


Thurs 19 Mar		Lecture Theatre 2	Lecture Theatre 3
10.00-10.45		Plenary lecture (sponsored by Lennox): Chair Mike Jones David Beerling - Fungal powerlines of plant energy: planetary regulators of CO <sub>2</sub> and climate?	
10.45-11.15	Tea/Coffee		
	Session 1: Chair Ewen Mullins		Session 2: Chair Fraser Mitchell
11.15-11.35	Doohan et al. Plant responses and resistance to a fungal toxin that inhibits protein synthesis: new insights into plant stress tolerance		Finn et al. Experimental increases in diversity and evenness improve productivity and reduce weed invasion in grassland swards ...
11.35-11.55	Le Martret et al. Generation of plastid transformants to examine the role of ROS scavenging enzymes in plant response to environmental stresses		Power et al. Botanical diversity in organic and conventionally managed grassland
11.55-12.15	Destefanis et al. Sequence based characterisation of the Gpa4 locus conferring resistance to PCN in potato		Delaney et al. Maritime Cliff-top Grasslands in Ireland.
12.15-12.35	Mullins et al. Genotype dependency within <i>Agrobacterium tumefaciens</i> in relation to the integration of vector backbone sequence in GM potato		Hochstrasser et al. Irish grasslands between 1965-1975: Austin O'Sullivan's dataset
12.35-14.00	Lunch		
	Session 3: Chair Phil Dix		Session 4: Chair J
14.00-14.20	Mina-Vargas et al. Phylogenetic relationships between the common bean <i>Phaseolus vulgaris</i> and closely related bean species.		Osborne Why does <i>Gunnera</i> do it and other Angiosperms don't?
14.20-14.40	Diekmann et al. Diversification patterns of chloroplast genes and genomes in the grasses (Poaceae)		Armstrong et al. Habitat-Related Mapping of <i>Gunnera tinctoria</i> Invasion at a Local Scale
14.40-15.00	Hodkinson et al. Phylogenetics of the grass family (Poaceae)		Ugoletti et al. A comparison among <i>Impatiens</i> species with different invasive capacity
15.00-15.20	Fornara et al. Mechanisms responsible for positive plant diversity/composition effects on critical ecosystem services		Dietzsch et al. Density effects of alien invasive plants on native pollination processes
15.20-15.40	Tea/Coffee		
	Session 5: Chair Paul McCabe		Session 6: Chair Mike Jones
15.40-16.00	Ali et al. Cellulosic-ethanol: Simultaneous saccharification and fermentation of wheat straw and bran		Chambers et al. An inducible, modular system for spatio-temporal control of gene expression in stomatal guard cells
16.00-16.20	Hennessy et al. Optimising the capacity of <i>F. oxysporum</i> for degrading lignocellulose wheat straw and bran into ethanol and added value ...		Lanigan et al. Carbon Isotope Fractionation during Photorespiration and Carboxylation
16.20-16.40	O'Donoghue et al. Assessment of <i>Medicago truncatula</i> as a model for transcriptome analysis of white clover undergoing cold stress		Abdalla et al. Modeling the Impacts of Climate Change on Irish Cereal Crops
16.40-17.00	Reardon et al Evolution of flower shape in <i>Plantago lanceolata</i>		Stengel A botanical excursion to Bologna – results from a survey of Irish and German academics in 2008
17.00-17.30	Jebb Plant Conservation Network for Ireland Forum discussion		
17.30-19.30	Poster Session		

<b>Fri 20 Mar</b>	<b>Lecture Theatre 2</b>	<b>Lecture Theatre 3</b>
	Session 7: Chair Frank Wellmer	Session 8: Chair Daniel Kelly
09.00-09.20	Besse et al. Aquaporins in developing barley ( <i>Hordeum vulgare</i> ) leaves	Campbell et al. Conservation and monitoring of rare and legally protected bryophyte species in Ireland
09.20-09.40	Cotecchia et al. Investigating the correlation between the fatty acid levels and the expression of candidate genes involved in the fatty acid pathway in perennial ryegrass	Higgins et al. Contribution of lichens to biodiversity and productivity in different habitats in Swaziland, southern Africa
09.40-10.00	Knipfer et al. Whole-plant water flow in barley and the impact of root aquaporins on shoot water supply	O'Hanlon et al. An investigation of fungal diversity in Irish managed and semi natural forests
10.00-10.20	Petti et al. Towards the understanding of the molecular mechanisms determining the activation of plant defences by non-pathogenic biocontrol bacteria	Kildea et al. Irish potato late blight populations in 2008
10.20-11.10	<b>Tea/Coffee</b>	
	Session 9: Chair Tommy Gallagher	Session 10: Chair
11.10-11.30	Meade et al. An empirical gene flow model for Ryegrass	Mitchell et al. Reconstructing raised bog water tables over the last 5,000 years from proxy methods
11.30-11.50	Chikkaputtaiah et al. Molecular and functional characterization of the Arabidopsis ESCRT-I complex	Saunders et al. Assessing the impact of forest age on net ecosystem carbon exchange
11.50-12.10	McKeown et al. Genome dosage effects in isogenic polyploids of Arabidopsis thaliana	Albanito et al. Automated soil profile respiration measurements: new information, opportunities and challenges
12.10-12.30	Plesters et al. Genetic variation of dormancy-related traits in European trees	Lanigan et al. The carbon balance of European croplands: the influence of gross primary production during non-optimal growth periods
12.30-13.00	<b>Prize giving and meeting close</b>	

**Modeling the Impacts of Climate Change on Irish Cereal Crops**

**Abdalla, Mohammed<sup>1</sup>; Jones, Mike<sup>1</sup>; O'Reilly, S.<sup>2</sup>; Holden, N. M.<sup>3</sup>**

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The impacts of future climate change on Irish spring barley, winter wheat and silage maize production were assessed using the CERES-model (Crop Environment Resource Synthesis) which is part of DSSAT (Decision Support System for Agro-technology Transfer) package. A baseline (1961-1990), and two future climate scenarios 2055 (2041-2070) and 2075 (2061-2090), each of a 30 year period, from the HadCM<sub>4</sub> (Hadley Centre Global Climate Model) were investigated. The objective were to assess the impacts of climate change on yield of these crops and variability between three main regions of crop production in Ireland (Mid-east, south-east and border region). Predicted low spring and summer precipitations, due to climate change, would negatively affect spring barley. By the end of this century, spring barely grain yield in south/ mid-east of the country would decrease by 10% however, that in border area would be less affected (-4%). This would lead to spring barley growth to be shifted to the border area and water irrigation to be essential in the south/ mid-east regions. Winter wheat grain yield would increase by up to 6% until 2055; however by the end of this century would reduce by up to 14%. This reduction is mainly due to high temperature and would be partly compensated by increasing CO<sub>2</sub> concentration. Silage maize biomass production would significantly increase (34-97%) and the crop would be viable in places which are less suitable at present.

**Automated soil profile respiration measurements: new information, opportunities and challenges**

**Albanito, Fabrizio; Jones, Mike B.**

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In recent years soil CO<sub>2</sub> emissions has been the subject of intense investigation because (i) its potential role in amplifying global warming; and (ii) gaseous compounds formed in the soil environment are, in general, good indicator of soil biology and biochemistry. Accurate techniques used to monitor soil CO<sub>2</sub> profile concentrations offers the opportunity to identify localized carbon dioxide sources and potential sinks in the soil, and to understand the processes that control CO<sub>2</sub> production and emission. In this study, we developed an automated method to continuously monitor soil CO<sub>2</sub> concentration, by using a new type of soil diffusion chamber. We estimated soil CO<sub>2</sub> efflux using a new model to determine the vertical CO<sub>2</sub> gradient across the soil profile up to 80 cm depth, in conjunction with models to determine the soil CO<sub>2</sub> diffusion coefficient. Furthermore, we assessed vertical CO<sub>2</sub> production rates within the soil profile. Automated soil profile respiration measurements provide valuable information that is often missed with less frequent manual measurements, and presents the opportunity to move beyond empirical models towards a predictive understanding of the key mechanisms that determine soil respiration fluxes. However, these continuous measurements present new challenges in that they require the additional management of complex equipment and large data sets as well as novel analytical approaches.

**Cellulosic-ethanol: Simultaneous saccharification and fermentation of wheat straw and bran.**

**Ali, Shahin<sup>1</sup>; Khan, Mojibur<sup>1</sup>; Fagan, Brian<sup>1</sup>; Mullins, Ewen<sup>2</sup>; Doohan, Fiona<sup>1</sup>**

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*Fusarium oxysporum* is a good candidate for simultaneous saccharification and fermentation (SSF) of lignocellulosic-agricultural waste to ethanol. We studied 18 *F. oxysporum* strains collected from Ireland to evaluate their SSF ability in solid state fermentation of wheat straw and bran. Based on preliminary studies, 5 strains were selected and their solid state cultural conditions were optimised in terms of media, pH, moisture content and temperature. Among the six different standard fungal growth media tested, production of ethanol was highest in the media earlier described by Mishra *et al.* (1984). The best pH, moisture content and temperature for ethanol production by these fungi were found to be 7, 90% and 35<sup>0</sup>C respectively. Under these optimum growth conditions, the *F. oxysporum* isolates produced up to 57 and 136 mg ethanol per gram of wheat straw and bran, respectively. We studied the inter-strain variation in the activity of the enzymes involved in saccharification of cellulose and hemicellulose with lignocellulosic material. Of the 5 strains tested, *F. oxysporum* strain 11C had highest  $\beta$ -glucosidase, endoglucanase and exoglucanase activities. But *F. oxysporum* strain 27E had highest  $\beta$ -xylosidase and endoxylanase activities. Using these 2 fungal strains we also showed that the quantity of ethanol yielded by SSF of straw is dependent on the wheat cultivar used.

Mishra *et al.* (1984) *Applied and Environmental Microbiology*, 48(1): 224-228.

### Habitat-Related Mapping of *Gunnera tinctoria* Invasion at a Local Scale

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Predicting the future spread and impact of plant invasions is limited because of the absence of detailed information required to identify the habitats and localities most susceptible to invasion. Habitat-related mapping on a local scale can provide information on the areas most susceptible to invasion as well as providing information on the potential means of spread. These data are vital for the implementation of effective management programmes. We have mapped the distribution of the invasive species *Gunnera tinctoria* on Achill Island, Co Mayo, and examined the distribution of this species in relation to rivers, roads, human habitation and vegetation cover using the CORINE land cover map. As well as recording the presence of *G. tinctoria*, the percentage cover was estimated and categorised into a scale, from individual plants to large populations, to better understand the process of invasion. It is the first map produced of invasive species distribution at this scale in Ireland, and has given important insight into the means of spread, the habitats it invades and produced a baseline for future monitoring. The range of habitats where *G. tinctoria* was found included: grasslands, coastal cliffs, waterways, roadsides and bog.

### Aquaporins in developing barley (*Hordeum vulgare*) leaves

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It is not known along which pathways water moves within growing leaf tissues. The membrane intrinsic proteins (MIP) or aquaporins are likely to play a key role, allowing the passage of water or small-molecular weight solutes through membranes. Previous microarray studies on the developing barley (*Hordeum vulgare* L.) leaf suggested that several aquaporins are expressed in either a growth-specific manner or specific to photosynthesizing, transpiring leaf tissue. In the present project this idea has been tested in detail using real-time PCR (qPCR), and also by comparing expression of aquaporins between day and night periods (differing in leaf transpiration and growth). Several candidate aquaporins have been cloned and tested for functionality (water

channel activity) through expression in *Xenopus laevis* oocytes. Currently, the subcellular localization of candidate aquaporins is studied using transient expression in onion epidermal cells.

