

## Plant sessions

Steven Yates (AU)	Genetic dissection of drought tolerance in red clover
Barend de Graaf (CU)	Pollen proteomics to elucidate recognition phenomena during pollen-pistil interactions
Karen Harper (BU)	How to keep an Orange fresh and healthy
Gemma Cook (CU)	Investigating the role of protein-protein interactions in the post-translational regulation of WEE1 kinase
Walter Dewitte (CU)	A role for D-type cyclins in somatic cell proliferation in <i>Arabidopsis thaliana</i>
Carlos Rodrigues (AU)	Because we are not all top models: Pret-a-porter epigenetics from model organisms to non-model crops
Catherine Withers (AU)	Reducing disease resistance associated yield reductions in cereals caused by stomatal dysfunction using <i>Arabidopsis thaliana</i> as a model
Danilo Aros (CU)	Characterization of a novel Alstroemeria monoterpene synthase (ALSTER)
Michal Mos (AU)	Characterisation of senescence in the energy grass <i>Miscanthus</i>
Adel Mahgrabi (CU)	Acclimation of embryogenic calli and cell suspensions of <i>Medicago truncatula</i> under long term abiotic stress
Jeroen Nieuwland (CU)	The function of D-type cyclins in root growth and development
Fazah Mohd-Salleh (CU)	Regulation of stress and senescence by a LEA protein; SAG21
Ben Cruickshank (Reading)	Using alternative substrates for herb production in the UK: impact on growth, yield and flavour profile
Trish Toop (AU)	Sustainable grasslands for biological energy production
Bob Lovitt (SU)	Large scale production of algae : the promise and the pitfalls
Emma Bennett (Reading)	Manipulating <i>Arabidopsis</i> Silique Development in Relation to Whole Plant Resource Allocation
Shereen Naz Baloch (BU)	Salt tolerance in cotton
Golnaz Rafiei (CU)	Studies on the interaction between WEE1 and a glutathione-S transferase in the DNA replication checkpoint
Anushin Sivakumarai (AU)	Ethylene plays a major role in conferring resistance to the economically important pathogen <i>Botrytis cinerea</i> , the causal agent of Gray Mould on soft fruits
Chincole Laxuman (BU)	Towards fine-mapping a QTL for root length in rice
Matthew Hegarty (AU)	When genomes collide: consequences of interspecific hybridisation in plants
Maciej Bisaga (AU)	Molecular characterisation of response to drought in a perennial species

## Micro sessions

Lovleen Joshi (CU)	How does <i>Clostridium difficile</i> stick around?
James McDonald (BU)	The molecular ecology of anaerobic cellulose-degrading microorganisms
Florence Prive (AU)	Mining the rumen metagenome for enzymes involved in lipid metabolism
Magdalena Markham (AU)	Are the Rpf proteins lytic transglycosylases?
Catrin Williams (CU)	Garlic: a possible cure for "Hole-in-the-Head" disease of fish?
Ed Dudley (SU)	Insects as a source of novel antimicrobial compounds
Ceri Gwyther (BU)	Survival of pathogenic organisms in a novel dead sheep disposal system
Victoria Gray (CU)	From the ocean to the field: bioluminescent bacteria as a biosensor for water toxicity
Jessica Adams (AU)	Biofuels from macroalgae
Paula Roberts (BU)	Importance of dissolved organic nitrogen to the nitrogen cycle of the Maritime Antarctic
Jen Hiscox (CU)	Monokaryons and dikaryons of <i>Trametes versicolor</i> have similar combative and decay abilities
Justin Pachebat (AU)	Illumina sequencing of <i>Dictyostelium discoideum</i> mutants
Naomi Cope-Selby (AU)	Bacterial endophytes of <i>Miscanthus</i>
Suzy Moody (SU)	Deciphering the role of biosynthetic cytochrome P450s in secondary metabolism pathways in <i>Streptomyces coelicolor</i>
Hans Göransson (BU)	Nutrient availability for plants and bacteria during early succession in the Alps
Sally Rangelcroft (BU)	Biochar: Greenhouse Gas Sink or Health Risk? Assessing the potential impacts of biochar on the fate of polyaromatic hydrocarbons (PAHs) in soil, and consequently agricultural crops
Richard Quilliam (BU)	Sporulation of arbuscular mycorrhizal fungi in organic-rich patches following host excision
Christoph Gertler (BU)	Will microbes save the Gulf? - Ecology and global distribution of bacteria for crude oil bioremediation
Roszaini Khadir (BU)	Heartwood extractives of different hardwood species and their relationships to termite and white-rot fungi
Kirsty Dougal (AU)	Is there a stable core microbiota in the horse?
Camilla Thorn (BU)	Effects of land use types on the activity of waterborne <i>Escherichia coli</i> O157:H7 within a UK catchment
Sharon Huws (AU)	Temporal ruminal biofilm formation on fresh perennial ryegrass

## Posters

- Poster1: Manipulation of meiotic recombination in barley (*Hordeum vulgare*) (Joanna Wnietrzak, AU)
- Poster2: Grass to Gas: Sustainable grasslands for biological energy production (Trish Toop, AU)
- Poster3: From catchment to coast: the activity of *E. coli* O157:H7 in watercourses is affected by land-use (Prysor Williams, BU)
- Poster4: On the role and evolution of phosphofructokinases in actinomycetes (Geertje van Keulen, SU)
- Poster5: Proteomic profiling: a novel approach to understanding the biological causes of soil water repellency (Geertje van Keulen, SU)
- Poster6: The search for salt tolerant crops for Libya (Khalifa El Tayef, BU)
- Poster7: The management of upland areas for biodiversity & bioenergy (John Corton, AU)
- Poster8: A study of the naturally occurring *xFestulolium* Hybrid- *xFestulolium loliaceum* and its progeny (John Harper, AU)
- Poster 9: Social predators of the bacterial world (Dave Whitworth, AU)
- Poster 10: Does the activity or amount of extractable compounds account for antitermitic activity in durable Malaysian hardwoods? (Roszaini K. and Mike D. Hale, BU)
- Poster 11: Towards a hybrid *T. occidentale* x *T. pallenscens*: the possible progenitor species of white clover (Charlotte Jones, AU)
- Poster 12: Title? (Alison Kingston Smith, AU)
- Poster 13: Improving Drought Tolerance In *Miscanthus* Genotypes (Richard Webster, AU)
- Poster 14: Variations of quality components and Gene Expression Analysis of Postharvest Senescence Marker in Baby Spinach Leaves (Valentino Baldassarre)
- Poster 15: Exploiting the diversity of form in *Miscanthus* for increased Biomass (Kerrie Farrar, AU)

## Plant Science Talks

### **Genetic dissection of drought tolerance in red clover**

Steven Yates

*Aberystwyth University*

Red clover (*Trifolium pratense*) is an increasingly popular forage legume. The advantages of this crop are soil improvement and protein rich forage for silage. Its major problem is low persistency, where satisfactory yields are only achieved in the first and second year after sowing. Improving persistency is thus a key objective in breeding programs. Persistency is a complex trait of which abiotic stress is a major component. This work focuses on drought, which together with cold, salinity and heat lead to water deficiency. Plants respond to different abiotic stresses with similar and distinct mechanisms. This project aims to dissect drought tolerance at a genetic level. The project uses a mapping population, created as part of the ERANET project, to identify drought associated quantitative trait loci (QTL). They are genetic regions which have a quantitative effect upon a given trait. Thus measuring water stress in a mapping family forms the basis of identifying QTLs related to drought tolerance. This information can potentially be used in future breeding programs to improve drought tolerance using genetic markers associated with the QTL. Secondly, it is a prerequisite for future positional cloning efforts to find genes underlying this important trait. Preliminary results of the first major drought experiment will be presented and discussed.

### **Pollen proteomics to elucidate recognition phenomena during pollen-pistil interactions**

Barend H. J. de Graaf

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Sexual reproduction in flowering plants involves species specific communication events. Upon pollen landing, pollen and pistil are being recognized as 'own' or 'foreign' which results in acceptance or rejection, respectively. To date very little is known about which pollen and pistil proteins play a role in the communication between both partners during compatible pollen pistil interactions. Moreover, nothing is known about how male and female partners of different plant species discriminate between own and foreign. Pre-fertilization barriers are of central importance to the biology of flowering plants. These barriers depend on various recognition processes between the growing pollen tube and the female pistil. Although their genetic basis and molecular mechanisms are still largely unknown and have proven recalcitrant to analysis, these interactions should all depend on signalling through pollen tube membrane-associated processes. We apply an innovative strategy of organelle-specific proteomics to identify novel pollen and pistil proteins involved in communication. The protein content of different pollen organelle compartments, isolated from pollinated pistils of *Nicotiana tabacum* and *Brassica napus*, will be subjected to mass spectrometry analysis. The function of candidate pollen membrane receptor-like proteins and endocytosed pistil proteins will be established by cell biology, reverse genetics and gene silencing strategies.

