctor (2003) Plant Physiol (in press)

[2] G Noctor, L Novitskaya, PJ Lea, CH Foyer (2002) J Exp Bot 53, 939–946

P2.6—Functions of leaf mitochondria during photosynthesis and senescence

P. Gardeström, Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå, Sweden

Mitochondrial functions in the light are closely linked to chloroplasts and photosynthesis. In fact, oxidation of photorespiratory glycine by the mitochondrial Glycine Decarboxylase Complex (GDC) is a main activity for leaf mitochondria of C₃ plants in the light. This reaction produces NADH in the mitochondrial matrix but in the photorespiratory glycolate pathway an equimolar

amount of NADH is consumed in the peroxisomal step when hydroxypyruvate is converted to glycerate. The peroxisomal NADH requirement can be met by shuttling of NADH from the mitochondria or from the chloroplast via the 'malate valve'. Due to flexibility between these alternatives photorespiration can function as an important redox shuttle in the photosynthetic cell. Taken together photorespiratory and respiratory carbon fluxes in the light form a highly flexible system to distribute supply and demand of energy (ATP), reducing equivalents (NADH, NADPH) and biosynthetic intermediates between different cellular compartments. Results to exemplify this will be presented from experiments using *Arabidopsis* plants with modified activities of GDC and mitochondrial alternative oxidase. When a leaf turn from active photosynthesis to senescence massive metabolic changes occur with an initial decrease in photosynthesis followed by degradation of chloroplasts. During this conversion the mitochondria maintain activity and become the main supplier of ATP for the cell. The role of mitochondria in the process of leaf senescence will be discussed based on an analysis of ESTs from control and senescing Poplar leaves.

P2.7—Conservation of control mechanisms for regulation of C and N metabolism

Nigel G. Halford, Rothamsted Research

The components of control mechanisms that regulate carbon and nitrogen metabolism in plants are being elucidated gradually. Several groups, including the Metabolic Signalling group at Rothamsted Research, have studied signalling pathways involved in sensing and responding to carbon metabolic status. These studies have revealed startling conservation of components of these pathways in yeast, animal and plant systems, but also intriguing differences. For example, components of the SNF1 complex, a yeast protein kinase that has wide-ranging effects on carbon metabolism, are all present in plant cells. Yet plants also contain proteins that interact with the SNF1 homologue, SnRK1, but which are not present in other systems. Furthermore, while SNF1 respond rapidly to changes in glucose availability, the exact nature of the signal that results in activation of SnRK1 is still not entirely clear. There are also differences in the types of genes that are regulated by SnRK1 and SNF1. Studies on SnRK1 are relatively advanced compared with those on another potentially extremely important regulatory protein that is homologous to the yeast protein kinase, GCN2. GCN2 enables yeast to respond to amino acid starvation, causing a general reduction in protein synthesis and initiating changes in expression of a huge number of genes in a process called

general amino acid control. *Arabidopsis* GCN2 will complement the *gcn2* mutation, appearing to support the controversial hypothesis that plants have a similar system of general amino acid control. However, other components of this system are not readily identifiable in the *Arabidopsis* genome.

P2.8—Reactive nitrogen and oxygen species impact on respiration function and carbon skeleton provision by mitochondria in plants

¹A.H. Millar, ¹N.L. Taylor, ²L.J. Sweetlove,
¹J.L. Heazlewood, ²C.J. Leaver, ¹D.A. Day

¹Plant Molecular Biology Group, School of Biomedical and Chemical Sciences, The University of Western Australia, Australia