**Conservation and monitoring of rare and legally protected bryophyte species in Ireland**

**Campbell, Christina<sup>1</sup>; Kelly, Daniel<sup>1</sup>; Smyth, Noeleen<sup>2</sup>**

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Ireland has one of the richest bryofloras in Europe, with over 50% of the European bryophyte flora represented in Ireland. Some species occur in specific habitats such as dune slacks, machair, fens and old metal rich mine sites. These habitats are under threat from land use change and other human activities. The project focuses on 10 bryophyte species and aims to develop both *in situ* and *ex situ* conservation methods. Suitable permanent monitoring will be set up for selected bryophyte populations to ensure that both the habitat and environment in the remaining Irish sites is recorded and to maintain their suitability into the future for the continued existence of dune slack, machair, fen and metal rich grassland species in Ireland. The project also aims to make a full genetic appraisal of the Irish populations of the species including *Petalophyllum ralfsii*, *Bryum uliginosum*, *Paludella squarrosa*, *Leiocolea rutheana*, and *Ditrichum cornubicum*. Germination trials and experiments will be carried out. Spores of *Bryum uliginosum*, a dune slack species, have successfully germinated and growth on the different media mixes trialed recorded. These initial results indicate that the production and germination of spores of this species is not the limiting factor influencing its rarity in Ireland.

**An inducible, modular system for spatio-temporal control of gene expression in stomatal guard cells**

**Chambers, John<sup>1</sup>; Xiong, Tou Cheu<sup>1,2</sup>; Hann, Cliona<sup>1</sup>; Surget, Marie<sup>1</sup>; Ng, Carl<sup>1</sup>**

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Stomata, flanked by pairs of guard cells, are small pores on leaf surfaces of plants and function to control gas exchange between plants and the atmosphere. Stomata will open when water is available to allow for uptake of carbon dioxide for photosynthesis. During periods of drought, stomata will close to reduce desiccation stress. As such, optimal functioning of stomata will impact on water use efficiency by plants. Here, we report the development of an inducible, modular system for robust and targeted gene expression in stomatal guard cells. We showed that application of ethanol vapour to activate the gene expression system did not affect the ability of stomata to respond to ABA in bioassays to determine promotion of stomatal closure and inhibition of stomatal opening. The system we have developed allows for robust spatio-temporal control of gene expression in all cells of the stomatal lineage, thereby enabling molecular engineering of stomatal function as well as studies on stomatal development.

**Molecular and functional characterization of the *Arabidopsis* ESCRT-I complex**

**Chikkaputtaiah, Channakeshavaiah<sup>1,2</sup>; Shahriari, Mojgan<sup>2</sup>; Huelskamp, Martin<sup>2</sup>; Schellmann, Swen<sup>2</sup>**

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Recently the *Arabidopsis* *ELCH* gene, a homolog of Vps23/ TSG101 and the key component of the plant ESCRT-I complex has been functionally characterized. The *elch* mutant shows multiple nuclei

in various cell types, indicating a role in cytokinesis. VPS28 and VPS37 are other known components of ESCRT-I complex that in combination with *elch* have synergistic phenotypes in double and triple knock-outs suggesting their involvement in ELCH-dependent regulation of cytokinesis. Cytokinesis regulation is therefore a function of the whole ESCRT system in plants. In addition to ELCH and VPS23-2 a third, dicot-specific Vps23 homolog, VPS23-3 (At2g38830) has been identified. It shows a lesser degree of homology (47%) to *Arabidopsis* ELCH compared to the homology of ELCH to *Oryza sativa* ELCH (66%) and VPS23-2 (72%). Similar to ELCH, VPS23-3 is ubiquitously expressed, localized on endosomes, binds to ubiquitin and part of a high molecular weight complex suggesting that VPS23-3 is a component of the plant ESCRT system. Surprisingly however, VPS23-3 did not rescue the *elch* phenotype indicating that it has a cellular function different from the other two Vps23 genes. ELCH-HA expressed in *vps23-3* knock-out showed a shift in the molecular weight of the complex and VPS23-3 show differential interaction patterns suggesting that VPS23-3 might serve as an additional, fourth component of the *Arabidopsis* ESCRT-I complex.

### **Investigating the correlation between the fatty acid levels and the expression of candidate genes involved in the fatty acid pathway in perennial ryegrass**

**Cotecchia, Luca<sup>1,2</sup>**; Boland, Tommy<sup>2</sup>; Lynch, Bridget<sup>2</sup>; David McGilloway<sup>3</sup>, Mullins, Ewen<sup>1</sup>; Byrne, Stephen<sup>1</sup>; Susanne Barth<sup>1</sup>

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In Ireland approximately 90% of the total agricultural area is devoted to grassland, and perennial ryegrass (*Lolium perenne* L.) is the predominant grass species. Polyunsaturated fatty acid (PUFA) intake has been positively associated with human health as well as cow fertility. High levels of fatty acids in perennial ryegrass increase the levels of PUFAs. Focussing on the candidate genes involved in the fatty acid pathway in perennial ryegrass, degenerate primers were designed from available cDNA sequences of *Poaceae* using the web program PriFi. Amplification in perennial ryegrass was achieved for Acetyl-coenzyme A carboxylase (Accase) = catalyzes the first step in the synthesis of long chain fatty acids; Lipocalin = fatty acid-binding proteins, which function similarly by binding small hydrophobic molecules; Oleate desaturase = catalyze the insertion of a double bond at the delta position of fatty acids. The transcriptional activity of each gene was assessed in 12 ryegrass varieties, previously field grown in 2008. These results will be discussed in regard to increasing our understanding of the biological and genetic control mechanisms for the variation of fatty acid levels in ryegrass.

### **Maritime Cliff-top Grasslands in Ireland.**

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The Irish Survey of Semi-natural Grasslands surveyed 250 sites in counties Cork and Waterford in 2008. The results of this survey highlighted semi-natural grassland communities primarily associated with coastal areas, including maritime cliffs. Coastal grasslands are clearly recognised in the UK but, with the exception of dune grasslands, are not included in the Heritage Council's Guide to Habitats in Ireland. Relevés recorded within 200m of the coast were re-analysed using Ellenberg indicator values calibrated for Britain and Ireland. The saline character of these grasslands is evinced by the frequency of species with a high salt tolerance including *Armeria maritima*,



*Plantago maritima* and *Plantago coronopus*. This type of grassland may occur naturally in Ireland, unlike many others which are maintained by anthropogenic factors. The maritime cliff-top grassland type differs clearly from those currently described in the most frequently used classification in Ireland. It is therefore likely to be under-recorded, and may suffer due to a lack of conservation.

**Sequence based characterisation of the Gpa4 locus conferring resistance to PCN in potato**  
**Destefanis, Marialaura**<sup>\*1,2</sup>; Jones, Peter<sup>2</sup>; Bryan, Glenn<sup>3</sup>; Griffin, Denis<sup>1</sup>; Milbourne, Dan<sup>1</sup>

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The potato cyst nematode *Globodera pallida* Pa2/3 is one of the most relevant soil-borne pests of potato in Europe. A quantitative trait locus, called Gpa4, that confers resistance to *G. pallida* has been identified on the short arm of potato chromosome IV, in a region which harbours a resistance hotspot conferring resistance to this nematode and to unrelated pathogens. The main objective of this study is the identification of the genes involved in conferring the resistance phenotype at the Gpa4 locus by producing a high quality DNA sequence of the region. A number of BAC clones from this region were identified using markers genetically linked to Gpa4. The BACs were sequenced and assembled, in part by exploiting the synteny between the potato and tomato genomes. Putative genes expressed in the BAC sequences were identified using an *ab initio* gene prediction package, and further analysis revealed that one of the BACs contains 2 R gene candidates, belonging to the NBS-LRR sub-class of resistance genes. Their sequences revealed a high degree of similarity to *Rpi-Blb3*, a late blight resistance gene that clusters in the same resistance hotspot on chromosome IV. Physical mapping and sequencing of BAC clones to elucidate the complete sequence of the Gpa4 region is ongoing.

**Diversification patterns of chloroplast genes and genomes in the grasses (Poaceae).**

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Recent years have witnessed a major acceleration in the sequencing of whole chloroplast genomes of monocots and grasses in particular. To date 18 complete chloroplast genome sequences of monocots are available. Thereof thirteen sequences are from Poaceae taxa including the world's most important agricultural species like *Oryza sativa*, *Saccharum officinarum*, *Triticum aestivum* and *Zea mays*. Our research group sequenced the entire plastid genome of *Lolium perenne*, the most important forage grass in many parts of the temperate world. Here we compare the chloroplast genome of *Lolium* with its close relatives in Pooideae and also its more distant relatives in the grass family and related families. We discuss structural features of the chloroplast genome of Poaceae in comparison to other monocots and present studies of chloroplast gene evolution and diversification. We compare known and newly generated information on RNA editing sites and how these are related to phylogenetic pattern in grasses. The potential of these whole genomes for phylogenetic inference using Bayesian and parsimony based methods is also explored.

**Density effects of alien invasive plants on native pollination processes**

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Alien invasive plants can alter native communities as well as ecosystem processes and are therefore recognised as a major threat to biodiversity. In recent years, a growing body of research has investigated their impacts on mutualisms, such as plant-pollinator interactions. Alien plants can benefit native plants and pollinators by attracting a surplus of pollinators or offering valuable resources. Alternatively, they may disrupt native plant-pollinator interactions by monopolising pollinators, producing toxic compounds or displacing native plants. Most studies on invasive plants and pollination focus on heavily invaded communities. However, the impacts of invasive plants are thought to be a function of their density within a habitat. We therefore investigated insect flower visitation, pollen deposition and seed set of native focal plants in response to different density levels of *Rhododendron ponticum* and *Impatiens glandulifera*, two severely invasive species in Ireland. In addition, we studied pollinator behaviour and pollinator population characteristics. Bumblebees, the main pollinators, were attracted by the aliens and benefited from their plentiful rewards. The impacts of the aliens on native co-flowering plants were species-specific and density-dependent. Although flower visitation and pollen deposition was disrupted by the aliens at medium or high densities, these impacts were not always mirrored by a decrease in fruit or seed set.

**Plant responses and resistance to a fungal toxin that inhibits protein synthesis: new insights into plant stress tolerance**

**Doohan, Fiona;** Walter, Stephanie., Arunchalam, Chan; Erard, Guillaume; Jiang, Shumei; Doyle, Siamsa; Egan, Damian; Ng, Carl; McCabe, Paul.