### **How to Keep an Orange Fresh and Healthy**

Karen Harper (bspa35@bangor.ac.uk)

*Biological Sciences, Bangor University, KESS Project Partner: Agrisense*

A series of experiments were conducted with the view to looking at the postharvest environment of oranges (*Citrus sinensis*). These were initially investigations into weight/water loss of the fruit stored under different temperature and relative humidity (RH) conditions. All experiments involved plastic boxes (16 L) of oranges stored at 20 and 5°C. The two temperature regimes resulted in significantly different rates of weight loss throughout all experiments. Techniques were developed to regulate and maintain RH in the boxes. Initially, various concentrations of sodium chloride (NaCl) in 300 ml glass beakers were tried, but these did not give a sufficient range of RH. Next, saturated solutions of different salts were tested, combined with the introduction of an air-circulation system. These were much more successful and, once the factor of solution surface area was also resolved by pouring them directly into the bases of the boxes, the final assay for temperature and RH was achieved.

Chitosan, produced by the deacetylation of chitin, has been reported to illicit natural plant defence responses (Bautista-Banos et al., 2006). Oranges were dipped in solutions of various concentrations (0.2 – 2%) before being placed in the experimental boxes. Preliminary results suggested that the higher the concentration of chitosan the lower the cumulative percentage weight/water loss of the fruit. Initial analyses of the structure of chitosan using both MALDI-TOF and H<sup>+</sup> NMR techniques were also conducted. In contrast, trans-2-hexenal, reported to have antifungal activity (Utama et al., 2002), had no effect. The temperature/RH assay was also used to investigate weight/water loss of lettuces and strawberries – the results of this were obtained within a few days, rather than the weeks it required to achieve those using oranges.

Bautista-Banos, S., Hernandez-Lauzardo, A.N., Velazquez-del Valle, M.G., Hernandez-Lopez, M., Barka, E.A., Bosquez-Molina, E. and Wilson, C.L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection* 25, 108-118.

Utama, I.M.S., Wills, R.B.H., Ben-Yehoshua, S. and C., K. (2002). In Vitro Efficacy of Plant Volatiles for Inhibiting the Growth of Fruit and Vegetable Decay Microorganisms. *Journal of Agricultural and Food Chemistry* 50, 6371-6377.

## **Investigating the Role of Protein-Protein Interactions in the Post-Translational Regulation of WEE1 Kinase**

Gemma S. Cook<sup>1</sup>, Robert J. Herbert<sup>2</sup>, Hilary J. Rogers<sup>1</sup>, Dennis Francis<sup>1</sup>

<sup>1</sup>Cardiff University, <sup>2</sup>Worcester University.

In animals and yeasts, WEE1 negatively regulates progression of the cell cycle from G2 phase into mitosis by phosphorylating the key cell cycle regulators cyclin dependent kinases. It also has an important role in inhibiting entry into G2 when cells are subjected to DNA damage or DNA replication is stalled. In higher plants WEE1 may be functionally redundant in a normal cell cycle, but it has a clear role in the response to DNA damage and the stalling of DNA replication. This work aims to further elucidate the role of plant WEE1 by investigating its regulation by other proteins. WEE1::GFP fusions have been expressed in Arabidopsis under the 35S promoter and showed that WEE1 localises to the nucleus and chloroplasts. Tobacco BY-2 cells were also transformed with this construct in order to study the subcellular localisation of WEE1 during the cell cycle. A yeast two-hybrid screen in the Cardiff lab revealed that over 60 proteins interact with Arath;WEE1 and several of these interactions have been confirmed in vivo through transient bimolecular fluorescence complementation (BiFC). BY-2 cells have also been stably transformed with these BiFC vectors to study the dynamics of the interactions during the cell cycle. One interactor is Arath;SKIP1, an F-box protein, and we are analysing the root phenotype and WEE1 protein levels of Arabidopsis seedlings knocked-down for SKIP1 to verify a functional role for this interaction.

## **A role for D-type cyclins in somatic cell proliferation in Arabidopsis thaliana**

Walter Dewitte

Cardiff University

## **Because we are not all top models: Pret-a-porter epigenetics from model organisms to non-model crops**

Carlos Marcelino Rodriguez Lopez

Aberystwyth University

Plants experience a multitude of environmental stresses and react by adjusting their global gene expression profiles to amend phenotype, cell biology and developmental progression. This process is known as phenotypic plasticity and allows plants to tolerate a wide amplitude of variation for abiotic and biotic stress factors. Such plasticity is mediated by targeted control of gene expression and is known as epigenetics. Gene silencing or down-regulation via the *de novo* methylation of cytosine residues in regulatory regions plays a key role in epigenetic regulation. In the absence of a genome sequence, it is difficult to apply such information from model species into non-model crops such as cocoa (*Theobroma cacao* L.). Many non-model crops nevertheless possess an extensive EST data set. For cocoa, a recent international research project generated 149,650 valid EST sequences from different organs, genotypes and from plants grown under different environmental conditions (Argout et al. 2008). The present study aims to exploit data from *Arabidopsis thaliana* methylome to target candidate genes from cocoa that may be under epigenetic control and also implicated in stress response pathways. The *Arabidopsis thaliana* methylome has been deciphered using sequencing-by-synthesis technology and microarrays (Zhang et al., 2006; Zilberman et al., 2007; Lister et al., 2008). From this we initially selected almost 2000 genes as possible targets for methylation analysis. The combined gene data bases from the three papers mentioned above were cross referenced with the software Geneinvestigator to determine which of them were 1) responsive to 81 different stresses, 2) differentially expressed during plant development and 3) differentially expressed in different plant tissues. The *A. thaliana* genes that gave a significant change in expression under any of the above situations were then blasted against the cocoa EST database. Thus, in this study we used an interspecific reverse genetics approach to find stress response, development and tissue specific genes in the *T. cacao* genome regulated by DNA methylation. This strategy may also be of use in allowing efficient transfer of known methylomes from model organisms into not sequenced genomes from wild or cultivated species.

## **Reducing disease resistance associated yield reductions in cereals caused by stomatal dysfunction using Arabidopsis thaliana as a model**

Catherine Withers

Aberystwyth University

Infection of barley by the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (Bgh) causes major losses in yield in the UK. The breeding of resistant crop plants is an effective method of combating disease and barley varieties with resistance based on papilla production or the hypersensitive response have been produced. However, it has been observed that stomata of resistant varieties showing the hypersensitive response infected with Bgh failed to close at night ('lock-up').

Stomata are microscopic pores found mainly in the leaf epidermis and are surrounded by two specialised epidermal cells known as guard cells. They balance desiccation from water loss with uptake of CO<sub>2</sub> for photosynthesis. Disruption of stomatal function by 'lock up' will affect processes such as photosynthesis and transpiration and increases water use, exacerbating drought effects during periods of low water availability. These increased drought effects will reduce photosynthesis and shorten the growing season, both of which will reduce yield. Thus the processes involved in stomatal lock up are being studied to allow future breeding to minimise the costs to the plant of this valuable resistance to disease.

There are a number of similarities between the signalling pathways of pathogen induced defence mechanisms and ABA induced stomatal closure and I am currently focussing on these to try and gain an understanding of the molecular basis behind this pathogen induced stomatal dysfunction. I am working with the model plant

Arabidopsis thaliana as there are a number of mutants with lesions in relevant biochemical pathways. Initial studies suggest that there may be a role for nitric oxide.

### **Characterization of a novel Alstroemeria monoterpene synthase (ALSTER)**

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Terpenoids comprise a large number of primary and mostly secondary metabolites with a wide variety of structural types and their biosynthesis is controlled by a group of enzymes called terpene synthases (TPS). Using as a starting point four ESTs previously found in petals of *A. cv 'Rebecca'* and described as homologous to a *Q. ilex* chloroplast TPS, rapid amplification of cDNA ends (RACE) was performed in order to obtain the full length ORF of a novel *Alstroemeria* TPS (ALSTER). Characterizations of the nucleotide (genomic organization) and amino acid sequences (phylogenetic analysis) were performed. Furthermore, ALSTER was expressed in bacteria in order to investigate its enzymatic activity.

ALSTER comprised a genomic region of 2323 bp containing five introns and six exons. This unique genomic organization classified ALSTER as a member of the class III terpene synthases according to a recent phylogenetic classification system with a merged 5-6 exon and a sesquiterpene synthase-like first exon. The deduced amino acid sequence of 567 aa was classified into the subfamily TPS-b according to published phylogenetic analysis of angiosperm monoterpene synthases. Moreover, the conserved domains R(R)X8W and DDXXD were observed at amino acids 28 and 321 respectively. The functional analysis performed showed enzymatic activity of ALSTER with geranyl diphosphate (GPP) and the monoterpene myrcene was the only product obtained. Thus ALSTER can be described as a novel *Alstroemeria* myrcene synthase.