²Department of Plant Sciences, The University of Oxford, UK

During oxygen evolution and oxygen consumption a series of reactive oxygen species are produced in plants. Through successive reduction of nitrate to ammonium a series of reactive nitrogen species are also produced in plants. The concentrations of these reactive chemicals are increased during both biotic and abiotic stress. We have been investigating the impact of these chemicals on plant mitochondria during the provision of TCA cycle intermediates, the flux of the electron transport chain and oxidative phosphorylation and the operation of the photorespiratory cycle through oxidation of glycine. We aim to identify the exact molecular targets and the means of their inhibition in order to firmly establish the nature of mitochondrial susceptibility to oxidative stress. These studies have investigated reversible enzyme inhibition, post-translational modification of mitochondrial proteins, and changes in the mitochondrial proteome. Nitric oxide (NO) operates as an O₂ analogue and specifically inhibits electron transport chain flux leading to ATP generation through cytochrome c oxidase. Lipid peroxidation plays a critical role in reactive oxygen based damage to mitochondrial through rapid modification of lipoic acid containing enzymes including pyruvate and 2-oxoglutarate dehydrogenases and glycine decarboxylase. Thioredoxin-based enzyme systems for disulphide repair and H₂O₂ reduction are induced by oxidative stress in plant mitochondria. Meanwhile, susceptible enzymes are often selectively degraded, presumably following unrepairable damage, but an inducible mitochondrial proteolysis system. These data suggest mitochondria are susceptible to reactive oxygen and nitrogen chemicals in plants and their protection will be vital for plant cell maintenance, growth and development in stressful environments.

P2.9–Nitrogen management during leaf aging

C. Masclaux-Daubresse, E. Carrayol, K. Pageau, M. Lelandais, M. Reisdorf-Cren and J-F. Morot-Gaudry, Unité Nutrition Azotée des Plantes, INRA Versailles

Along its main axis, the leaves of vegetative tobacco plants can be divided in two main sections with respect to the expression of the enzymes involved in primary N-assimilation (Nitrate Reductase NR and plastidial Glutamine Synthetase GS2) or N-remobilisation (Glutamate DeHydrogenase GDH and cytosolic Glutamine Synthetase GS1). The decrease in NR and GS2 activities, together with the appearance of new GS1 and GDH enzymes characterise the occurrence of a sink to source transition at a particular leaf stage. At this stage, carbohydrate contents reach a peak whereas both organic and inorganic nitrogen bottom out. Both *gs1* and *gdh* induction and *nia* and *gs2* repression were then used as markers to facilitate the study of signals possibly involved in the occurrence of the transition zone. Source leaves, where GDH is expressed, were more widely studied, revealing that *gdh* mRNA fluctuated in an opposite way compared to that of *nia* transcripts (which encode NR) along the day/night period. As GDH activity can reversibly convert glutamate to alpha-ketoglutarate, its involvement in source leaf tissues was also investigated through isotopic ¹⁵N tracing and by studying the amino acids contents in source and sink leaves, along the day/night period.

Leaf disk incubation experiments allowed us to characterise metabolic, hormonal or abiotic stress-inducing signals, involved in the differential regulation of NR, GS2, GS1 and GDH encoding genes. A pharmacological approach facilitated the study of cognate signalling pathways and revealed the existence of an opposite control pathway for *gdh* and *nia* gene expression depending on sugar availability.

P2.10–Physiology and molecular genetics of nitrogen assimilation applied to crop improvement

B. Hirel¹, M.A. Limami² Thérèse Tercé-Laforgue¹, Marie-Hélène Valadier¹ and A. Gallais³

¹ Unité de Nutrition Azotée des Plantes, INRA, R.D. 10, Versailles Cedex, France. hirel@versailles.inra.fr

²UMR Physiologie Moléculaire des Semences, Université d'Angers, 2 Bd. Lavoisier 49045 Angers cedex, France. limami@sciences.univ-angers.fr

³Station de Génétique Végétale du Moulon, INRA-UPS-INAPG, Ferme du moulon, 91190 Gif/Yvette, France. gallais@moulon.inra.fr