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The *Fusarium* mycotoxin deoxynivalenol (DON) is a potent inhibitor of eukaryotic protein synthesis and acts as a virulence factor during infection of wheat heads. Using both *Arabidopsis* and wheat, we show that the plant response (cell death and defence gene expression) to DON is light-dependent, suggesting that chloroplasts and cell death regulation are an important components of the host response to this toxin. This was further validated by wheat transcriptome studies. Some wheat genotypes can tolerate DON and resist its deleterious effects; others cannot. Transcriptome studies identified DON-responsive wheat genes and highlighted those that were up-regulated in spikelets of the DON-resistant wheat cultivar CM82036 in response to DON treatment. Their functional characterisation, coupled with *in silico* studies of *Arabidopsis* homologs, suggest that the promotion of cell survival and alleviation of oxidative stress are important components of DON resistance. Functional studies have led to the characterisation of novel wheat genes and these characteristics and the contribution of this work to the understanding of plant stress tolerance will be discussed.

**Experimental increases in diversity and evenness improve productivity and reduce weed invasion in grassland swards over three years across 12 European sites.**

**Finn, John<sup>1</sup>;** Kirwan, Laura<sup>1,2</sup>; Brophy, Caroline<sup>3</sup>; Connolly, John<sup>3</sup>

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We manipulated the relative abundance of four agronomic species (2 legume and 2 grasses) to create a gradient of plant diversity in 30 plots at each of 12 European sites. Over three years, the average yield of mixtures exceeded that of monocultures. The average weed biomass of the monocultures increased from 15% in year 1 to 40% in year 3, whereas weeds consistently contributed only about 5% of the total yield of the mixtures. By year 3, the average yield of the



original sown species in mixture was more than double that in monoculture. Within harvest years 1-3, the average total annual yield of mixtures was estimated as being 6%, 20% and 16% greater than that of the highest yielding monoculture.

### **Mechanisms responsible for positive plant diversity/composition effects on critical ecosystem services**

**Fornara, Dario<sup>1</sup>; Tilman, David<sup>2</sup>**

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The importance of conserving plant diversity has been recognized by a large community of ecologists and this mainly because greater species diversity contributes to the delivery of multiple ecosystem services such as primary production and soil carbon (C) sequestration. Complementarity in resource use between plant species and/or functional groups may be a cause of these diversity effects but the presence of key species and/or key functional groups within the plant assemblage may also contribute. Here we focus on a related issue that has received much less attention, the proximate ecological mechanisms associated with both the positive effect of plant diversity/composition on productivity and on soil C sequestration. Using data of a long-term grassland experiment we show how the increased presence of C4 grasses, C3 grasses, legumes and forbs at higher species diversity affect seasonal nitrogen (N) supply and use, seasonal soil moisture, root production and the total root N pool to maximize aboveground productivity. We also show how increased plant diversity and the joint presence of C4 grass and legume species are key causes of greater soil C and N accumulation which occurs via higher root biomass and from greater root biomass accumulation to deeper soils.

### **Optimising the capacity of *F. oxysporum* for degrading lignocellulose wheat straw and bran into ethanol and added value by-products**

**Hennessy, Rosanna<sup>2</sup>; Khan, Mojibur R.<sup>1</sup>; Doohan, Fiona M.<sup>1</sup>; Mullins, Ewen<sup>2</sup>**

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The filamentous fungus *F. oxysporum* is capable of degrading low-value lignocellulosic wheat straw and bran into sugars which are subsequently converted to ethanol in a single step. This process of simultaneous lignocellulose-residue saccharification and fermentation (SSF) is considered a major advantage of *F. oxysporum* over other microbial fermenters. *Agrobacterium-tumefaciens* mediated transformation is being used to generate a library of *F. oxysporum* gene disruptant mutants from which individuals with an increased or decreased ability to produce ethanol and added value by products will be selected. The binary vector used for ATMT is a pBht2 vector containing the hygromycin resistance gene (*hph*) and EGFP markers to select for transformants. Putative transformants can be confirmed by GFP microscopy and a triplex PCR using primers for *hph*, EGFP and an ITS sequence specific to *F. oxysporum*. The *A. tumefaciens* strain AGL1 is grown in selective media and co-cultivated with fungal spores in the presence of acetosyringone. Transformants are identified on selective media and eventually single spores are isolated and stored. Each fungus or isolate requires its own optimal conditions to obtain optimal transformation efficiency. Several factors influence ATMT and therefore optimisation of the ATMT protocol is essential as this technique will be used to identify genes intrinsic to bioethanol production. Ultimately, the aim is to enhance the ability of *F. oxysporum* for lignocellulose bioconversion.

**Contribution of lichens to biodiversity and productivity in different habitats in Swaziland, southern Africa**

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Lichens play an integral role in the ecological value of a habitat and are commonly accepted as reliable indicators of environmental quality. As a result, the mapping of lichen abundance, diversity and distribution is becoming routine across the globe, as such surveys are generally fast and inexpensive. However, large parts of Africa remain unexplored in terms of lichenological research. For example, no published records of lichen distribution, diversity or abundance exist for Swaziland, southern Africa. This current study aims to document the diversity and distribution of epiphytic lichens in two protected nature reserves of Swaziland to serve as a baseline database for epiphytic lichens for the country. Lichens were collected across an altitudinal gradient in Swaziland. Over 170 lichen samples were collected and are currently being identified; distribution throughout the country was mapped and species restricted to Highveld and Lowveld sites were noted. The importance of lichens for passerine birds was also analysed by examining nests for the presence/absence of lichens, whilst paying particular attention to the types of nests which commonly incorporate lichen into their design. Distinct trends were noticed in the type of nests that included lichens; specific traits suggested that birds more than likely use lichen as a form of camouflage, deterring predators as opposed to ascertaining any structural benefit from the lichen thallus.

**Irish grasslands between 1965-1975: Austin O'Sullivan's dataset**

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Grassland habitats occupy about 70% of the Irish landscape and are of great cultural and economic importance. However, despite this importance, little attention has been paid to the botanical composition of lowland grassland habitats, to a formal classification of these plant communities, and to changes in their composition over time. In the period 1965 to 1975 Dr. Austin O' Sullivan carried out a survey of Ireland's grasslands completing approximately 3,000 relevés according to the standard Braun-Blanquet method. We wish to report on how these relevés have been inputted into a standardised vegetation database, to summarise some of Dr. O'Sullivan's data, and to outline the classification work that will be undertaken using these data. Since Dr. O'Sullivan's survey many of these grasslands may have been lost to intensification. We will discuss the relevance of these data in terms of current landscape management at the national and regional scale.

**Phylogenetics of the grass family (Poaceae)**

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Phylogenetic analyses were used to study patterns and processes of evolution in the Poaceae. Analyses included a broad representation of diversity (84% of tribes and 42% of genera) and sequences of three plastid DNA regions *rbcL*, *matK* and *trnL-F*. Maximum parsimony, Bayesian and molecular dating methods were applied. Using this 'backbone' tree we subsequently reconstructed the phylogeny of all 800 grass genera by incorporating morphological characters. We used these trees to study patterns of diversification, the evolution of C<sub>4</sub> photosynthesis, co-evolution with ungulates in relation to herbivory, and biogeographic diversification. The ancestral origin of grasses was found to be in Africa. The PACCMAD clade, including all C<sub>4</sub> grasses, originated in Africa. We found that shifts from C<sub>3</sub> to C<sub>4</sub> photosynthesis occurred at least 12 times starting 30.9 mya. However, the ecological dominance of C<sub>4</sub> grasses coincides with recent phylogenetic diversification events in the late Miocene suggesting that once the grasses were C<sub>4</sub>, factors other than photosynthetic type drove diversification. To investigate co-evolution of grasses and ungulates, we combined fossil and phylogenetic data for ungulates and measures of the density of silica bodies in grass leaves. We provide evidence that C<sub>4</sub> grasses have evolved higher silica densities in response to grazing.

### **Irish potato late blight populations in 2008**

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In late 2007 an all Ireland study of potato blight populations was initiated. As part of this study an extensive collection of *Phytophthora infestans* was made from blighted material obtained from commercial potato crops throughout the country and their mating type determined. A total of 230 single lesion isolates were successfully obtained (203 from the Republic of Ireland and 27 from Northern Ireland). Of these 58 (or 25% of the collection) were characterised as the A2 mating type. The A2 isolates were not confined to a specific cultivar or region, although the majority were retrieved from the East of the country. Further phenotypic and molecular characterisations of these isolates are ongoing. The dramatic re-emergence of the A2 mating type within Irish *Phytophthora infestans* populations is however a worrying development as the pathogen has now the capacity to sexually recombine.

### **Whole-plant water flow in barley and the impact of root aquaporins on shoot water supply**

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The present study shows a detailed analysis of water transport in barley (*Hordeum vulgare*) with focus on the role of the root-system in regulating shoot water supply. The hypothesis is tested that the root-system can limit shoot water supply and that the root/shoot junction is a bottleneck for axial water transport. A possible role of root aquaporins in the regulation of root (and shoot) water transport is investigated. Hydraulics of seminal and adventitious roots are analysed, and measurements are combined with transpiration measurements at the whole-plant level. Analogue to an electric circuit the hydraulic resistance of the root-system, root-shoot junction and shoot is determined. The data show that the root-system is not limiting shoot water supply but that barley plants can, in theory, transpire at 200-times higher rates before root hydraulics become limiting. Similarly, the root/shoot junction does not limit axial water transport from root to shoot. Since the main resistance to water flow through the plant is located in the shoot (stomata), regulation of aquaporins in roots is unlikely to impact on whole-water flow, at least under the growth conditions

tested.

### Carbon Isotope Fractionation during Photorespiration and Carboxylation

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The magnitude of fractionation during photorespiration and the effect on net photosynthetic <sup>13</sup>C discrimination ( $\Delta$ ) were investigated for three *Senecio* species, *S. squalidus*, *S. cineraria*, and *S. greyii*. We determined the contributions of different processes during photosynthesis to  $\Delta$  by comparing observations ( $\Delta_{\text{obs}}$ ) with discrimination predicted from gas-exchange measurements ( $\Delta_{\text{pred}}$ ). Photorespiration rates were manipulated by altering the O<sub>2</sub> partial pressure (pO<sub>2</sub>) in the air surrounding the leaves. Contributions from <sup>13</sup>C-depleted photorespiratory CO<sub>2</sub> were largest at high pO<sub>2</sub>. The parameters for photorespiratory fractionation ( $f$ ), net fractionation during carboxylation by Rubisco and phosphoenolpyruvate carboxylase ( $b$ ), and mesophyll conductance ( $g_i$ ) were determined simultaneously for all measurements. Instead of using  $\Delta_{\text{obs}}$  data to obtain  $g_i$  and  $f$  successively, which requires that  $b$  is known, we treated  $b$ ,  $f$ , and  $g_i$  as unknowns. We propose this as an alternative approach to analyze measurements under field conditions when  $b$  and  $g_i$  are not known or cannot be determined in separate experiments. Good agreement between modeled and observed  $\Delta$  was achieved with  $f = 11.6\text{‰} \pm 1.5\text{‰}$ ,  $b = 26.0\text{‰} \pm 0.3\text{‰}$ , and  $g_i$  of  $0.27 \pm 0.01$ ,  $0.25 \pm 0.01$ , and  $0.22 \pm 0.01 \text{ mol m}^{-2} \text{ s}^{-1}$  for *S. squalidus*, *S. cineraria*, and *S. greyii*, respectively. We estimate that photorespiratory fractionation decreases  $\Delta$  by about 1.2‰ on average under field conditions. In addition, diurnal changes in  $\Delta$  are likely to reflect variations in photorespiration even at the canopy level. Our results emphasize that the effects of photorespiration must be taken into account when partitioning net CO<sub>2</sub> exchange of ecosystems into gross fluxes of photosynthesis and respiration.