Keywords: *Alstroemeria*, TPS, RACE, ALSTER, myrcene.

### **Characterisation of Senescence in the energy grass *Miscanthus***

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There are very ambitious targets for reductions in carbon dioxide emissions and the use of renewable energy. Biomass crops have the potential to make a major contribution toward achieving these targets. Perennial grasses such as *Miscanthus* are highly promising bio fuel crops because they are high yielding with low inputs. For combustion in power stations *Miscanthus* stems are harvested dry once senescence is complete. Remobilisation of resources from aerial parts to the rhizome (below ground storage organ) and subsequent leaching during the winter means that very little nitrogen is removed from the field. Most plant nitrogen is contained within proteins; furthermore, approximately 30% of the leaf protein is associated with chlorophyll which, during senescence, is degraded and remobilised. The loss of chlorophyll and therefore green colour is a functional marker for senescence. Senescence with co-ordinated protein and chlorophyll breakdown is different to rapid cell death caused by late season frosts. The ability to mobilise resources via senescence before cell death processes occur is an important goal for sustainable energy crop production. The timing of senescence will impact on yield and compositional characteristics of the crop. Senescence has been extensively studied in other plant species but very little is known about this process in *Miscanthus*. We will use basic knowledge from other species to understand more about this important process in *Miscanthus*. We aim to characterise senescence in *Miscanthus* and determine the molecular determinants and morphological consequences of variations in natural senescence identified in different *Miscanthus* genotypes. We will also study a number of senescence-associated processes in a range of genotypes and to determine the impact on yield and nutrient remobilisation and also the potential for optimising *Miscanthus* as a 2nd generation bio fuel crop through manipulating senescence.

### **Acclimation of embryogenic calli and cell suspensions of *Medicago truncatula* under long term abiotic stress**

Adel Mahgrabi (CU)

### **The function of D-type cyclins in root growth and development**

Jeroen Nieuwland (CU)

### **Regulation of stress and senescence by a LEA protein; SAG21**

Fazah Mohd-Salleh (CU)

### **Using alternative substrates for herb production in the UK: impact on growth, yield and flavour profile**

B. Cruickshank<sup>1</sup>; C. Wagstaff<sup>1</sup>; A. Daymond<sup>1</sup>; M. O'Reilly; C. Müller<sup>2</sup>

<sup>1</sup> University of Reading, <sup>2</sup> Cardiff University

This project aims to provide a holistic profile of the effects of recycled household compost (RHC) on several aspects of basil (*Ocimum basilicum*) variety 'Sweet Aroma 1', including physiology, flavour profile and sensory properties. It is hoped that findings from these experiments will improve the potential for basil to be grown in the UK with improved environmental responsibility and equal flavour characteristics of a Mediterranean grown product. RHC is obtained from the composting of garden waste and is therefore a highly sustainable and low cost alternative substrate to inorganic nitrogen fertilizer or peat, with great potential for commercial application. The

basil plants were grown from plugs in protected beds with either 25, 50 or 75% RHC mixed with loam. Samples were taken at weekly intervals up until the commercial harvest point to assess physiological differences, with yield being a primary focus. Fully grown samples were collected after eight weeks and subject to CGMS analysis to examine variation in their volatile aroma profiles. Sensory analysis was also conducted to establish if changes identified instrumentally could be detected by the consumer. Our findings show clear differences between plants grown in the different RHC substrates and the implications for RHC use in a commercial application will be discussed.

### **Sustainable Grasslands for Biological Energy Production**

Authors: Trisha Toop<sup>1</sup>, Richard Dinsdale<sup>2</sup>, Iain Donnison<sup>1</sup>, Joe Gallagher<sup>1</sup>

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The combination of increased energy consumption and bold targets for the reduction of CO<sub>2</sub>, means that there is a great need for new sources of clean sustainable energy. Energy crops must be found that are acceptable both socially and environmentally. Perennial ryegrass (*Lolium perenne*) and other such grassland cover around two thirds of UK agricultural land is able to grow on marginal land not used for primary food production. The combination of its availability and favourable traits for fermentation indicates that grassland may be considered as an important UK energy crop of the future.

The aim of the project is to investigate the use of grassland in the UK as a biomass feedstock for the production of hydrogen by fermentation. Fermentations both at laboratory scale and using a novel biohydrogen pilot plant installed in IBERS Aberystwyth by Glamorgan University will be used to collect data linking grass composition to hydrogen production. A model for biomass production and conversion in the UK will be produced for present climatic conditions and also using climate change scenarios. The data produced from the model will be used to develop a data set for the life cycle assessment (LCA) of the bioenergy chain. This presentation will explain the project objectives, plan and results to date.

### **Large scale production of algae : the promise and the pitfalls**

Bob Lovitt (SU)

### **Manipulating *Arabidopsis* Silique Development in Relation to Whole Plant Resource Allocation**

Emma J Bennett<sup>1</sup>, Frances Gawthrop<sup>3</sup>, Harriet Trewin<sup>3</sup>, Jeremy A Roberts<sup>2</sup> and Carol Wagstaff<sup>1</sup>

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The ability to manipulate where resources are ultimately stored within a plant could positively impact both the yield and nutritional value of seeds. An important component of seeds are the seed storage proteins (SSPs), which represent the main plant protein consumed by man and, in various parts of the world, constitute an essential part of human and livestock diets. SSPs primarily act as a source of amino acids for growth upon germination; hence early plant development and seed nutritional quality are intrinsically linked.

The current study investigated how manipulating the number of *Arabidopsis* reproductive structures per plant via selective stem removal impacted upon pod physiology and protein concentration. Analysis showed that whilst fewer siliques per plant resulted in fewer seeds per pod these seeds were of an increased weight and size; therefore there remains the potential for yield enhancement in Brassicaceous species. Such alterations to seed physiology also correlated with delayed leaf senescence, a likely consequence of having fewer pods acting as sinks for resource allocation. Contrary to theoretical predictions the protein concentration per pod remained unaffected by selective stem removal, indicating that other storage compounds such as lipids might be responsible for the increased seed weight and size.

These findings demonstrate the highly plastic nature of *Arabidopsis* resource allocation, a process which if further understood could be used to increase the yield and nutritional quality of commercial Brassicas.

### **Salt tolerance in Cotton**

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Cotton is the world's most important fibre crop. It is also the second oil seed crop of the world (Khan et al., 1999). It is a major foreign exchange earner in Pakistan, where it is cultivated mostly in Sindh and Punjab provinces. Cotton is a halophytic crop, which is generally grown in mildly saline soil (Ashraf, 2002). However, its yield and quality is reduced at high salt levels. Some 6.67 million hectare in Pakistan are seriously affected (Alam et al., 2000).

Introduction of salt tolerant cotton varieties will play a key role in increasing the agriculture productivity of cotton under saline condition. The object of this project was the identification of genetic markers associated with the salt tolerance of cotton. 32 genotypes were tested with high salt (150 mM) and the response of nutrition; photosynthesis and basic geometry were measured. On the basis of sodium accumulation three genotypes were selected to represent salt tolerant and salt sensitive genotypes Salinity influenced most of the measured parameters. Meanwhile, microsatellite DNA sequences (SSR; potential genetic markers) were sought. Seventeen pairs of primers were used on the three selected lines and on 32 different cotton genotypes. Very little polymorphism that could be correlated to the phenotypic effects was found.

Alam, S. M. et al. 2000. Saline Agriculture and Pakistan. Nuclear Institute of Agriculture, Tando Jam. Pakistan. 2. Ashraf, M. 2002. Crit. Rev. Plant Science. 21:1-30. 3. Khan, M. A. et al. 1999. Crop Sciences. 39: 253- 258.

### **Studies on the interaction between WEE1 and a glutathione-S transferase in the DNA replication checkpoint**

Golnaz Rafiei (CU)

### **Ethylene plays a major role in conferring resistance to the economically important pathogen *Botrytis cinerea*, the causal agent of Gray Mould on soft fruits**

Anushin Sivakmarai

*Aberystwyth University*

We are examining how ethylene interacts with various defence signal pathways to confer disease resistance. We here describe evidence demonstrating that NO is generated rapidly following challenge of tomato and Arabidopsis with *B. cinerea* and plays a key role in initiating ethylene production. Examination of Arabidopsis lines with lesions in haemoglobin genes (*glb1* and *glb2*) and transgenic lines over-expressing *Glb1* and *Glb2* demonstrated a causal link between NO generation, the production of ethylene and resistance to *Botrytis*. Further, examination of the hormone mutants *sitiens* and *lazy2* in tomato suggested contributions by ABA and IAA to the pattern of NO generation following host attack by *Botrytis*.

Taken together our data demonstrates the importance of NO to a range of plant defence signalling pathways.