Improved nitrogen-use efficiency (NUE: N fertilizer taken up by plants and assimilated into organic N) has the

potential to enhance yield under low N and thereby improve crop nutritional quality while reducing ground water contamination by nitrates. In order to realize potential benefits with respect to sustainable agriculture, we need to identify the physiological, biochemical and molecular mechanisms controlling component processes, including inorganic N uptake, N reduction, N partitioning between roots and shoots and N-incorporation into organic molecules as a function of carbon availability. Moreover, sustained decreases in fertiliser input and improved or stabilised yield require an improved understanding of the transition between N assimilation and N remobilisation during development. Recent advances in plant molecular biotechnology, combined with modern physiological and biochemical studies, have expanded our understanding of the regulatory mechanisms controlling the primary steps of inorganic nitrogen assimilation and the subsequent biochemical pathways involved in nitrogen supply for secondary metabolism. In this lecture, we will describe how the understanding of the molecular controls of nitrogen assimilation has increased through the use of quantitative genetic approaches. By ascribing metabolic functions and agronomic traits to DNA markers it is now possible to understand both the genetic and physiological basis of NUE during the plant life cycle. Current knowledge prospects for future development and applications in crop improvement will be explored.

P2.11–The effect of nitrogen form and supply rate on the interaction between *Septoria nodorum* and wheat

A. Taylor, S.A. Rolfe & J.D. Scholes, Dept. Animal and Plant Sciences, University of Sheffield, S10 2TN

Septoria nodorum, (glume blotch of wheat), is a necrotrophic pathogen that kills host tissue prior to abstracting nutrients. The nutritional status of the host, particularly with respect to the form and amount of nitrogen that a plant receives can have a profound effect on disease development although the underlying mechanisms are poorly understood. The aims of this study were (1) to use real-time, non-invasive imaging of host chlorophyll fluorescence and the vital reporter system Green Fluorescent Protein in *S. nodorum* to simultaneously examine changes in host metabolism and the location of mycelia within the leaf when plants were grown under different nutrient regimes and (2) to determine how inactivation of *S. nodorum* nitrate reductase (by gene disruption) affected disease development. Wheat leaves infected with *S. nodorum* rapidly developed necrotic lesions and these correlated with the location of fungal mycelium within the leaf. Chlorophyll fluorescence imaging showed that photosynthesis was inhibited throughout the infected leaf when compared with the

control leaf but was most severe within lesions. The development of *S. nodorum* was affected by both the form and amount of nitrogen supplied to the host although inactivation of *S. nodorum* nitrate reductase activity did not alter disease development. These results are discussed in relation to alterations in host nitrogen metabolism and defence gene expression.

P2.12—Possible roles of reactive nitrogen and oxygen species in the legume-Rhizobium symbiosis

Pierre Frendo¹, Didier Hérouart¹, Judith Harrison¹, Emmanuel Baudouin¹, Florence Alesandrini¹, Alexandre Jamet¹, Marie Le Gleuher¹, Fabiola Bastian², Danièle Touati² and Alain Puppo¹

¹Laboratoire de Biologie Végétale et Microbiologie, CNRS FRE 2294, Université de Nice-Sophia Antipolis

²Laboratoire de Génétique Moléculaire des Réponses Adaptatives, CNRS-Universités Paris 6-Paris 7

The first steps of the *Sinorhizobium meliloti*–alfalfa symbiosis involve the formation of reactive oxygen species. Superoxide radicals and hydrogen peroxide have been detected in infection threads and there is also evidence for the presence of nitric oxide in young alfalfa nodules. Moreover, rhizobial mutants, with a reduced antioxidant defence (superoxide dismutase or hydroperoxidase mutants), exhibit an impaired capacity to nodulate. The oxidative burst generated in response to symbiotic infection can be consistent with rhizobia being initially perceived as invaders by the plant. However this long-lasting burst is also associated with successful infections. The burst could trigger the expression of plant and/or bacterial genes which are essential for the nodulation process. In this framework, glutathione (GSH) and homogluthathione (hGSH) could be key intermediates for gene expression, via the modification of the redox balance: indeed, transgenic roots with lowered GSH/hGSH levels exhibit an altered nodulation capacity. On the other hand, when nitrogen fixation begins to decline, accumulation of important amounts of hydrogen peroxide is observed in the periphery of the central infected tissue of soybean nodules. Concomitantly, expression of a cysteine protease gene and events related to programmed cell death are observed in this zone, which progressively enlarges towards the centre of the organ; this can be viewed as the onset of the nodule senescence. Thus, reactive species appear to play important roles in the development of the symbiotic interaction.