### The Carbon Balance of European Croplands: The influence of gross primary production during non-optimal growth periods

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Carbon fluxes from European croplands are the least well quantified and most uncertain of all fluxes. Whilst some studies have suggested a net loss of carbon from European croplands, predominantly as a soil C loss, other studies have suggested that croplands are currently close to equilibrium. The large uncertainty arises from a) the effects of management, including annual changes in rotations and tillage practices and b) climatic inter-annual variability. In particular, the extent of the fallow season in contributing to ecosystem C loss and the effect of winter cover on ameliorating these losses are vital to the final C balance of the system. This study examines 12 arable sites throughout Europe and quantifies both fallow season and winter cover fluxes and explores the mitigation potential of maximizing winter gross primary production on net C balance.

## **Generation of plastid transformants to examine the role of ROS scavenging enzymes in plant response to environmental stresses**

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One approach to understanding the role of Reactive Oxygen Species (ROS) scavenging systems in plant stress tolerance is to manipulate the levels of antioxidant enzyme activities. In this study, we expressed in the chloroplast genes encoding three enzymes of the antioxidant pathway: Dehydroascorbate Reductase (DHAR), Glutathione-S-Transferase (GST) and Glutathione Reductase (GR). GST catalyses the conjugation of the glutathione to a variety of cytotoxic substrates, whereas the DHAR and GR are involved in the regeneration of oxidized ascorbate and glutathione. These genes were introduced into tobacco chloroplasts via biolistic transformation. Chloroplast transformation presents several attractions including the targeted integration of the gene of interest, a high protein expression directly at one of the main site of ROS production, and finally the possibility of co-expressing different genes as an operon. Transformants containing either DHAR or GST, or a combination of DHAR/GR and GST/GR were generated and confirmed by molecular and biochemical analysis. In addition to enzyme activities, the metabolic consequences of these genes expression, in terms of glutathione and ascorbate levels, were investigated. These plants were then placed under environmental stresses such as high salinity and low temperature. Preliminary results indicate enhanced seedling growth under these stress conditions.

## **Genome dosage effects in isogenic polyploids of *Arabidopsis thaliana***

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The genomes inherited from maternal and paternal parents can affect organismal development in distinct ways related to their parental origins, even when these genomes are genetically identical. These effects are important for understanding the role of hybridisation or wide crosses in crop breeding programmes, and in naturally-occurring speciation. We are investigating parent-of-origin effects in genetically identical dosage series of the model plant, *Arabidopsis thaliana* L.. In triploid *Arabidopsis* of different accessions (genetic backgrounds), increased dosages of maternally- or paternally-derived genomes affect many aspects of plant phenotype, from germination rate to plant growth habit. Microarray analysis indicates that these changes are accompanied by significant alterations to gene expression across a dosage series of *Arabidopsis* polyploids. We also demonstrate that isogenic lines with different genome dosage (ploidy) have altered nuclear organisation, suggesting a mechanistic basis for these epigenetic effects at the level of plant chromatin. These findings highlight fundamental roles and effects of genome dosage in relation to plant development, evolution and breeding.

## **An empirical gene flow model for Ryegrass**

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Perennial Ryegrass (*Lolium perenne* L.) is the dominant organism on the Irish landscape, covering some 91% of all agricultural land. Recently the crop has become the target of genetically-modified improvement programmes around the world. As an obligate outbreeder Ryegrass is regarded as a major challenge in terms of biosafety and co-existence of GM and Non-GM varieties. We present



the first molecular-marker based empirical model of gene flow between ryegrass cultivars in the field. Generated using microsatellite allele scoring of F1 paternity, our pollination data more or less match an inverse exponential pollen distribution pattern, however it suggests significant wind-mediated stochasticity in these patterns, particularly beyond 96m from the pollen source. We conclude that co-existence management of pollen-mediated gene flow from outbreeding GM ryegrass will not be possible in the open pasture landscape of Ireland.

**Phylogenetic relationships between the common bean *Phaseolus vulgaris* and closely related bean species.**

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The common bean, *Phaseolus vulgaris*, is the most widely cultivated species of bean, representing 90% of all bean production worldwide. Valuable genetic variation for bean breeding programs is held within the secondary gene pool of *Phaseolus vulgaris*. The secondary gene pool consists of the closely related species of *P. vulgaris*, comprising *P. albescens*, *P. coccineus*, *P. costaricensis*, and *P. dumosus*. Understanding the phylogenetic relationships between these species is important in order to assess and prioritise efforts for inter-specific crosses between the secondary and primary gene pools for crop improvement. Phylogenetic relationships have been estimated amongst *P. vulgaris* and closely related species based on combined morphological and molecular markers (Delgado-Salinas et al 1999). In the former study, *P. vulgaris* forms a sister clade to the remaining species, which cluster together, while in a latter study (Delgado-Salinas et al 2006), the phylogenetic relationships between the species remain mainly unresolved. We have conducted phylogenetic analysis using the chloroplast trnK sequence data available in GenBank and also have generated DNA sequence data from the chloroplast genome for almost 10,500 bp across 12 highly variable loci using several individuals for each species within the *P. vulgaris* clade. The samples were obtained from the *Phaseolus* germplasm collection in CIAT, Colombia, and represent wild relative samples collected across Central and South America. Initial chloroplast sequence analysis indicates that *P. dumosus* seems more closely related to *P. vulgaris* than *P. coccineus*, indicating that *P. dumosus* could be one the closest relatives of *P. vulgaris*. We will discuss the implications of this and ongoing sequence analysis for understanding the phylogenetics of common bean and its close relatives.

**Reconstructing raised bog water tables over the last 5,000 years from proxy methods**

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The reconstruction of mire water tables has been widely used in northern and western Europe as a proxy for past net precipitation. Plant macrofossils are one of several proxies used in this respect. The two most widely used approaches have been the summation of subjective qualitative weightings applied to taxa based on broad ecological ranges (e.g. the Dupont index) and the use of ordination axis scores from detrended correspondence analysis (DCA) when an axis appears to correlate with a moisture gradient. There are several problems with these approaches; they are subjective, they generate a relative index of mire surface wetness rather than actual water table depths and their precision is not apparent. In this paper we explore alternative approaches to quantitative water table reconstructions from plant macrofossils and compare them to these traditional methods. The EU funded ACCROTELM project has delivered a detailed macrofossil



analysis of a midlands raised bog covering the last 5,000 years. These proxies will be compared to water tables derived from testate amoebae and peat humification analysis from the same core. This comparison aptly illustrates the added benefit of the multiproxy approach over reconstructions based on a single proxy.

**Genotype dependency within *Agrobacterium tumefaciens* in relation to the integration of vector backbone sequence in GM potato**

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In this study the effect of *Agrobacterium tumefaciens* genotype of two strains AGL1 and LBA4404 was investigated in regard to the propensity for backbone integration during the transformation of potato for blight tolerance conferred by the resistant to blight (RB) gene carried by the vector pCLD04541. A PCR based walking approach was employed to identify left and right backbone sequences as well as for selected genes carried on the plasmid backbone. It was found that adjacent to the left border insertion site, the integration of backbone sequence was greater for AGL1 than for LBA4404; however, the opposite was observed with regards to the right border T-DNA junction. Considering both T-DNA borders LBA4404 was found to have a two fold greater integration potential for backbone than the AGL1. The possibility of only backbone integration in T-DNA negative plants was also investigated with the average rate of integration between the two strains calculated at 4.2% with LBA4404 recording a three fold greater occurrence of backbone integration than AGL1. In summary, LBA4404 has greater potential to integrate non-T-DNA vector sequence than the AGL1 and this should be taken into account when utilising the listed *A. tumefaciens* genotypes in generating transgenic potato.

**Assessment of *Medicago truncatula* as a model for transcriptome analysis of white clover undergoing cold stress**

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To determine the most cold tolerant white clover cultivar available at Teagasc, twenty white clover cultivars were exposed and ranked according to resistance to cold stress. The transcriptome of the most cold resistant white clover cultivar (cv. W980108) was analysed under cold stress. Affymetrix DNA chips from the closely related species *M. truncatula* were used for the transcriptome analysis. The expression profiles of a number of genes were validated using quantitative Real Time-PCR analysis. It was found that *M. truncatula* was more appropriate as a model for white clover than hybridising cDNA from cold stressed white clover to *Medicago* Genome affymetrix chips to analyse white clover gene expression undergoing cold stress. The expression of differentially expressed genes across eight cold susceptible/resistant cultivars for 6 candidate genes of interest was analyzed to gain an understanding of gene expression effects during long term stress within white clover cultivars.

**An investigation of fungal diversity in Irish managed and semi natural forests**

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The current forested land in Ireland is 10% (650,000ha) and is set to increase to 17% (1.2million ha) by 2030. The majority (>60%) of this area will be composed mainly of the exotic conifer species Sitka spruce. The ecology and diversity of Sitka spruce plantations in Ireland is receiving much research interest from state funded bodies like COROFD with regard to Sustainable forest management. This project seeks to investigate the diversity and functional diversity of Irish semi natural, and plantation (first and second rotation) forests. Ash, Oak, Scots pine and Sitka spruce forests are being investigated and the factors which effect fungal diversity are being identified. It was found that the fungal diversity of Irish forests is on par with that of U.K. forests. From two years sampling it can be deduced that (a) each tree type has its own associated fungi which are well suited to the microclimate it creates in the surrounding soil (b) Age class was found to have significant effects on the fungi appearing on site in the case of Sitka spruce forests.(c) In agreement with many past studies on fungal diversity three years is not enough time to totally quantify fungal diversity in a site.

### **Why does *Gunnera* do it and other Angiosperms don't?**

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The *Gunnera-Nostoc* symbiosis is an enigmatic plant-cyanobacterial symbiosis; the only known angiosperm-cyanobacterial symbiosis. I postulate that this symbiosis, together with perhaps all other plant-cyanobacterial symbioses, was more important in the geological past and was a response to a unique suite of environmental conditions that are uncommon today. Whilst we can only speculate on the evolutionary drivers that led to the establishment of the *Gunnera-Nostoc* symbiosis there is plausible evidence that this could have been due to low oxygen-induced combined nitrogen deficiency associated principally with inland seas or seaways that existed in the Cretaceous. Phylogenetic analyses indicate a distinct origin for the evolution of the *Gunnera-Nostoc* symbiosis within the angiosperms, although interestingly this symbiosis may share more common features with both rhizobia and *Frankia* symbioses than might have been expected. There is even some evidence that low oxygen conditions can induce a number of factors that could be related to the establishment of plant-bacterial symbioses (rhizobia and *Frankia*). An important goal for the future is the identification of the origin and development of specialised 'glands' that are the conduit through which cyanobacteria are able to enter cortical tissue prior to the establishment of a functional symbiosis. Currently we have little understanding of the functional or evolutionary significance of this structure. Far from being 'glands', in the strictest sense, there is evidence for an origin associated with adventitious root formation, a feature that also has parallels with nodule formation in legumes and this requires more detailed investigation.