### **Title: Towards fine-mapping a QTL for root length in rice**

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This work is part of a Royal Society Joint International Project between Bangor University and UAS, Bangalore, India. The aim is to fine map QTL9 (on rice chromosome 9) that has been previously identified in different rice populations and also used successfully as a target in marker-assisted breeding for upland rice. The QTL confidence interval spans 26 BACs and contains 426 genes. We are using a population selected from advanced backcrossing of Azucena into Kalinga III where the Azucena allele at QTL9 increases root length. These lines are segregating only for markers in the region of QTL9. We have used a unique system developed at UAS, Bangalore to study the interaction of the root system with drought. It uses PVC cylinders (1 m long, 180 mm diameter) filled with soil to closely model the field situation, yet allow precise measurements of roots to be taken. The results of fine mapping will identify candidate gene/s for the QTL from the rice genome and should reveal insights into the genetic control of interaction of rice roots with soil under drought conditions.

### **When genomes collide: consequences of interspecific hybridisation in plants**

Matthew Hegarty

*Aberystwyth University*

Interspecific hybridisation is relatively common in higher plants, and the merger of two divergent genomes within a hybrid nucleus has long been recognised as a key source of genetic novelty upon which selection can operate. Studies in a wide range of hybrid species have demonstrated that genome mergers, whether involving a change in ploidy or not, can result in a suite of genetic, transcriptional or epigenetic changes in order to allow the two (or more) genomes to coexist. Changes in the ploidy of hybrid plants have also been demonstrated to contribute to these modifications. An overview of known consequences of hybridisation and polyploidy will be presented, with data from systems such as Arabidopsis, cotton, bread wheat and Senecio (ragworts). The ramifications of these findings for agriculture and plant evolution will be discussed

### **Molecular characterization of response to drought in a perennial species**

Maciej Bisaga, Adriana Ravagnani, Leif Skøt and Glyn Jenkins

*Aberystwyth University*

Water deficit is one of the environmental factors most effecting crop productivity world-wide. Understanding the molecular mechanisms of the response to drought is of fundamental importance for the production of drought tolerant varieties by either genetic engineering or classical breeding methods.

In this project we investigate the genetic response to drought in white clover (*Trifolium repens* L.), a perennial and most important forage legume of temperate grassland system. Our aim is to identify the full spectrum of genes expressed in response to drought as well as those expressed while recovering from drought. This will be achieved by constructing Suppression Subtractive Hybridisation (SSH) cDNA libraries composed of sequences differentially expressed in stressed, unstressed and recovering plants. These data will also be complemented by a proteomics approach. Two-dimensional electrophoresis (2D-PAGE) will be carried out to identify the proteins involved in response to and recovery from drought

## Microbiology Talks

### **How does *Clostridium difficile* stick around?**

Lovleen Joshi

*Cardiff University*

*Clostridium difficile* is an anaerobic Gram positive bacterium implicated in causing antibiotic associated diarrhoea (CDAD). The ability of *C. difficile* to produce highly resistant endospores facilitates environmental survival and disease transmission. For this reason we set to determine the contribution of the spore to surface adherence and biocide resistance. Initially the ability of a diverse collection of 21 clinical isolates representing different ribotypes to form spores in brain heart infusion broth was determined. While spore yields varied between 10<sup>4</sup>-10<sup>8</sup>cfu/ml we found no obvious correlation between yield and ribotype. The surface properties of these spores were subsequently compared using a Hexadecane-based partition assay which determines relative hydrophobicity. The results revealed a relationship between hydrophobicity and ribotype, with hypervirulent isolates tending to be hydrophobic suggesting that they are more adept at binding to hydrophobic materials such as stainless steel. Indeed this hypothesis was confirmed using a novel replica plating method which found that hydrophobic spores bound more firmly to stainless steel discs than their less virulent (hydrophilic) counterparts. Subsequent examining of spore structure via electron microscopy found that hydrophobic (virulent) spores possessed a more pronounced exosporial layer than their hydrophilic counterparts. The exosporial layer has been found to play an important role in facilitating the adhesion of spores to human cell substrates, agar and glass surfaces during spore germination. The adhered exosporium also maintains the emergence of newly formed bacterial cells, ensuring surface colonization and spread. To determine if exposure to sub-lethal concentrations of biocide affect the ability of spores to adhere to surfaces such as stainless steel we exposed spores to 1000ppm of the biocide Sodium dichloroisocyanurate (NaDCC). While exposure to biocide had no effect on viability or hydrophobicity it did increase the ability of virulent spores to adhere to stainless steel. This is of particular interest as adherent bacteria show increased resistance to biocide. Given these results it is intriguing to speculate that exposure to sub-optimal concentration of biocide could potentiate the adherence of hypervirulent spores, thus aiding environmental contamination and onward patient transmission.

### **The molecular ecology of anaerobic cellulose-degrading microorganisms**

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Cellulose is the most abundant organic polymer in the biosphere, and its decomposition by natural microbial assemblages is therefore a key component of the global carbon cycle. Yet staggeringly, the identity and function of cellulolytic microbial communities under anoxic conditions in terrestrial and aquatic environments is barely understood, and is restricted to a small number of strains belonging to the genus *Clostridium*. This is largely due to difficulties associated with the isolation and cultivation of obligately anaerobic microorganisms, and we have consequently utilised a molecular biological approach to characterise those microbial communities that colonise and degrade cellulosic substrates in landfill sites and freshwater lakes. The design and application of specific 16S rRNA gene PCR and qPCR primer sets targeting known cellulolytic microbial taxa (*Clostridium*, *Fibrobacter* and Anaerobic fungi), most of which have only been previously detected in the herbivore gut, provided the first evidence that novel species belonging to the genus *Fibrobacter* are present in freshwater lakes and landfill sites, where they colonise cellulosic substrates incubated *in situ*. qPCR analysis of cDNA derived from colonised cellulose 'baits' incubated in landfill leachate microcosms indicated that *Fibrobacter* and *Clostridium* cluster III are the most abundant organisms in the biofilms of heavily-degraded cellulose substrates (relative abundance 28.8% and 17.4%, respectively) and both taxa had very low abundances on cellulose baits that were poorly degraded (relative abundance <0.1%), providing the first evidence for a role of *Fibrobacter* spp. in cellulose degradation beyond the herbivore gut. This research highlights the need to further characterise saccharolytic microbial communities in the biosphere, particularly in light of current efforts to source new organisms and enzymes for biotechnological applications such as biomass processing in biofuels production.

### **Mining the rumen microbes for enzymes involved in lipid metabolism**

Florence Prive

*Aberystwyth University*

Conjugated linoleic acid (CLA) is a collective term for isomers of linoleic acid with conjugated double bonds. Among them, cis9,trans11-CLA and trans10,cis12-CLA are the main isomers found in human food and have been reported to prevent diseases like cancers, obesity and atherosclerosis. Ruminant milk and meat are the richest in CLA; however despite the fact that the ruminant diet is polyunsaturated fatty acid (PUFA)-rich, ruminant products are rich in saturated fatty acids due to bacterial lipolysis and subsequent biohydrogenation of ingested PUFA within the rumen. *Anaerovibrio lipolytica* is a well known rumen lipolytic bacterium, nonetheless data regarding its lipolytic capacity or those of other rumen microbes is scarce and the little that is available is based on a few culturable isolates. The project seeks to get a better understanding of the rumen bacterial lipases and the bacteria that possess them by creating metagenomic libraries of the rumen bacteria. Based on the direct cloning of environmental DNA for the construction of large clone libraries, metagenomics allow accessing microbial genomes and their functions, including the uncultured. Novel enzymes have been successfully isolated from various



environments, including the rumen, then the study of lipases, an important group of biotechnologically relevant enzymes with large applications in food, dairy, detergent and pharmaceutical industries is of interest.

### **Are the Rpf proteins lytic transglycosylases?**

Magdalena Markham  
*Aberystwyth University*

A family of secreted proteins called resuscitation promoting factors (Rpf) are common throughout the actinobacteria, including the corynebacteria. They control the growth and culturability of these organisms. Most of the Rpf molecules remain associated with the cell surface but after prolonged cultivation *in vitro* they are released into the culture supernatant. The secreted protein induces the resuscitation of dormant (temporarily non-culturable) cells after which they resume an actively growing state. Structure determination suggested that Rpf is a lytic transglycosylase. There is experimental evidence indicating that these proteins are peptidoglycan-hydrolyzing enzymes i.e. that they have muralytic activity. The muralytic activity of Rpf variants generated by site-directed mutagenesis correlated with their abilities to stimulate bacterial culturability and resuscitation, which suggested that their biological activity results either directly or indirectly from their ability to cleave bonds in bacterial peptidoglycan.