P2.13—Nutrient dynamics in the rhizosphere

P.R. Hirsch and A.J. Miller, Agriculture and the Environment Division, Rothamsted Research, Harpenden

The rhizosphere defines the extent of chemical and bacteriological influence of the roots. Microbial numbers, especially those of heterotrophic bacteria, may be several orders of magnitude higher in the first millimetre of soil surrounding a root, compared to the bulk soil. Soil is relatively low in nutrients and the major influence of roots on soil bacteria not in immediate contact with the root surface (the rhizoplane) or sloughed-off cells and mucilage, is due to soluble exudates which include simple sugars, organic acids and amino acids. The composition and activity of bacterial populations in the rhizosphere is distinct from that in bulk soil and varies in different plant species. On the root surface, distribution of bacterial cells and micro-colonies is not uniform and is assumed to reflect sites where nutrients are most readily available due to cellular lysis or exudation. Better methods to study the spatial distribution of bacteria in the rhizosphere and on the root surface will lead to an improved understanding of their relationships. This is relevant to specific plant–microbe interactions such as the symbiotic nitrogen fixing associations of rhizobia and legumes. Also, rhizosphere and rhizoplane bacteria may affect plant nutrition by modulating the availability of nutrients, for example many bacteria can convert nitrate to ammonia, which is less readily leached and has a lower energy requirement for assimilation by the plant. To study these phenomena, we are developing microsampling techniques combined with PCR, to study the distribution of bacteria on and around roots.

P2.14—Metabolic and regulatory networks in carbon and nitrogen metabolism

M. Stitt, Max Planck Institute for Molecular Plant Physiology, Am Mühlenberg 1, 14476 Golm, Germany

Carbon–nitrogen interactions provide a good system to explore the use of system-orientated approaches to improve understanding of complex biological systems. It is a relatively complex area, in which many basic structural features of the network are known, which greatly aids experimental planning and the interpretation of the results. Crucially, it is affected by many inputs, and interacts with a wide range of downstream processes, in metabolism but also in growth-related processes and development. I will discuss a range of genomics-based approaches being used to characterise these responses. (i) The systematic use of reverse genetics to produce series of dosage mutants in order to define

control patterns within metabolic pathways. (ii) The application of broad phenotyping platforms, in particular metabolite determinations, to unravel the implications of changes in the expression of one enzyme on other metabolic pathways and other processes. (iii) The use of multilevel phenotyping programmes to provide an in depth view of metabolic responses. (iv) Use of systematic measurements of metabolites to uncover key sites for regulation in complex metabolic systems and (v) to define experimental systems to characterise regulatory mechanisms. The talk will highlight recent work revealing an important role for redox-dependent post-translational modification in the regulation of starch metabolism, and the use of multilevel metabolite, enzyme activity and transcript profiling to uncover regulatory responses in carbon–nitrogen interactions.