### **Towards the understanding of the molecular mechanisms determining the activation of plant defences by non-pathogenic biocontrol bacteria.**

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Biocontrol bacteria, including strains of *Pseudomonas* and *Bacillus* sp., can reduce the severity of plant diseases. *Pseudomonas fluorescens* strain MKB158 reduced the severity of head blight disease of wheat and barley caused by the fungal pathogen *Fusarium culmorum*. This fungus produces the mycotoxin deoxynivalenol in infected cereals and the biocontrol bacterium reduced

DON contamination levels in grain. Seedlings tests showed that *P. fluorescens* potentiates the host resistance response to *F. culmorum*. Transcriptome analysis of barley heads co-inoculated with *P. fluorescens* and *F. culmorum* identified a large number of transcripts involved in the potentiated host response. This includes genes associated with cell rescue and defense, signalling and transport of metabolites, metabolism, transcription and protein synthesis. The functional importance of a select number of genes is currently under investigation.

### **Genetic variation of dormancy-related traits in European trees**

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Climate change is causing changes in phenology, putting trees under selective pressure, as their reproductive success and ultimately their survival are affected. It has been shown that rapid responses to climate change are already widespread in natural populations of forest trees. However, the genetic basis of this response is currently unclear. In temperate latitudes dormancy is a critical phenophase for the survival of many tree species. Temperature and photoperiod seem to be the most important triggers for dormancy-associated traits (initiation and release), therefore the interactions of the genes involved in these traits are most likely to play a role in the response to climatic change. We are focusing on *Populus tremula* and *Betula pubescens* to assess variation in genes related to budburst. Plant material from 40 natural populations from a broad latitudinal range across Europe will be used to determine (1) clinal variations of targeted temperature and photoperiod response related genes across the proposed geographical area, and (2) the diversity of these genes that is present within certain populations, showing the capacity of such populations to adapt to future changes in climate. We aim to analyse single nucleotide polymorphisms and patterns of DNA sequence variation in the candidate genes between the different populations.

### **Evolution of flower shape in *Plantago lanceolata***

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Wind pollination is mainly a derived condition that has evolved many times in flowering plants. *Plantago lanceolata* produces small actinomorphic (radially symmetric), wind pollinated flowers that have evolved from a zygomorphic, biotically pollinated ancestral state. To understand the developmental mechanisms that might underlie this change in flower shape and associated change in pollination syndrome we analyzed the role of *CYC*-like genes in *P. lanceolata*. Related zygomorphic species have two *CYC*-like genes that are expressed asymmetrically in the adaxial region of developing flowers where they affect the rate of development of adaxial petals and stamens. *Plantago* has a single *CYC*-like gene (*PICYC*) and there is no apparent asymmetry in the pattern of *PICYC* expression during flower development. This suggests that the evolution of actinomorphy in *Plantago* has involved loss of *CYC*-like gene function in adaxial floral regions. *PICYC* is expressed in the inflorescence stem and pedicels and throughout stamen development suggesting a novel role for *PICYC* in compacting the inflorescence and retarding stamen development in protogynous flowers. We propose that the evolution of actinomorphy and protogyny in *Plantago* may be correlated through *CYC*-like gene loss and/or changes in *CYC*-like gene regulation.

**Botanical diversity in organic and conventionally managed grassland.**

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There is growing concern about the declining species diversity in agro-ecosystems caused by agricultural intensification. Organic farming is expected to maintain higher species richness than conventional management as chemical pesticides and mineral fertilisers are not applied. A large volume of work has been completed on the effects of agrochemicals and farming system on botanical diversity in various arable crop systems, yet relatively few focus on intensive grasslands. Intensive grasslands are the dominant type of grassland in lowland regions, representing millions of hectares in Europe. Our study examines whether organically managed grasslands in Ireland have a higher plant species diversity than conventionally managed grasslands. In addition, the question of whether farmer attitude to, and knowledge of, wildlife has a greater influence on the species diversity of their farm than the management system they employ is also addressed. Plant species diversity was investigated in field edge and centres of three permanent grassland fields in each of 10 organic and 10 conventional farms. Results indicate that plant species number was higher in organic than in conventional farms. Plant species number was also higher in the field edges than in the field centres of conventional farms, but there was no difference in plant species number between field edge and centre on organic farms. These results are discussed with reference to maintaining both agricultural productivity and biodiversity in farm ecosystems.

**Assessing the impact of forest age on net ecosystem carbon exchange.**

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During the first commitment period of the Kyoto Protocol carbon sequestration through afforestation and reforestation activities since 1990 may be used to offset national greenhouse gas emissions. It is therefore important to quantify the carbon source or sink strength of a forest through the various stages of stand development, including thinning cycles and other management effects. Eddy covariance techniques can be used to verify net ecosystem changes in carbon stocks at the stand scale that are associated with forest growth and management. However, in order to make comparative measurements of Net Ecosystem Exchange (NEE) within ecosystems or replicated treatments over comparable time scales, using eddy covariance techniques, multiple flux towers would normally be required. This is generally an unfeasible approach due to financial constraints however an alternative approach is to use mobile or roving towers that can readily be moved between sites over various measurement periods. This approach does present additional problems, including the reduced amount of data collected and the increased reliance on modelled estimates of NEE. Furthermore the timing of tower movements and the intervals between successive periods of data collection may be crucial, particularly in ecosystems with fast growing vegetation. In this paper we provide a critique of this approach using ecosystem experiments based on the use of a mobile flux tower to investigate the impact of forest age on net ecosystem carbon exchange.

**A botanical excursion to Bologna – results from a survey of Irish and German academics in 2008**

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Results are presented of survey of academics conducted in 2008 focusing on current practices in

botany/plant science teaching in Higher Education Institutions in Ireland and Germany. The survey aimed to compare national and institutional differences with regard to the following topics: Respondents' profiles; Botany/Plant Science degree structures; General awareness of, and opinions on, the Bologna Process; Bologna-compliance of courses at home institution and changes that had occurred to achieve this; Personal experiences and attitudes towards teaching; Skills and employment of graduates; of the Bologna Process. Whilst respondents' profiles were very similar in both countries, attitudes towards teaching practices and changes related to the Bologna Process differed greatly. Despite recent moves to harmonise European undergraduate degree programmes, considerable variation in degree structures, and teaching practices existed within and between the two countries. Besides considerable discrepancies between the scale of (dis)satisfaction regarding the implementation of the Process in the two countries, the awareness of the degree of implementation also differed. Irish degrees appeared to be more compliant although Irish staff were not always aware of key elements of Bologna but appeared to have accepted relevant recent changes, whilst German academics considered their degrees compliant even though key elements were not included.

### **A comparison among *Impatiens* species with different invasive capacity**

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Phenotypic plasticity is believed to play an important role within exotic species, as it may increase their invasiveness. To test this hypothesis we germinated seeds of five plant species of the genus *Impatiens* that are both invasive and native to Europe. *Impatiens noli-tangere* is native to continental Europe and Britain. *Impatiens glandulifera* is currently one of the dominant invasive plant species in Europe. In addition, we selected other exotic *Impatiens* that are naturalized in Europe and vary in their invasive capacity (*I. balfourii*, *I. parviflora* and *I. capensis*). *I. glandulifera* showed the highest germination rate. No germination occurred in *I. noli-tangere* seeds. Seedlings of the four germinated species were potted and measurements of growth were taken weekly. The photosynthetic capacity of 10 individual plants per species was assessed using an infra-red gas analyzer. Light response curves for each individual of each species were obtained weekly for 5 weeks, which followed the leaf's life cycle. CO<sub>2</sub> response curves also were obtained for 10 individuals per species. Plants had been harvested and growth parameters had been determined. These results will be combined with results of a further experiment where individuals of different *Impatiens* species will be grown in different levels of water stress.

**Resolving species relationships between *Adonis* spp. undergoing domestication**

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*Adonis palaestina*, a plant originating from the middle-east, is a potential source of the red pigment astaxanthin, which has uses both as a high value antioxidant nutraceutical and as a feed additive for farmed salmonid fish species. The species is not established as a crop and a programme of domestication is underway at UCC to obtain lines that can be cultivated to exploit the production of the secondary metabolite. Two groups of lines, exhibiting distinctly different flowering morphologies are available at UCC, both of which have been described as *Adonis palaestina*, the first is a small flowered type with a double row of petals, the second a large flowered type with a single row of petals. An apparent barrier to sexual hybridisation of the two forms exists, and this has previously been assumed to be due to a ploidy difference between the two sets of lines (the large flowered lines being assumed to be tetraploid variants of the diploid small flowered lines). Using a combination of flow cytometry, to compare the nuclear genome sizes of the lines, and DNA sequencing of the ITS region of the 16s ribosomal rRNA subunit, we have found that it is unlikely that this crossing barrier results from a difference in ploidy, and in fact, that the two flower type groups probably represent different species, a key piece of information in the domestication process for this plant.

**Investigation of self-incompatibility in white clover (*Trifolium repens* L.)**

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White clover (*Trifolium repens* L.) is a legume (family Leguminosae) which is highly important in agriculture, mainly as a pasture species and as a means of fertilising the soil. *T. repens* is self-incompatible, meaning that a plant cannot be fertilised by its own pollen. Self-incompatibility (SI) is known from approximately 60 percent of all angiosperm species, and the mechanism has been investigated in some families, e.g. Solanaceae, Rosaceae, Plantaginaceae, Papaveraceae and Brassicaceae. In Papaveraceae, Solanaceae and Rosaceae, and in *Antirrhinum* (Plantaginaceae), the specificity of the SI reaction has been shown to be determined by the products of a single multi-gene region in which the genes are tightly linked, the self-incompatibility (S) locus. The results of cross-pollination experiments show that the SI reaction in *T. repens* can also be explained as the effect of one locus. Members of the Solanaceae, Rosaceae, and Plantaginaceae share closely related ribonucleases (S-RNases) as female receptors in SI. In terms of evolutionary relationships and what is known of the SI mechanism in *T. repens*, it is very likely that the female receptor is related to those in Rosaceae, Solanaceae, and Plantaginaceae. Here details are presented of ongoing efforts to characterise the S locus in *T. repens*.

**Developing marker assisted selection for combining potato cyst nematode resistance from several sources into individual potato genotypes**

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The objective of this research is to establish a small scale experimental breeding programme and



use marker-assisted selection to combine multiple traits into single genotypes in high dosage state. The availability of genetic markers, which are easy to score, inexpensive and diagnostic for resistance to desirable traits would greatly speed up variety development. The two species of potato cyst nematodes (PCN), *Globodera pallida* (pathotype Pa2/3) and *Globodera rochochiensis* (pathotype Ro1) are major pests of potato crop in the UK and Europe. Two potential sources of partial resistance to *Globodera pallida* Pa2/3 derived from *S. tuberosum ssp. andigena* accession CPC 2802 and *Solanum vernei* respectively, are widely available to breeders. Both are controlled by quantitative trait loci (QTL) which have previously been characterised. Complete resistance to *G. rochochiensis* from the H1 gene derived from *S. tuberosum ssp. andigena* Hawkes CPC1673. Genetic markers exist for all of the above traits, but in their current format, they may not conform to all of the characteristics required to deploy them for marker assisted selection (MAS). Adaptation of these markers for suitability for use in MAS will enable us to efficiently combine these various PCN resistance loci into single potato genotypes.