Very little is currently known about the specific role of the corynebacterial Rpf proteins. All 3 Rpf proteins of *C. jeikeium* were expressed as recombinant proteins in *E. coli* and obtained in native form. Their biological activity as muralytic enzymes was demonstrated. HPLC has been used to separate the muropeptide products of Rpf action on bacterial peptidoglycan. The work so far has demonstrated the cross species activity of the proteins (release of muropeptides from heterologous peptidoglycan) and an element of protein specificity (release of different muropeptides by two different *Corynebacterium jeikeium* Rpf proteins). The main investigation is looking into the cleavage specificity of the Rpf proteins. The initial results suggest that Rpf it is not a muramidase since the muropeptide profile produced is distinctly different from that produced by a commercially available muramidase. The peaks obtained with the muramidase shift following reduction with borohydride, owing to the presence of a carbonyl group associated with the muramic acid residue. However, the mobility of those obtained following digestion with Rpf is not affected by borohydride treatment. This behaviour is characteristic of anhydromuropeptides, characteristically produced by lytic transglycosylases. Mass Spectrometry is currently being employed to confirm that the products of Rpf action are anhydromuropeptides.

### **Garlic: a possible cure for 'hole-in-the-head' disease in fish?**

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The *Spironucleus* genus of diplomonad flagellates is well-known amongst aquaculturists as the causative agent of 'hole-in-the-head' disease in fish. The stressful conditions of aquaculture are a major contributing factor for intestinal colonisation of the host and subsequent systemic infection. As a result, high mortalities are often associated with outbreaks of spironucleosis, causing huge economic losses to the industry. Due to the ban on use of the traditional drug of choice metronidazole on food fish, as a result of its carcinogenicity and non-biodegradable properties, there is currently no effective therapy to eradicate this potentially devastating parasite. *Allium sativum* (garlic) was chosen as a possible antiparasitic agent against *Spironucleus vortens*, a parasite of ornamental fish. Automated optical cell density monitoring as well as membrane inlet mass spectrometry were employed to monitor, respectively, parasite growth and gas metabolism *in vitro*. Whole garlic extract as well as its derivative compounds displayed an inhibitory effect against exponential growth rates, total growth yield and gas metabolism of the parasite in Keister's modified TYI-S-33 culture medium. Of the compounds tested, the ajoene-free mixture of dithiins and thiosulphinates showed the highest degree of inhibition (MIC = 83 µg. ml<sup>-1</sup>, IC50% = 56 µg. ml<sup>-1</sup>); followed by impure ajoene oil (MIC= 107 µg. ml<sup>-1</sup>, IC50%= 58 µg.ml<sup>-1</sup>); with whole garlic extract (MIC>20 mg. ml<sup>-1</sup>, IC50%=7.9 mg. ml<sup>-1</sup>) and allicin (MIC and IC50% >160 µg. ml<sup>-1</sup>) having the lowest inhibitory effect. These inhibitory concentrations are generally higher than usually observed in related protozoa, e.g. *Giardia intestinalis* (MIC allicin = 30 µg. ml<sup>-1</sup>), indicating an unusually high level of tolerance for these compounds in *S. vortens* or a possible alternative mechanism of garlic action. However the ajoene oil and dithiin mixture, as well as preliminary data on other allium-derived compounds show great scope for future work.

### **Insects as a novel source of antibiotics**

Ed Dudley  
*Swansea University*

The talk will discuss the potential of factors derived from insects as potential antibiotics and elaborate on work ongoing to purify and characterise such an entity from the *Lucilia sericata* larvae, which has been shown to contain antimicrobial peptides and separate low molecular weight compounds with activity against bacteria and fungi and hence have potential therapeutic value.

### **Survival of pathogenic organisms in a novel dead sheep containment system**

Ceri Gwyther, Prysor Williams, Davey Jones, Gareth Edwards-Jones, Peter Golyshin ([c.i.gwyther@bangor.ac.uk](mailto:c.i.gwyther@bangor.ac.uk))  
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Livestock mortalities represent a major waste stream within agriculture. Although their disposal is controlled by stringent legislation within the European Union (EU), the legal disposal options currently available to farmers (i.e. centralised collection and subsequent rendering or incineration) are frequently negatively perceived on both practical and economic grounds. Bioreduction can reduce the reliance on these options by providing an on-farm storage option that is both simple and cheaper than using the central collection service. When livestock mortality occurs the farmer must make an incision into the animal's abdomen to release the internal microorganisms and the carcass is immediately placed into a bioreduction vessel containing water which is both heated to 40 °C and aerated. The internal microorganisms degrade the carcass as would happen naturally with the benefits of the optimised conditions provided within the vessel. It has already been proved that bioreduction will reduce the volume of waste produced, however in order to satisfy biosecurity concerns within the EU and therefore present bioreduction as a viable storage option, field scale trials have been designed to establish the fate of potentially pathogenic micro-organisms within. Three bioreduction vessels have been seeded with *Salmonella*, *E. coli* O157 and *Enterococcus faecalis*. Bacteriological, viral, and physicochemical parameters were determined over a four month period. Results show that *E. coli* O157 and *Salmonella* numbers will be sufficiently reduced to satisfy the European Union requirements for a 5-log reduction in indicator organisms, although *E. faecalis* numbers increased somewhat as addition of fresh carcasses increased the loading of this particular pathogen in the liquor.

### **From the ocean to the field: bioluminescent bacteria as a biosensor for water toxicity**

Gray, V. L.<sup>1</sup>, Randles, N.<sup>1</sup>, Lloyd, D.<sup>2</sup> and Russell, S.<sup>3</sup>.

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A continuous on-line water toxicity monitor, utilizing the bioluminescent bacterium *Vibrio fischeri* NRRL-B-11177 as a biosensor, provided reproducible detection of aqueous toxicity events. The analyser employs photo-multiplier tubes (PMT) to detect a decrease in bioluminescence of the *Vibrio* in response to toxic events. The system is able to run un-aided for one month on continuously recording data and alerting the customer to a toxic event in less than 1 min. This is a significant achievement in comparison with time consuming laboratory based batch tests.

The challenge of developing an instrument from a laboratory operated rig, to an automated analyser able to run in the field for one month, has generated novel methodologies and improved understanding of the basic microbiological principles of continuous culture. These principles have subsequently been applied to the maintenance and monitoring systems of the analyser. Problems overcome include: fermentor contamination, continuous flow and mixing rates, sterile consumable installation, and reliable cleaning regimes.

The analyser consists of a fermentor, a nutrient reservoir, a bioreagent/water sample mixing point and a section of residence tubing for various exposure times to occur. As it is widely noted that different chemical types cause most notable effects at different contact times, multiple PMTs were placed at various time points along the residence tubing. This can be seen below in Figure 1, where Zinc (5 mg/L) gives a significantly more marked effect after a 15 min contact time in comparison with 3 min.

### **Biofuels from macroalgae**

Jessica Adams

Aberystwyth University

Climate change, fuel security and economics are creating a requirement for alternative, renewable fuels. The majority of the 'renewable' energy generators – solar-, wind-, hydro- etc generate electricity or heat directly or indirectly; only biomass has the potential to provide a liquid fuel for storage or for use as a transport fuel. This talk will consider the various conversion routes and products for biomass to biofuels using macroalgae (seaweed) as the feedstock example.

### **Developing the DON paradigm: the role of high molecular weight proteins and peptides in plant and microbial nutrition in Antarctic soils**

Paula Roberts<sup>1</sup>, Davey L. Jones<sup>1</sup>, Kevin Newsham<sup>2</sup>, Richard Bardgett<sup>3</sup>

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Until recently the focus of research into plant uptake of N has focused on inorganic species (i.e. NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>). Current research focused on the role of amino acids in plant and microbial nutrition has confirmed that higher plants can capture N from amino acids in cold climate and other soils (e.g. Schimel and Chapin 1996). This direct uptake of organic N provides an effective short circuit of the N cycle removing the need for ammonification and nitrification (e.g. Schimel and Chapin 1996; Jones et al. 2005; Bardgett et al. 2003). It was thought that in order for DON uptake to occur, all proteins and peptides undergo enzymatic cleavage into constituent amino acids. Results in this laboratory suggest that scientists may have overlooked the most important N uptake pathway, that of direct peptide uptake (i.e. amino acid chains of ca 2-20 unit lengths) by plants and soil microorganisms. Peptide transport in microorganisms is well documented and recent evidence has demonstrated the presence of a range of peptide transporters in plants (Williams and Miller 2001). Our results indicate that peptide uptake rates by soil microorganisms are of the same order or faster than that of amino acids and that under controlled conditions plant roots can take up exogenously applied peptide as sole source of N for growth. In this paper we suggest a short circuit whereby plants and microorganisms may directly utilise complex organic N sources.