P2.15–Global aspects of C/N constraints determining plant–environment interactions and their implications for agriculture

JA Raven, Life Sciences, University of Dundee, L L Handley, Scottish Crop Research Institute, Invergowrie, M Andrews, School of Sciences, University of Sunderland

The major involvement of C and N in plant metabolism and biogeochemical cycles is permitted by their production in stars and subsequent partitioning into the Earth's crust, hydrosphere and atmosphere as well as by their chemical properties. The atomic C:N ratio in photolithotrophs is a function of their content of nucleic acids, proteins, lipids, polysaccharides and other organic materials, and varies from about 5 in some protein-rich microalgae to much higher values in macroalgae and in higher plants with relatively more structural and energy storage materials. These differences in C:N ratios among organisms means that there is more N assimilation by photosynthetic organisms in the oceans than on land despite the near equality of global photosynthetic C assimilation rates in the two environments. The aquatic organisms obtain inorganic C and inorganic N from the surrounding water. Terrestrial photolithotrophs obtain inorganic C, dinitrogen (by diazotrophy) and some combined N from the atmosphere, with the remaining combined N coming from the soil. The nitrogen cost of growth (biomass production rate per unit plant N) varies with the C:N ratio and specific growth rate of the organism. The water cost of growth (water lost per unit biomass gain) in terrestrial plants is a function of N supply and of C supply; water cost is lower with higher N and

C availability. Water supply is also important in determining denitrification rates on land and on N (and C) fluxes from terrestrial to aquatic systems.

P2.16–Components of nitrogen use efficiency in Barley genotypes

L.J. Irving & D. Robinson, University of Aberdeen

Global nitrogen use efficiency (NUE) for crop production averages 33%. Approximately one million tonnes of fertiliser are applied to cereals annually; therefore 660 000 tonnes are not recovered in biomass. Low NUE is a result of available N being lost from the soil, the plant or both, by physical or biochemical means. I am investigating N loss associated with biochemical processes, particularly the turnover of Rubisco. Rubisco (ribulose-1,5-bisphosphate carboxylase /oxygenase) is probably the most abundant enzyme in the world. It accounts for 15–30% of the N in well-fertilised plants, has a high molecular weight (550 kDa) and occurs far in excess of requirements for maximal photosynthetic rate, but turns over slowly. Thus it is believed that Rubisco is the major long term N store in C₃ plants.

Using eight genotypes from a mapping population of barley with varying NUE's, I am investigating genotype × environment interactions for a number of key features, such as biomass distribution, chlorophyll content, Rubisco content and turnover, nitrate content and nitrate reductase activity. Current work concentrates on how N fluxes through the Rubisco pool are affected by differential synthesis and degradation of the protein. It is possible that turnover of this pool represents a major opportunity for N loss and that there is a genetic basis for differences in turnover. This has implications for crop NUE since grain N is predominantly derived from this pool, rather than directly from soil N whose availability is restricted to the first few weeks post-germination, long before grain formation begins.

P2.17–Carbon and nitrogen metabolism in the carnivorous plant *Sarracenia purpurea*

K. Clark, R.C. Leegood, Animal and Plant Sciences, University of Sheffield

Carnivorous plants tend to grow in sunny nutrient poor environments. They have evolved to supplement the uptake of nutrients through their roots by trapping invertebrates, mainly insects, in modified leaves digesting them and absorbing the products of this digestion. *Sarracenia purpurea* (the Northern pitcher plant) is a native plant of Northern America inhabiting mainly acidic sphagnum dominated peatlands that are nutrient poor and low in available nitrogen. *S. purpurea* pitchers both

photosynthesise and absorb nitrogenous compounds from digested prey, making them an intriguing system in which to study the links between the assimilation and metabolism of carbon and nitrogen.

S. purpurea pitchers were fed various amino acids, ammonium hydroxide, BSA (Bovine serum albumin) and insects, giving a range of different forms of nitrogen. Amino acid and ammonium ion absorption appeared to occur rapidly whereas the absorption of proteins was a slower process. Feeding nitrogenous compounds to the pitchers also appeared to stimulate the release of proteases into the pitcher fluid by the plant. SDS/PAGE and Western blotting also showed that on feeding, enzymes involved in carbon and nitrogen assimilation and transformation, such as GS (Glutamine synthetase), were induced within the pitcher tissue. *S. purpurea* appears to have an inducible response to prey, with digestive and assimilative enzymes only produced in response to the presence of nitrogen within the pitcher.