### Phylogeography of Irish Arctic-Alpine Caryophyllaceae

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Conventional biogeographic theory suggests that animal and plant colonization of Ireland occurred at the end of the Pleistocene glaciation via a landbridge across Britain. Recent analysis of native mammalian populations however shows marked deviations from British genotypes, bringing this supposition into question. Extant Irish arctic-alpine species were likely among the earliest colonizers at this juncture and two of these species (*Arenaria ciliata* and *Minuartia recurva*) do not occur in Britain at all, having their closest neighbour populations respectively in the Jura mountains and Iberia. In this project we are testing the putative postglacial colonization routes for *A. ciliata* and *M. recurva* using a comparative phylogeographical analysis involving closely related but more widely distributed Caryophyllaceae (*Arenaria norvegica*, *Silene acaulis*, *Minuartia verna*, *M. rubella*, *M. sedoides* and *Arenaria serpyllifolia*). Populations from these species are being sampled where they occur in Ireland, Scotland, Norway, Portugal, the Pyrenees and the Alps using direct field collection and ex-situ genebank accessions to generate c. 25 individual DNA samples per population. Individual genotypes are being characterized using both AFLP and cpDNA markers. A variety of Genetic diversity/ similarity indices will be calculated based on within and between population variance and historical migration routes will be inferred and tested based on nested phylogeographic analysis of these species datasets.

### Development of cpSSR markers in *Miscanthus* (Poaceae)

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*Miscanthus* is a highly important genus of forage and horticultural perennial grasses (Poaceae) primarily native to South East Asia. *Miscanthus* is under intense global investigation as a biomass source for renewable energy production and breeding initiatives are underway to develop new genotypes optimized for improved biomass and tolerance to a range of environmental stress conditions. *Miscanthus* possesses a basic chromosome number of 19 and is highly polyploid, with ploidy ranging from diploid to hexaploid. From a plant breeding perspective, chloroplast and mitochondrial genome sequence information is required to develop simple and accurate marker

systems for the identification/designation of cytoplasmic gene pools in *Miscanthus*. Organelle genome information will be essential for understanding the evolution of this group especially the role of hybridisation and polyploidy. Although cpDNA generally has lower variability than nuclear DNA, chloroplast simple sequence repeat (cpSSR) loci have been shown to be polymorphic particularly at mononucleotide repeat loci. We present results of cpDNA characterisation of a range of *Miscanthus* genotypes with a broad range of ploidy (diploid to possible x ploid).

### **Soil $^{13}\text{C}$ dynamics in aggregates and soil profile under an established *Miscanthus* system**

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World soils play an important role in carbon (C) cycling. Being a principal terrestrial C pool, soils contain more than twice the C than in the atmospheric, land plant or biotic pool. Carbon sequestration implies transferring atmospheric  $\text{CO}_2$  into long-lived pools and storing it securely so it is not immediately re-emitted. Particularly, perennial bioenergy crops, established on cultivated land, seem to be able to sequester carbon in the soil. Studies have begun in *Miscanthus*, which is a bioenergy crop, as it is a convenient replacement of conventional cropping system and it is likely to increase the soil organic matter (SOM). Therefore, the principle aim of this study is to measure the amount of C sequestered under a *Miscanthus* stand following the movement and partitioning of carbon. Soil organic matter is a complex entity consisting of different fractions characterized by different physical and chemical properties, microbial degradability and turnover time. Therefore, to evaluate changes in soil C and SOM dynamics correctly, it is necessary to distinguish the different SOM fractions in relation to their functionality. For that reason in this study, we have decided to fractionate SOM by size and density. Moreover, stable C isotope studies have been successful in elucidating belowground C dynamics. We will then combine SOM fractionation techniques with the  $^{13}\text{C}$  natural abundance technique to investigate small shifts in soil C stores that would be significant in the long term, but that might not be detected by conventional methodologies. As the largest pool of terrestrial organic carbon, soils interact strongly with atmospheric composition, climate, and land cover change. Our capacity to predict the consequences of global change depends in part on a better understanding of the distributions and controls of soil organic carbon (SOC) and how vegetation change may affect SOC distributions with depth. For that reason, we will analyze the carbon content.

### **Evolution of stomatal function in land plants**

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Plant stomata control two of the most important physiological processes of vascular plants: uptake of carbon dioxide ( $\text{CO}_2$ ) for photosynthesis and loss of water via transpiration. The evolution of stomatal guard cell function in land plants, from basal gymnosperms to the most advanced angiosperms, will be investigated by mapping key traits onto land plant phylogenies. The traits concerned are cellular (guard cell chloroplast number), functional (rate of stomatal opening/closing), anatomical (level of guard cell lignification) and developmental (stomatal density response to atmospheric  $\text{CO}_2$ ). This novel approach will explore whether stomatal response to changing atmospheric conditions has been modified over evolutionary time. In particular, we will investigate whether the developmental response (stomatal density adaptation) has moved towards a control response, where rates of stomatal opening and closing have

become more finely-tuned. This innovative research will be vital in predicting future physiological and biodiversity responses of vegetation to climate change and rising atmospheric CO<sub>2</sub>.

**An Evaluation of the Effects of Predicted Climate Change on *Ascophyllum nodosum***

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*Ascophyllum nodosum* (Linnaeus) Le Jolis (Phaeophyceae) is probably the most important intertidal keystone species on sheltered rocky shores on the Irish west coast, but is also harvested commercially. The overall objective of this project is to predict potential changes in productivity due to environmental pressures including climate change and to ensure sustainable exploration of the seaweed. The present study thus assesses productivity of *A. nodosum* under current conditions *in situ*, and attempts, in lab and field experiments, to predict change in productivity under different environmental conditions, focusing in particular on responses to temperature, CO<sub>2</sub> and UV.

Using sites in Cos. Clare and Galway, *in situ* growth in harvested and non-harvested sites is measured, and morphological and structural changes in *Ascophyllum* populations in response to harvesting assessed. Following harvesting events, the regrowth of *A. nodosum* and as well as changes in vegetation structure of harvested *A. nodosum* beds, are assessed. Physiological differences between plants from harvested and non-harvested sites are investigated on a seasonal basis using CO<sub>2</sub>-assimilation and PAM-fluorescence.

***Gaultheria mucronata*: Biology and Distribution within Glenveagh National Park, County Donegal**

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Like many invasive exotic species, *Gaultheria mucronata* was introduced from South America for its ornamental value. To date, *G. mucronata* still abounds in Irish garden centers. In its native habitat, *G. mucronata* inhabits moraines, densely forested areas, open fields, as well as margins of forests within Argentina and Chile in regions such as Tierra del Fuego, Bahia Loredó and Chiloé Island. Habitats also include permanently and intermittently inundated areas and nutrient poor acidic soils. Since its introduction to Ireland, *G. mucronata* has since been recorded in counties Kerry, Galway, Dublin, Mayo, Down, and Antrim. *G. mucronata* has also colonized a wide variety of habitats, including heathlands and woodlands, within Glenveagh National Park, Co. Donegal. Dispersal within the Park can be linked to management activities, aquatic dispersal and possibly endozoochory. Most importantly, suckering roots, fragmentation, a late flowering period and year-long fruit production, further enhance the plant's successful distributive capabilities. Based on this research, as well as literature reviews and comparisons with invasives such as *R. ponticum*, *G. mucronata*'s status as an invasive alien species within Ireland is presented. Records of its distribution would be welcomed by the authors.

**Effects of Climate Change on Net Ecosystem Exchange of Irish Grasslands**

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The aim of this project is to predict the impact of climate change on the productivity of grasslands, and or whether grassland ecosystems are a carbon sink or source. To access the

future climatic conditions in our experiment, we used the climatic data from the downscaling of the Global Circulation Model, the Hadley Climate Model (HadCM3). In brief the resulting downscaling provided 852 10 km X 10 km grid cells for Ireland. For each of these cells monthly climate data are defined. In particular we extrapolated the data specific for Carlow County where the experimental field is located. Using a multiple mesocosm enclosure facilities such as monoliths, which permit realistic comparisons of ecosystem responses to climatic factors, we sowed *Lolium perenne* cv. Cashel in monocultures and exposed them to either current climatic or future climatic scenario. A timed series of step wise changes in temperature and precipitation was used to create the control (near current climatic conditions) and treatment (near 2055 climatic conditions). We therefore aim to report differences between transient and equilibrium climate responses of *Lolium perenne* and attempt to quantify the combined effects of the climatic variables that are known to have an influence on the Net Primary Productivity (NPP) and quality of *Lolium perenne* growing in Ireland.

### **Micropropagation and rooting of *Hebe* 'Pink Fantasy'**

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The genus *Hebe* (*Scrophulariaceae*) provides many important commercial cultivars for the nursery industry. They are propagated vegetatively, but an efficient method of producing healthy stock plants by *in vitro* culture is required. Earlier work on *Hebe speciosa* resulted in the establishment of successful cultures. Actively growing shoots of *Hebe* 'Pink Fantasy' were excised from greenhouse grown plants, sterilized, established *in vitro* and multiplied on a WPM culture medium enriched with N<sup>6</sup>-benzyladenine (BA, 0.2 mg l<sup>-1</sup>), α-naphthalene acetic acid (α-NAA, 0.001 mg l<sup>-1</sup>) and sucrose (2% w/v), with or without activated charcoal (AC, 0.3% w/v). Two explants were cultured per glass jar (replicate) containing 30 ml of medium and there were ten replicates. The cultures were incubated at 22 °C ± 1 °C under a 16-hour photoperiod (photon flux density of 58.6 μmol m<sup>-2</sup> s<sup>-1</sup>). When AC was included in the medium, the length of the shoots obtained increased significantly by three fold (p<0.01). However, the number of shoots and nodes produced per explant and the micropropagation rate were not significantly affected in the same treatments. AC resulted in a highly significant increase in rooting rate (from 15% to 100%), the number of primary roots induced and also root extension growth (p<0.01).

### **Holocene vegetation dynamics and human impact in north-west Sligo, western Ireland: first results from investigations at Lough Dargan, near Ballygawley**

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The first results from palaeoecological investigations centred on north-west Sligo will be presented. The project aims at reconstructing woodland dynamics and human impact in a part of Ireland where the archaeological evidence for human settlement is particularly strong. Results from earlier palaeoecological investigations in the region will be considered and the first results from investigations of a long lake core from Lough Dargan, near Ballygawley will be presented. Particular attention will be paid to local woodland dynamics and the effects of early farming on the natural environment.

### **The response of the energy crop *Miscanthus* to fungal pathogens: A preliminary study**

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There is increasing interest in the use of biomass crops for energy production. *Miscanthus* is a perennial rhizomatous C<sub>4</sub> grass that has remarkable adaptability to different climatic environments. As the acreage of the biofuel crop *Miscanthus* expands in Ireland and the EU, we can expect that pests and pathogens will have a significant impact on both biomass yield and biomass quality. However, little is known about diseases of novel biofuel crops such as *Miscanthus*. The aim of this research was to assess the response of *Miscanthus x giganteus* towards fungal pathogens including some of the main Irish cereal pathogens using an *in vitro* detached leaf assay and *in vivo* whole plant test. Visual disease symptoms observed on the *Miscanthus x giganteus* leaves in this experiment varied among the 19 pathogens (37 isolates) assessed and symptoms included brown lesions, premature necrosis, presence of pynidia and mycelial growth. In general, all 19 pathogens (37 isolates) assessed caused some level of disease *in vitro*. The pathogen *Rhizoctonia solani* caused the greatest visual disease symptoms on the *Miscanthus x giganteus* leaves (mean LGR = 3.5 cm day<sup>-1</sup>), while *R. secalis* caused the least visual disease symptoms (mean LGR= 1.5 cm day<sup>-1</sup>) (P< 0.05). While of the 6 pathogens assessed in the *in vivo* whole plant test only *F. culmorum*, *F. graminearum* and *M. nivale* caused visual disease symptoms (mean LGR= 0.3 – 0.5 cm day<sup>-1</sup>).