Bardgett RD, Streeter T, Bol R, (2003). Soil Microbes compete effectively with plants for organic N inputs to temperate grasslands. *Ecology* 84:1277-1287.  
Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A, (2005). DON uptake by plants – an important N uptake pathway? *Soil Biol. Biochem.* 37:413-423.  
Schimel JP, Chapin FS, (1996). Tundra plant uptake of amino acid and  $\text{NH}_4^+$  in situ: Plants compete well for amino acid. *Ecology* 77:2142-2147  
Williams LE, Miller AJ, (2001). Transporters responsible for the uptake and partitioning of nitrogenous solutes. *Ann Rev Plant Physiol Plant Mol Biol* 52:659-679

### **Monokaryons and dikaryons of *Trametes versicolor* have similar combative and decay abilities**

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Abstract: *Trametes versicolor* is a common wood decay basidiomycete, which fruits prolifically, producing a high proportion of viable basidiospores that germinate readily. The 'fitness' of mono karyotic *T. versicolor* mycelia was compared to that of dikaryons in terms of extension rate, decay rate, combative ability, and production of ligninolytic enzymes in agar culture and during growth on beech wood blocks. Eight monokaryons, four natural dikaryons and four artificially synthesised dikaryons (created by pairing monokaryotic cultures) were compared, and paired against 11 wood decay species to assess combative ability. There were no significant differences between monokaryons and dikaryons of *T. versicolor* in any of the characters examined, with as much variation within the karyotic groups as there was between them. When artificial dikaryons and their component monokaryons were considered individually, the dikaryon resembled one of the component mono karyons rather than being intermediate. This implies that dikaryons behave as functional diploids.

### **Illumina sequencing of *Dictyostelium discoideum* mutants**

Justin Pachebat (AU)

### **Bacterial Endophytes in *Miscanthus***

Naomi Cope-Selby

*Aberystwyth University*

*Miscanthus* is a perennial C4 tall woody grass from Asia, which has excited considerable interest in recent years due to the potential for its use as a non-food biofuel crop. *Miscanthus* requires little additional nutrient inputs, including nitrogen, yet grows rapidly ( $3.5\text{m y}^{-1}$ ) and produces a high biomass yield.

Bacterial endophytes live inside internal plant tissues and have a mutualistic relationship with their host. Known advantages to the plant host from the bacterial endophytes presence include pathogen defence, increased nitrogen fixation and plant hormone production.

This work forms the start of a comprehensive study on the species of endophytes present in *Miscanthus* and whether these mutualistic relationships are influenced by factors such as host species. This work is carried out using the unique germplasm collections directly imported from Asia at IBERS (Gogerddan), University of Aberystwyth.

### **Deciphering the role of biosynthetic cytochrome P450s in secondary metabolism pathways in**

#### ***Streptomyces coelicolor***

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*Streptomyces* are Gram-positive soil bacteria with the ability to produce many useful biologically active molecules, such as antibiotics, through secondary metabolism. In 2002, 18 cytochrome P450 (CYP) sequences were revealed from the *Streptomyces coelicolor*A(3)2 genome sequencing project, presenting an unexploited wealth for novel P450 enzymology. Four of the P450s were proposed to be involved in the production of novel secondary metabolites as observed from genetic analysis of putative gene clusters. CYP158A1 and A2 are involved in flaviolin polymerisation. Our work has focussed on CYP170A1 and CYP105N1. CYP170A1 is a bifunctional enzyme with one active site catalysing the production of albaflavenone, and the other producing farnesene. The roles of both antibiotic and signalling molecule in streptomycetes have yet to be understood. Bioinformatics has shown that unlike the other antibiotics produced by *S. coelicolor*, the albaflavenone two gene operon is conserved across at least 8 species of streptomycetes. The least related homolog was found in *Streptomyces albus*. The CYP170 homologous gene was cloned and the protein heterologously expressed in *Escherichia coli*, to further understanding of its role in streptomycetes. Characterisation work is ongoing, looking both at activity and differences in protein folding. CYP105N1 has been cloned, expressed and crystallised for structural analysis. We have initiated the characterisation of CYP105N1 in order to elucidate its biological function in the putative biosynthetic pathway of a zinc chelator, coelibactin. Our work will enhance understanding of streptomycete biology, and the contribution CYPs make to secondary metabolism, with the possibility of harnessing this for biotechnological applications.

### **Nutrient availability for plants and bacteria during early succession in the Alps**

Hans Göransson

*Bangor University*

During stages of primary succession there are only small amounts of nutrients available for bacteria and plants. We measured total soil N and P and adsorption of N and P to ion exchange resins *in situ* as well as bacterial growth response to nutrient addition along the 137 year long chronosequence created by the retreat of the Damma glacier in Switzerland. *In situ* resin-adsorbed N decreased from the pioneer stage (< 16 years since deglaciation) to the middle part of the forefield (57-80 years), and increased again in the older part (> 108 years) whereas adsorbed P was stable. The total N increased over time whereas total P was unchanged. The decrease in N availability from the pioneer stage to the middle part of the forefield likely results from increased plant N uptake due to increased vegetation cover. The bacterial response to nutrients was done by adding labile sources of C, N and P to soil samples and measuring the bacterial growth response (Leucine incorporation) after a 40 h incubation period. The bacteria were never found to be limited by N or P. On the youngest soils nor C, N or P induced a bacterial growth which indicate co-limitation. On older soils the bacteria was found to be C-limited. The shift from where the bacteria did not respond to C to being C-limited was related to the C:N ratio in the soil where the C-limitation occurring only on soils with a C:N ratio below 13.4 (w/w). The increase in growth response to C addition with increasing soil age suggests that N becomes relatively more available to bacteria as compared to C as the soil develops. The strongest N limitation of bacteria was thus not at the same part of the forefield as the lowest availability of N to plants.

### **Biochar: Greenhouse Gas Sink or Health Risk? Assessing the potential impacts of biochar on the fate of polyaromatic hydrocarbons (PAHs) in soil, and consequently agricultural crops**

Sally Rangecroft

*Bangor University*

Currently there is a significant gap in the literature and knowledge surrounding the environmental effects of applying biochar to land. It has been seen that biochar can lead to the sequestration of carbon (C) and can be linked to a reduction in methane and nitrous oxide emissions (Rondon et al., 2005; Spokas et al., 2009), providing a possible strategy for climate change mitigation. However, the production of biochar carries the risk of carcinogenic Polycyclic Aromatic Hydrocarbon (PAH) formation (Ledesma et al., 2002; Garcia-Perez, 2008). Therefore it is hypothesized that biochar could increase the uptake of PAHs by plants via the soil solution following biochar addition to soil. Conversely, biochar may initially reduce the uptake of PAHs due to its supersorbent properties. However, these processes are likely to change over time, as, due to its resistance to degradation, the biochar may turn from a PAH sink to a source.

The current study aims to analyze this potential impact of biochar on the uptake of PAHs as part of a PAH pathway risk assessment. Using a <sup>14</sup>C labelled PAH compound (phenathrene), the potential fate of PAHs in biochar amended soils can be quantified. The four identified pathways include: biodegradation; abiotic degradation (leaching); accumulation in plants; and volatilization (USDHHS, 1995). The impact on the uptake of PAHs is of great importance when considering the possible risk for crop contamination following biochar addition. These findings could be of use to Governments assessing any policy for the application of biochar to agricultural land as a method of C sequestration.

### **Sporulation of arbuscular mycorrhizal fungi in organic-rich patches following host excision**

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Soil organic matter is known to directly influence nutrient capture and affect the growth of arbuscular mycorrhizal fungi (AMF). The effect of organic-rich patches in soil on subsequent sporulation and the persistence of spores will have important implications for sustainable agricultural practices particularly as AMF spores are likely to play a significant role in soil carbon dynamics and ecosystem productivity. We have used a mesh-exclusion approach to quantify the temporal dynamics of AMF sporulation in organic-rich patches following host shoot excision. Wheat plants were grown in pots, (containing three 25 cm<sup>3</sup> bags (35 µm mesh) of farmyard manure (FYM), soil or sand) filled with sterile sand inoculated with either *Glomus intraradices* or *G. mosseae* or non-mycorrhizal control. At six weeks post emergence the host shoot was excised and AMF spores were quantified in each of the mesh bags and again at 24, 52, 66 and 80 days after host excision. Spore numbers were far higher in FYM than in patches of soil, although by 24 d after shoot excision the number of spores had dramatically decreased in patches of both FYM and soil; however this was followed by a significant increase in spore numbers of *G. intraradices* in the FYM patches. Extractable P, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the mesh bags were all significantly higher in the FYM patches than in the patches of soil, although there were no differences between the control and the mycorrhizal treatments. However, the concentration of soluble phenolics in the FYM colonised by both of the AMF species significantly decreased between 0-24 d after host excision compared to the control. This finding provides novel insights into the nature of asymbiotic sporulation in organic-rich patches and has important implications for sustainable agricultural practices, including FYM application enhancing the 'mycorrhizal potential' of soil following a harvest, by increasing the amount of early colonisation in the next crop.