P2.18—Light-regulated and nitrate-inducible isoforms of ferredoxin-nadp⁺ oxidoreductase (FNR) in the primary wheat leaf: some aspects of their regulation

J. Gummadova and C.G. Bowsher, School of Biological Sciences, Manchester University, 3.614 Stopford Building, Oxford Road, Manchester M13 9PT, UK

Ferredoxin-NADP⁺ oxidoreductase (FNR) is an FAD-containing enzyme that catalyses the reversible electron transfer between NADP(H) and ferredoxin. There are two forms of FNR in plants: heterotrophic FNR (hFNR) and photosynthetic FNR (pFNR). Each isoform may function in different metabolic pathways and tissues. Light regulates the rate of synthesis and protein activity of the photosynthetic enzyme. The hFNR isoform is induced by nitrate. Little is known about the presence and functional relationship of both differently regulated isoforms in the same organ. A wheat leaf blade represents an ideal model-organ for such studies. There is a gradient of photosynthetic activity between the base and tip. Segmentation of the wheat leaf allows the temporal and spatial monitoring of pFNR and hFNR. Our hypothesis is that, depending on the presence/absence of light and exogenous nitrate, a putative gene switch between pFNR and hFNR may be a flexible functional interface between carbon and nitrogen assimilation in plants.

A cDNA library was constructed using mRNA prepared from the illuminated leaves of nitrate-fed wheat seedlings. Two distinct groups of pFNR clones were identified – pFNRI and pFNRII. A partial hFNR clone (252 bp) has also been amplified by RT-PCR technique. These newly isolated pFNR and hFNR clones have been used in Northern blot analysis to quantify the amount of the mRNA along a wheat leaf grown in the presence or absence of light and/or nitrate. In addition, studies of the impact of light and nitrate on protein levels and enzyme activity will be presented.

P2.19—Short circuiting photorespiration in tobacco

P.J. Madgwick, A.J. Keys, J.F.C. Carvalho, P.J. Lea, M.A.J. Parry

Crop performance and improvement, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK
Biological Sciences, Lancaster University, Lancaster, LA1 4YQ, UK

Transgenic tobacco (*Nicotiana tabacum*) plants have been generated in which the part of the photorespiratory cycle that converts glycine to serine has been bypassed. The plants have been transformed with the genes *gcl* and *hyi* encoding respectively two enzymes isolated from the bacterium *Escherichia coli*, glyoxylate carboligase (*gcl*; EC 4.1.1.47), which converts glyoxylate to tartronate semialdehyde and CO₂, and hydroxypyruvate isomerase (*hyi*; EC 5.3.1.22), which converts tartronate semialdehyde to hydroxypyruvate. The two enzymes should short circuit photorespiratory metabolism and avoid the decarboxylation of glycine and the generation of ammonia. The new pathway should decrease the energy requirements of C₃ photosynthesis, by avoiding the necessity of reassimilating the NH₃ released in the mitochondrial reactions. The transgenic lines 32, 33 and 37 expressing the *E. coli* gene for *gcl* modified by the addition of a peroxisome targeting sequence and the lines 79, 84 and 92 bearing both *gcl* and *hyi* transgenes appeared to grow normally under low light or elevated CO₂ concentrations. Under bright light and at ambient CO₂ white lesions developed on the leaves. Under photorespiratory conditions, less ¹⁴C-glycolate was metabolised to glycine and serine and more to sucrose in the transgenic line than in the wild type plants. The amounts of glutathione in the leaves were variable in *gcl* and *gcl-hyi* lines but were always greater than the wild type. Amino acid quantification revealed that the glutamine: glutamate and the glycine:serine ratios were altered in the *gcl* and *gcl-hyi* lines. Further characterisation of the transformed plants is underway.