#### **Promoter analysis of a novel toxin-responsive wheat gene**

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Deoxynivalenol (DON) is a toxic secondary metabolite (i.e. mycotoxin) produced by the wheat pathogen *Fusarium graminearum*. It acts as a virulence factor, facilitating the spread of the fungus within plant tissues. We identified a novel wheat gene that is not expressed in healthy tissue, but is expressed in response to DON treatment. Gene expression studies and promoter analysis were conducted in order to better understand the transcriptional regulation of this gene. Gene expression studies showed that this gene was not activated in response to the plant hormones typically involved in plant defence and signalling. Promoter fragments derived from a region upstream of the transcription start site were fused to the GUS gene and heterologously expressed in *Arabidopsis thaliana*. Seedling studies, in which wild type and mutant roots were treated with DON or water, identified a promoter region associated with gene repression in healthy plant tissue. This region is currently being characterized.

#### **Investigating Rare Willows in Ireland - Remnants of an Arctic Past**

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Arctic-alpines represent less than 2% of the Irish flora and have restricted distributions mainly in northern and western mountain areas. The arctic-alpine habitats are under threat due to climate change and the prediction is that they will be greatly diminished or completely altered within this century. These plants are often at their range limits in Ireland or have disjunct European distributions. *In situ* conservation of these species may not be an option, and so *ex situ*



collections are being developed. Ideally, this collection should encompass the diversity across the country. The aim of this study is to investigate genetic diversity of populations of two montane willows: *Salix herbacea* and *S. phylicifolia*, so as to inform the creation of an *ex situ* collection of plants. The project is assessing the conservation status of the species based on population demographics and genetic diversity. A pilot study has been undertaken and sites have been sampled across Ireland. Cuttings from wild material have been propagated. The initial analysis utilized microsatellite markers to assess genetic diversity and clone size in the populations. Results are presented of the genetic analysis and the potential utility of microsatellites for assessing genetic diversity for *ex situ* populations is discussed.

### **Insect and wind mediated gene flow in Potato**

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Potato has become the focus of many GM improvement programmes worldwide, especially in relation to fungal disease resistance. Sustainable field release of GM potato will rely on a clear understanding pollen-mediated gene flow between GM and non-GM cultivars. To this end we have embarked on a three-year field study of gene flow in potato. Two varieties have been employed for duplicate experimental gene flow trials over three years beginning Summer 2008; the fully fertile var Desirée (as pollen donor) and the male sterile British Queen (pollen receptor). Gross pollen dispersion studies have been conducted for year 1. For year 2 and 3 four different pollination experiments will be applied to the potato plots to determine the respective role of insect and wind pollen movement between plots. In parallel we will investigate seed and tuber mediated gene flow over time in individual plots. Initial analysis of year 1 data suggests that wet weather negatively impacts on successful pollination, however paternity analysis in F1 seedlings in British Queen plots confirms a negative correlation between gene flow and distance from pollen donor (Desiree). At completion of the trial we expect to understand the ecological parameters that govern a manageable coexistence between GM and non-GM potato crops in the Irish landscape.

### **Assessing the suitability of commercial seed mixes in the establishment / restoration of native, species rich meadows in Ireland**

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'Native' wild-flower seed mixes are increasingly used in large-scale landscaping schemes. Best practice guidelines indicate the establishment of native meadow habitats, on for example motorway verges is optimised by broadcasting locally sourced seed or simply allowing natural colonisation from the surrounding vegetation or the existing soil seed bank. This research will assess the value of using commercially available seed mixes in the establishment of native wild flower meadows. Using standard vegetation survey techniques, changes in species diversity over time and the influence local seed rain on meadows established using commercial seed mixes will be examined. In addition levels of genetic diversity contained within selected 'native' species available through commercial seed suppliers will be compared with the level diversity contained in their wild relatives. Genetic analysis will be carried out using SSR markers and Amplified Fragment Length Polymorphism (Vos et al. 1995). Results will be used to inform best practice guidelines and will contribute to promoting the value and conservation of native meadow



species.

### **Assessing the Conservation Status of *Simethis planifolia* (Kerry Lily) in Ireland**

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The Kerry Lily, *Simethis planifolia* is a beautiful, diminutive white flowered member of the lily family (Asphodelaceae), and is listed under the Flora Protection Order, 1999. In Ireland, it is only known from three locations, occurring in the Derrynane and Lamb's Head regions of South-West Kerry and Dereenacush in West Cork. *S. planifolia* is found throughout Southern Europe in Spain, Portugal and extending northwards to France and eastwards to western Italy. In Britain it was formerly recorded near Bournemouth in Dorset, but became extinct around 1925. Very little is known about the Irish populations of *S. planifolia*, its detailed distribution has never been mapped and most records for the species were made pre 1940. The project aims to provide a conservation assessment for this species in Ireland by undertaking the following research: an associated vegetation survey, population census, an examination of seed production and population genetic diversity analyses. The species will be introduced into cultivation for the first time in Ireland. Plants will be grown at the National Botanic Gardens, Glasnevin and will be used to conduct future research. The living collection at NBG Glasnevin will be used to promote public awareness of issues relating to the conservation of rare Irish plants.

### **Conservation and monitoring of Killarney fern (*Trichomanes speciosum*) in Ireland**

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*Trichomanes speciosum* (Killarney fern) is considered one of Ireland's rare and threatened plant species. It is protected by both local (Flora Protection Order 1999) and European legislation (Habitats Directive Annex 1). Dr. Whitley Stokes made the first record of Killarney Fern in Ireland at Powerscourt Waterfall around the 1800's, this population is now considered extinct. The gametophyte generation of this fern species was discovered growing in Ireland as recently as the 1990's. A total of 66 sites with this species remain in Ireland (NPWS 2008). This project aims to set up suitable permanent monitoring for Killarney Fern populations in Ireland and to investigate the developmental stages of Killarney Fern and to elucidate reasons for their continued existence in mostly small-scattered populations. Germination trials of fertile material in the ex-situ collections at the National Botanic Gardens, Glasnevin and Trinity College Botanic Gardens were carried at Kinsealy Research Station during 2007 and 2008. Spores of these ex-situ collections were found to germinate freely in agar plates. Further research will be carried out into the genetic make-up of this species in Ireland using molecular fingerprinting techniques.

### **Fine-spatial palaeoecological investigations towards reconstructing late Holocene environmental change, landscape evolution and farming activity in Barrees, Beara peninsula, south-western Ireland**

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Long-term environmental change and human impact have been reconstructed at fine spatial and temporal resolutions in an archaeologically rich part of south-western Ireland, namely the Beara peninsula, Co. Cork. Detailed pollen and macrofossil analyses, and radiocarbon dating have been

carried out on several short peat monoliths, and on a peat core and a lake core from small basins. Landscape evolution, vegetation dynamics and farming activity from the end of the Neolithic (c. 2500 B.C.) to the present day, have been reconstructed. While significant opening-up of the landscape began relatively early in the Bronze Age (between c. 2400–2100 B.C.), the main woodland clearances took place in the later Bronze Age (beginning c. 1400 B.C. and continuing into the Iron Age, i.e. to c. 400 B.C.). The linear stone-wall system, now partly obscured by shallow peat, was laid out towards the end of the Iron Age (c. A.D. 400) in the context of a largely open landscape. While the initial foci of bog growth appear to relate to the late Neolithic/beginning of the Bronze Age, widespread development of blanket bog was essentially a phenomenon of the late 1<sup>st</sup>/early 2<sup>nd</sup> millennium A.D.

### **Nitrogen fixation on dryland steppe in Tierra del Fuego**

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In Patagonian grasslands, sustainable sheep production depends on improvement of lambing, increased lamb carcass weight and satisfying the nutritional requirements of sheep. Legumes can help in resolving these, because they are the cheapest sources of protein for animals on these grasslands and are a key component of organic production systems. In this project we investigated how soil humidity on dryland steppe (250–300 mm/year) affected the nitrogen fixation of *Trifolium repens*. In a field experiment in Tierra del Fuego (53°09'south; 68°44'west), grazing exclusion cages were used in established reseeded (on wet and dry areas) to measure the yield, chemical composition and nitrogen fixation during the growing season. To measure how much nitrogen is being fixed we used the <sup>15</sup>Nitrogen isotope dilution technique. The experimental design was randomized block analysis with four replicates. The model included area as sources of variation, analysed by analysis of variance. The mean level of nitrogen fixation was 115 kgN/ha (SD+/-36). Significantly more (P≤0.05) nitrogen was fixed on wet area (147 kgN/ha) than dry area (83 kgN/ha) of the farm. Depending on an economic analysis, these results show that legumes have great potential to improve dryland steppe in southern Patagonia.

### **Effect of calcified seaweed on yield of two forage legumes**

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Calcified seaweed (CS) is an organic material which can be applied to soil to raise soil pH and minerals (calcium, magnesium and traces) and enable forage legumes to be grown on acid soils in the Falkland Islands (FI). The overall aim of this work was to investigate the effect of different rates of CS in an acid soil on yield of two legume species (*Lotus uliginosus* and *Trifolium repens*). CS samples were randomly taken from an extraction site in the FI. In a controlled environment at Queen's University Belfast, a pot experiment was carried out (at 11°C) on an local organic soil (from an upland site at Glenwherry, Co Antrim), with five different rates of CS plus a control without CS (0; 0.8; 1.6; 3.2; 5.1 and 6.4 gCS/kg). The experiment ran for 120 days. The experimental design was split plot with three replicates. The model included rate, legume species and the interactions of rate x species as sources of variation, analysed by analysis of variance. CS improved top and root DM production in both legume species, but *Lotus* grew better than *Trifolium*. These results should encourage the use of calcified seaweed as a soil ameliorant to

improve legume growth on FI acid soils.

**Desoxynivalenol and Zearalenone in wheat and barley: Developing molecular tools to monitor toxin production of major *Fusarium* strains contaminating grain in field and storage.**

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A range of strains of the filamentous fungus *Fusarium* is associated with phytopathogenicity and small-grain cereal diseases like *Fusarium* Head Blight (FHB). Various *Fusarium* species have a great potential to produce mycotoxins during plant infection in the field and on grain in storage. These fungal secondary metabolites are harmful to human and animal health. Two major representatives are the trichothecene deoxynivalenol (DON), produced by frequent FHB causatives *Fusarium graminearum*, *F. culmorum* and *F. poae*, and zearalenone (ZON), that is not produced by *F. poae*. We are developing molecular tools to enable simultaneous detection of DON and ZON production by *Fusarium* species. We generated a transgenic *F. culmorum* strain that expresses fluorescent proteins under the control of promoters of genes that are essential for the biosynthesis of DON (trichodiene synthase; Tri5) and ZON (a promoter that drives two polyketide synthetases, Zea1 and Zea2). We developed triplex qPCR assays for the concurrent analysis of Zea1 and Tri4 (another gene essential for DON production), relative to the wheat/barley actin gene (Act1). This assay can be used to detect DON and ZON producers in wheat or barley grain and to quantify the amount of fungal contamination relative to the utilised plant material. Besides, we generated a multiplex RT-PCR assay to analyse Zea1, Tri4 expression levels in *F. culmorum*, *F. graminearum* and *F. poae* contaminated material relative to their constitutively expressed beta-tubulin (B-tub) gene. Incorporation of the B-tub enables normalization of Tri4 and Zea1 transcript expression, relative to fungal abundance.