### **Will microbes save the Gulf? - Ecology and global distribution of bacteria for crude oil bioremediation**

Christoph Gertler

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After three months of futile attempts to stop it, the current Gulf of Mexico disaster already represents the second largest oil spill in the history of this planet. More than 9,000,000 litres of toxic crude oil are flowing into this highly vulnerable part of the ocean in a single day, endangering both wildlife and livelihoods of thousands of people. A potential solution to the disaster, the large scale application of highly adapted marine oil degrading bacteria so far has been left out of the oil spill mitigation attempts, entirely wasting the potential of an ocean self-cleaning mechanisms which has kept our planet's oceans tidy for millions of years.

A whole range of microbes belonging to the Gammaproteobacteria - the marine obligate hydrocarbon degrading bacteria (OHCB) - are highly adapted to fast degradation of any kind of oil in the Sea. Naturally occurring on microalgae, they are ubiquitous and ideal candidates for environmentally friendly clean-up of marine oil spills. Yet, they depend upon the additions of vital elements such as nitrogen and phosphorus to multiply and perform their task quickly enough to prevent damages caused by oil slicks. To prove the feasibility and applicability of biotechnological methods for in situ – bioremediation prototypes, a comparative study of both oil degrading microbes and their predators was conducted in the North Sea, Irish Sea and Mediterranean with a standardised experimental design.

Results of the study show that similar microbial communities emerge at all three sites and undergo highly similar population dynamics. Exclusion of protozoan predation furthermore led to a decrease in biodiversity including the lack of vital members of the oil degrading microbial consortium. This indicates that the naturally occurring micro-ecosystem of OHCB and their predators is perfectly adapted and self sustaining, making them the method of choice for tackling oil spills on a large scale and in an environmentally friendly way.

### **Heartwood Extractives of Different Hardwoods Species and Relationships to Termite and White-rot Fungi**

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This study was conducted to determine the mechanisms for the natural durability of twelve commercial Malaysian hardwoods against subterranean termites and white-rot wood decay fungi, with the aim of providing future environmentally sound preservation in susceptible species. Two subterranean termites of Malaysian origin (*Coptotermes curvignathus* and *C. gestroi*) were used in a modified ASTM D3345 wood consumption test. For the white rot test *Pycnoporus sanguineus*, *Trametes versicolor* and *Lentinus sajor-caju* were used in a modified BS EN 6009 test assessed by percentage weight loss. Wood properties were correlated with durability including density, final moisture content and the quantity of solvent extractables (both Toluene: IMS and hot water using TAPPI standard methods). The results showed that the high quantities of extractable components were responsible for high durability (total termite mortality, low termite wood consumption and negligible weight loss against termite and fungi). Toxicity has been examined and this does not totally explain the good performance of the extractives as natural preservatives.

Key words: Natural durability, termite, white-rot fungi, heartwood extractives

### **Is there a stable core microbiota in the horse?**

Kirsty Dougal

*Aberystwyth University*

The horse is a hind gut fermenter that relies on the presence of a diverse symbiotic microbial population to assist in the breakdown of food. Other roles of the bacterial population have been identified such as links with immune function and health status of the horse. Current knowledge about the bacteria found within the horses' hindgut is extremely limited and has tended to be obtained by culture based methods or basic molecular study focused on specific bacteria which have been previously identified.

The project aims to obtain more detailed information regarding the groups/ species of bacteria found in the large intestine of the horse, including identifying whether or not there is a core microbiota present in most healthy horses. This will provide essential information to underpin further research regarding topics such as laminitis, colic and nutrition of the horse.

The initial stages of the project have been examining faeces as a potential sample source to reflect the large intestine. Terminal restriction fragment length polymorphism (T-RFLP) has been used to compare populations from faeces and two regions of the horse large intestine to gain an impression of how closely faeces match the other regions. Initial results suggest that proximal regions may not closely match faeces but distal regions are more similar. Further work will be carried out with more extensive sampling of different regions of the large intestine to answer the question about the use of faeces as a sample source more definitively. 454 pyrosequencing will then be used to gain more detailed information about the bacterial populations in the different regions of the large intestine.

### **Effects of land use types on the activity of waterborne *Escherichia coli* O157:H7 within a UK catchment**

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Contamination of watercourses with faecal matter from sewage outlets, as well as from surface run-off and leachate originating from agricultural land, can lead to the introduction of enteric pathogens, such as *Escherichia*

*E. coli* O157:H7, into these aquatic systems. This poses risks to public health and can also lead to re-infection of livestock and perpetuation of the cycle of infection. To understand the activity and behaviour of waterborne *E. coli* O157:H7, we generated microcosms using river water samples taken from areas of different land use types within the Conwy catchment, UK, such as forestry, peat moorland and agriculture. Half of these mesocosms were filtered to remove the native microbial community. Chemical properties were analysed and autochthonous populations of heterotrophic bacteria and coliforms enumerated. Filtered and unfiltered microcosms were inoculated with a chromosomally *lux*-marked strain of *E. coli* O157:H7 and kept in a constant environment. Activity was measured using a luminometer and expressed as relative light units (RLU). Results showed markedly different levels of *E. coli* O157:H7 activity in water from areas of different land use types. Further investigations will be carried out to simulate effects of increased nutrient fluxes, e.g. from heavy rainfall events, on the activities of *E. coli* O157:H7. It is hoped these results will help to elucidate for the first time how agricultural run-off can affect both the persistence and infectivity of this important human pathogen in watercourses downstream.

### **Temporal ruminal biofilm formation on fresh perennial ryegrass**

Sharon A. Huws

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Due to a growing population and increased demand for livestock products from developing countries, projections estimate that global demand for meat and milk will have doubled by 2050 compared with that at the start of the 21st century (FAOSTAT, 2009). This presents a great challenge to find novel strategies of increasing ruminant productivity given that land is also at a premium due to increasing bioenergy crop production. A major problem in increasing ruminant productivity is that the conversion of plant to microbial protein is inefficient. Thus it is crucial that we gain a better understanding of microbial colonisation and degradation of ingested plant material in order to develop novel strategies to sustainably increase ruminant nutrient use efficiency. Temporal colonisation studies on fresh perennial ryegrass incubated in sacco within cannulated Freisian x Holstein dairy cows have shown that attached bacteria produce EPS which reaches a maxima 2 h post-incubation before receding. Post 2 h of incubation, denaturing gradient gel electrophoresis also revealed a change in the attached bacterial community occurs. This suggests that a biofilm community predominates up to 2 h and thereafter these populations slough allowing colonisation by secondary bacterial colonisers. EPS characterisation revealed a predominance of carbohydrates followed by protein and then DNA. Whilst the DNA component was comparatively small it was comparable to the DNA quantity obtained from the attached bacteria post removal of EPS. Genomic comparisons of the DNA composition within EPS and attached bacteria with EPS removed showed that the EPS DNA likely originates from lysis of some of the attached bacteria. It is known that bacteria attached to forages within the rumen concentrate degradative enzymes, therefore prolonging biofilm longevity may offer a novel strategy of improving ruminant nutrient use efficiency.

## Posters

### **Manipulation of meiotic recombination in barley (*Hordeum vulgare*)**

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Reciprocal recombination is one of the most important factors responsible for generating genetic diversity among organisms. This exchange between two non-sister chromatids of homologous chromosomes starts at the onset of meiosis, and culminates in the formation of chiasmata. Chiasmata are not evenly spread through the genome in species such as wheat, barley, rye and ryegrass, as they occur more frequently in distal regions of the chromosomes. It is estimated that in barley about 50% of genes are confined to cold regions of recombination, and are effectively locked into large linkage groups. The aim of this project is to shift the sites of recombination into these backwaters, with the aim of releasing trapped genetic variation. The initial stage of the project is to accurately map the distribution and frequency of recombination events in barley through the use of immunolocalisation and fluorescence in situ hybridization (FISH). Detection of meiotic proteins using immunolocalisation with antibodies raised against meiotic proteins involved in the late stages of recombination in *Arabidopsis*, in conjunction with antibodies that bind to the synaptonemal complex will reveal the pattern of recombination for each chromosome pair. In order to identify each chromosome of the complement, FISH with single-locus bacterial artificial chromosomes (BACs) in conjunction with rDNA probes have been used, thus enabling the assembly of recombination maps for specific chromosomes. To preserve three-dimensional structure, meiocytes were embedded in polyacrylamide and optically sectioned using a wide-field fluorescence microscope equipped with a motorised stage. The development and application of this new cytological method for describing both the frequency and distribution of recombination will be discussed.