**Effect of Agronomic Factors on Levels of Phytochemicals in Irish Grown Vegetable Crops**

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Over recent years a range of epidemiological and other studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits. Numerous case control and cohort studies have indicated a significant inverse relationship between fruit and vegetable consumption and the risk of developing many forms of cancer, including cancers of the lung, prostate, pancreas, bladder and breast; as well as cardiovascular and other diseases. These beneficial health effects are due to the presence of bioactive phytochemicals which may be defined as “non-nutrient chemicals found in plants that have biological activity against chronic diseases”. A number of agronomic studies have indicated that the range and level of individual phytochemicals within a crop can vary in response to cultivation practices including cultivar choice; nutrient supply; tissue type; plant maturity; plant spacing and season. Our research as part of the **Irish Phytochemical Food Network** is focused on field trials to evaluate the effect of agronomic factors on accumulation of key phytochemicals in Irish grown carrot, broccoli and onion varieties.

**Characterisation of Plant Growth Promoting Bacteria**

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Plant growth promoting bacteria are considered an 'eco-friendly alternative' to chemical fertilisers. This study determined the ability of 14 bacteria (isolated from cereal crops) to promote the growth of wheat (cultivar Raffles) seedlings. Of the 14 bacteria, 5 significantly promoted coleoptile elongation (by  $\geq 11 - 21\%$ , relative to controls;  $P \leq 0.05$ ); these included strains of *Acinetobacter sp.*, *Pseudomonas fluorescens*, *Micrococcus luteus*. Two bacteria (*B. megaterium* strain MKB 41 and *P. fluorescens* strain MKB 100) significantly promoted primary root elongation (by 18 and 23 %, relative to controls;  $P \leq 0.05$ ). Possible modes of actions were tested; the ability to produce the plant hormone auxin, the extracellular enzyme 'ACC deaminase', siderophores production, phosphorus (P) solubilisation and sulphur (S) mobilisation. One bacterium, namely *Micrococcus luteus* strain MKB 24, had the ability to produce auxin; 11 bacteria produced ACC-deaminase; 12 produced siderophores, 7 solubilised phosphate and 5 mobilised sulphur. However, there was no correlation between any specific mode of action and plant growth promotion capacity of bacteria.

### **Sectoral Impacts on pollinators and pollination services - the impacts of bioenergy crops and road landscaping**

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Pollination is the main mode of gene transfer both within and between plant populations and is a vital ecosystem service required by approximately 90% of the angiosperms. However, pollinators and the services they provide are increasingly threatened by many factors including land use change and agricultural intensification. This project is focusing on two major sectoral land use changes at the landscape level in Ireland, the growth of the bioenergy crops (Oilseed rape and Miscanthus) and the building of roads and their associated landscape treatments. We are investigating how these land use changes contribute to changes in pollinator diversity and abundance, population structure and loss or enhancement of pollination services in agroecosystems. These impacts will be examined from genetic to landscape scales, using many pollinator species and a number of model plant species. This project is part of the SIMBIOSYS (Sectoral impacts on biodiversity) project which is focusing on the sectors of energy crops, road building and landscaping and aquaculture in Ireland, and their affects on associated ecosystem services - [www.simbiosys.ie](http://www.simbiosys.ie).

### **Molecular evolution of the grass subfamily Panicoideae (Poaceae): based on sequences of chloroplast and nuclear DNA**

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Phylogenetic relationships among major subfamilies in Poaceae and among major tribes within Panicoideae were evaluated using parsimony analysis of plastid *trnL-F*, *atpB-rbcL*, and nuclear ITS gene regions. A total of 132 grasses from six subfamilies and representatives from five tribes of Panicoideae (Andropogoneae, Arundinelleae, Eriachneae, Isachneae, Paniceae) were analysed. The PACCMAD clade was well resolved. The close relationship between Aristidoideae and Chloridoideae was found. The monophyly of Micrairoideae was resolved. Eriachne and Isachne

were resolved as monophyletic. Panicoideae were monophyletic and sister to a clade of Danthoniopsis + Tristachya. Paniceae were monophyletic. Within Paniceae, the bristle clade and the forest shade clade were found, but their circumscription remains ambiguous. Arundinelleae were polyphyletic. This study supported the separation of *Arundinella* and *Garnotia* from the remaining Arundinelleae and the inclusion of both genera in their own subtribes within Andropogoneae. Tribe Arundinelleae should be abandoned. Andropogoneae were monophyletic only if *Arundinella* and *Garnotia* were included. Five out of a total of 11 subtribes (Chionachninae, Coicinae, Dimeriinae, Germainiinae, Tripsacinae) were monophyletic. Five distinct groups, *Hackelochloa*, *Hemarthria* and *Mnesithea*, an agamic complex (*Bothriochloa*, *Capillipedium*, *Dichanthium*), a sub-basal awn group (*Arthraxon*, *Thelepogon*), the core Andropogoneae genera (*Hyparrhenia*, *Cymbopogon*, *Schizachyrium*, *Andropogon*), and a *Themeda* group) were also found as monophyletic.

### Hybrid alien ash: *Fraxinus excelsior* × *F. angustifolia* and its potential for interbreeding with native ash in Ireland

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*Fraxinus excelsior* L. is a native hardwood tree with important economic value. From 1993 to 2000, ash plants were imported from the continent to Ireland because of high demand but showed very bad stem form. It is hypothesised that poor growth may be due to the source of material, which could have been: a) hybrid trees of *F. excelsior* × *F. angustifolia*, b) pure *F. angustifolia* and/or c) provenances (sources) of *F. excelsior*, which were not well adapted for the Irish environment. The goals of this project are to examine the significance of hybrid plantations in Ireland, by analysing the key morphological characters in parental and hybrid trees, to determine how the breeding biology of both tree species may lead to the introgression of undesired gene pools and to estimate gene flow at the landscape level. The F<sub>1</sub> individuals of interspecific crosses showed an intermediate morphology. The flowering time of suspected hybrid material was monitored in two plantation sites and first results showed an overlap in flowering period of some late flowering *F. angustifolia*-like trees with some early flowering native *F. excelsior* trees. Molecular analysis of suspect hybrid plantations show indeed an important *F. angustifolia* component but morphology appears somewhat different from continental populations.

### Recent Advances in the Irish Phytochemical Food Network: Tracing Glucosinolates from Brassicas from Farm to Fork.

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A variety Irish grown fruits and vegetables contain phytochemicals in amounts which could have a beneficial effect to human health. However, the mechanisms of action, stability during food processing, effect of agricultural factors and stability in post harvest storage of phytochemicals,



such as glucosinolates from *Brassicaceae* family are not well understood. The *Irish Phytochemical Food Network (IPFN)* is a network funded by the Department of Agriculture Fisheries and Food, and it aims to address the knowledge gaps on phytochemicals from commonly produced/consumed Irish vegetables. To our knowledge this is the first time that this kind of study is conducted in Ireland. A main axe of research among the IPFN, is the study of glucosinolates profile among Irish grown broccoli cultivars. The glucosinolates profile can have a big impact in nutritious and sensory quality. A range of 10 cultivars of broccoli were grown in a randomised complete block field design at Teagasc-Kinsealy research centre and were analyzed in Ashtown Food Reaserch Centre. Glucosinolates from freeze-dried broccoli were extracted in a pressurized liquid extractor (PLE) using 70% aqueous methanol and glucosinolates profile was determined through ion pair high pressure liquid chromatography. Differences on glucosinolate profile from different cultivated varieties of broccoli are discussed.

### **An easy method to assess acidification of the apoplast in the growth zone of barley (*Hordeum vulgare*) leaves**

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The longer-term aim of this project is to test whether plasma membrane H<sup>+</sup>ATPase activity is higher in growing compared to non-growing leaf tissue to facilitate nutrient uptake (e.g. potassium) and wall expansion through acid growth. Previous work on developing barley leaves showed that the cuticle is very permeable in the lower two thirds (0-25 mm) of the elongation zone. This should make it possible to measure externally changes in apoplast pH. We have set up an in vitro assay to follow changes in pH, using the pH indicator bromocresol purple which is added to agar which is in direct contact with the leaf growth zone. Growth and changes in pH can be measured simultaneously and in response to reagents which either inhibit or activate plasma membrane H<sup>+</sup> ATPase.

### **Implication of Environmental Factors on Growth, Productivity and Pigment Composition of Algae**

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Algae are the base of most aquatic food chains and impacts on these organisms have enormous implications for the higher trophic levels. As part of an EPA STRIVE-funded project, this research aims to quantify algal responses to environmental conditions like UV-radiation, temperature and salinity, and to evaluate the release of chemicals like I<sub>2</sub>, Dissolved Organic Matter (DOM) and Particulate Organic Matter (POM) exudation from algae under different regimes. This work will provide input to the biological component of the ocean-atmosphere coupled climate model which will elucidate our understanding of air-sea exchange processes of aerosol and ozone and its impacts on climate. Optimum growth conditions for selected species of microalgae (including representatives of diatoms, dinoflagellates & coccolithophores) were determined. Impacts of different levels of irradiance, temperature and nutrient regimes on the growth cycle of two strains of *Emiliania huxleyi* were studied. Depending on the growth rate phase of the cultures, a relationship between the two biomass indices, namely cell number and chl *a* was investigated. In the case of macroalgae, significant species in terms of known iodine emission potential are being cultured under different environmental conditions. This study will also look at impacts of



PAR & UV, temperature and salinity on selected algal organic compounds, photoprotective pigments as well as productivity estimates. Furthermore, molecular iodine concentrations and releases from macroalgal cultures will be quantified.

**Sphingolipid  $\Delta$ 4-desaturase – a case of the riddle of the sphinx**

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Sphingolipids and their metabolites are emerging as important mediators of cellular processes and developmental processes in plants, and long chain base (LCB) phosphates have been implicated in plant programmed cell death and responses of stomatal guard cells to abscisic acid (ABA). In addition to the roles of LCB phosphates in mediating cellular processes in plants, sphingolipid metabolism has also been shown to regulate key developmental processes like gametophytic and sporophytic development, suggesting that regulated sphingolipid metabolism is fundamental to plant survival. Here, we report that the putative sphingolipid  $\Delta$ 4-desaturase is preferentially expressed in *Arabidopsis* pollen, in agreement with the recent observations for the importance of sphingolipid metabolism in pollen development. However, knockout mutants of the putative sphingolipid  $\Delta$ 4-desaturase gene did not display any observable phenotypic disruption in pollen morphology, germination and pollen tube growth. This raises interesting questions on the role, or lack of, of the sphingolipid  $\Delta$ 4-desaturase in *Arabidopsis*.

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