### **From catchment to coast: the activity of *E. coli* O157:H7 in watercourses is affected by land-use**

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Rapid transfer of potentially infective *E. coli* O157:H7 to watercourses may occur during periods of high rainfall due to overland flow and sub-surface carriage from soil or animal waste. In addition to causing human infections, contamination of water sources may also be important in the cycle of re-infection of livestock. Therefore, the objective of this study was to understand the activity of *E. coli* O157:H7 in freshwater environments associated with various land uses (e.g. mountains, heathlands, forest, farmland, and estuary). Water samples collected from areas of different land-use throughout the Conwy catchment (North Wales, UK) were used to generate mesocosms, which were then inoculated with a lux-marked strain of *E. coli* O157:H7 and incubated at 4 °C in diffuse light. Samples were also characterised in terms of their physico-chemical properties. The activity of bioluminescent *E. coli* O157:H7 cells was measured over 7 days and calculated as relative light units (RLU). Initial activity was high with a significant difference in the mesocosms representing areas of different land-use; however, by 7 days the activity had declined in all of the mesocosms and there was no difference between any of the samples. After 7 days a nutrient solution was added to all of the mesocosms, which resulted in a rapid increase in activity in all samples; however, the intensity and duration of this increase was significantly different between the land-use types. Clearly, water chemistry and the indigenous microflora associated with water from different catchment types are fundamental to the level of *E. coli* O157:H7 activity. These findings highlight the importance of catchment land-use on the persistence and infectivity of *E. coli* O157:H7 in the aquatic environment and have important implications for human health and livestock exposure, as activity is a more important indicator of infectivity than cell number alone.

### **The search for salt tolerant crops for Libya**

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Increased demand and salt intrusion into aquifers are having a significant effect on the crop production in Libya (1). This project will compare the relevant water relations parameters of wheat (a glycophyte) and a halophyte (*Suaeda maritima*) with a view to identifying physiological traits that could be the subject of future breeding in wheat and other crops. The poster presents data by which the physiological responses of wheat to hydroponic NaCl treatment can be assessed. It indicates an effect on final plant size, growth rate and plastochron index.

1. FAO 2005. Compendium of Food and Agricultural Indicators. (Statistical Division). Rome.

### **The management of upland areas for biodiversity & bioenergy**

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The aim of this research is assess the suitability of European upland areas for biofuel production and biodiversity conservation using a dual energy stream biorefinery concept. Sheep grazing is in decline in the European uplands

due to impaired economic viability. If these areas are abandoned, it is likely that dominant plant species will prevail with a resultant drop in biodiversity. If harvesting can replace grazing regimes the biodiversity of these upland areas may be preserved. It is envisaged that the harvests may be used to create bioenergy and support declining rural communities. The PROGRASS consortium is comprised of German, Estonian and Welsh academic partners. A pilot plant has been built in Germany and will be trialled in Germany, Estonia and Wales. The plant is mobile and designed for on-farm use. The PROGRASS group aim to take cuts from a selection of upland areas and following hydrothermal pre-treatment: subject the herbage to screw pressing. The fluid from the screw-press treatment will go into anaerobic digesters in order to produce methane. The remaining solid material is dried (using the methane produced from anaerobic digestion) and pelleted in order to produce fuel for combustion. IBERS at Aberystwyth University along with Aston University aim to subject the dried solids to fast pyrolysis in order to produce bio-oil as part of the SUPERGEN research syndicate. We aim to discover: the impact of biomass yield, chemistry and conversion of different grass species, present as different shares in the harvest; the impact of diversity upon biomass production in upland grasslands; and determine the types of processing and conversion routes that are most suited to this type of biomass.

This project is supported by SUPERGEN Bioenergy and the PROGRASS European Life project.

### **Does the activity or amount of extractable compounds account for antitermitic activity in durable Malaysian hardwoods?**

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Little is known about the reasons for durability against termite attack for Malaysian broadleaved trees and various properties (density, extractive content) are examined to explain durability. The heartwoods of four Malaysian broadleaved trees of different but substantial durability against termite attack (in decreasing order: *Neobalanocarpus heimii*, *Madhuca utilis*, *Cotylelobium lanceolatum* and *Shorea curtisii*) were quantitatively extracted in hot water to examine the compounds responsible for resistance to termite attack. High yields were obtained with all species (6.7%-9.6% w/w), especially *N. heimii*. The crude extracts of different concentrations (0%, 0.5%, 1% and 2%) were bioassayed on filter papers against two species of Malaysian subterranean termites (*Coptotermes curvignathus* and *C. gestroi*). The highest inhibitory activity against both termite species was found with *N. heimii* extracts. Other heartwood extracts showed strong but less activity. High durability / anti-termite ability can be explained by both the amount and composition of the extractives present in *N. heimii* and the least durable species, *S. curtisii*, showed the lowest activity when tested at similar concentrations, despite having a high extractive content.

Key words: Tropical species, extractives, bioassay test, toxicity, antitermite activity

### **Towards a hybrid *T. occidentale* x *T. pallescens*: the possible progenitor species of white clover**

Charlotte Jones

*Aberystwyth University*

White clover is an important agricultural crop, well adapted to cool damp climates. It is an ancient allopolyploid, with its alleged origin in the Eastern Mediterranean (Vavilov, 1941). The progenitor species are proposed to be *T. occidentale* as the male donor, and *T. pallescens* as the female donor (Ellison et al, 2004). Hand crossing has been performed, using plants from 3 centres of origin and one unknown. The embryos were rescued, and grown in Gamborg B5 media with or without Kinetin. When the hybrid has been produced it will be used in a study looking at the effect of hybridization and polyploidization on the fate of duplicated genes.

Alison Kingston Smith

### **Improving Drought Tolerance In *Miscanthus* Genotypes**

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Water scarcity is a reality for about one third of the world's population, and agriculture currently uses 70 per cent of the freshwater available for human use. Unless there is an increase in water-use efficiency, a 70 to 100 per cent increase in agricultural production will require roughly twice the amount of water in use today.

*Miscanthus* is a C4 perennial grass which has high biomass potential. However yields at many sites across Europe can be limited by insufficient water supply, and plant survival is endangered under extreme drought. The *Miscanthus* breeding program at Aberystwyth University has one of the largest *Miscanthus* germplasm collections in Europe. The collection contains accessions collected from diverse natural drought prone habitats. These are being screened for drought tolerance traits such as; fine stomatal control and exceptional WUE. These select accessions are included in our crossing program.

Yield potential from *Miscanthus* grown in Europe has been modelled using meteorological data. This model has been extended to include potential climate change scenarios up to 2050. Using modelling of predicted climate



change we have identified European zones where projected changes in rainfall will reduce productivity for presently available commercial germplasm, and where *Miscanthus* germplasm with improved water use efficiency will have greater yield stability.

### **Variations of quality components and Gene Expression Analysis of Postharvest Senescence Marker in Baby Spinach Leaves**

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Spinach leafy vegetables during postharvest stage undergo several mechanical processes that may induce tissue damage and quality losses. The membrane degradation may activate several postharvest problems with involvement of ascorbic acid degradation. Ascorbic acid is highly perishable and can be an indicator freshness or leaf senescence. However, though bioinformatics investigation were identified putative senescence postharvest markers. These genes were isolated using degenerate primers and cloned. Spinach leaves were harvested at commercial stage, cut in six pieces and stored at 20 °C. Control was represented by intact leaves stored at the same conditions of treatment. The ascorbic acid was measured in spinach leaves until 3 days of storage. Gene expression analyses of a putative cysteine proteases (SoSAG12) was determined in different sampling points. Results observed showed a decrease of ascorbic acid immediately after wounding and during storage. The expression of SoSAG12 was enhanced in wounded tissues as soon as after 6 h. Furthermore, the genes encoding for enzymes involved in the Ascorbate-Glutathione cycle were studied in response to wounding/cut in spinach leaves.

### **Exploiting the diversity of form in *Miscanthus* for increased Biomass**

Kerrie Farrar

*IBERS, Aberystwyth University*

There is an urgent need to breed new, higher yielding *Miscanthus* varieties in order to deliver lignocellulosic biomass whilst improving land use efficiency. Understanding the genetic control of biomass performance traits is of vital importance for the acceleration of breeding higher yielding varieties. Plant architecture is important for biomass yield and is under genetic control. IBERS curates a unique and comprehensive collection of *Miscanthus* which includes plants with very divergent architecture including *M. sinensis* which is compact with numerous thin stems and *M. sacchariflorus* which is tall with few thicker stems.

A thorough phenotypic characterisation of this UK *Miscanthus* collection is being carried out at IBERS in order to identify desirable idiotypes. The European *Miscanthus* Improvement (EMI) project demonstrated that no single genotype performed optimally at all latitudes in Europe (Agronomy Journal (2001) 93:1013-1019) and so different genotypes will be required for different locations. Linking genotype to phenotype and generating molecular markers for desirable traits will accelerate the breeding cycle and thereby allow more rapid development of lines adapted for their environments and end usage. To this end orthologues of candidate genes encoding morphological characteristics are being identified and cloned in *Miscanthus* with the aid of bacterial artificial chromosome (BAC) libraries, and association studies being performed to link genotype to phenotype. Alleles conferring improved characteristics for biomass will be identified and made available for use in the *Miscanthus* breeding programme based at IBERS.