Developing biomass crops for future climates

An international symposium
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Abstracts

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WATBIO

Biomass 2017: Developing sustainable bioenergy crops for future climates

This conference was organised by the WATBIO research consortium. The conference hosted 56 scientists from 12 countries. 45 scientific contributions were presented. The programme combined presentations from the WATBIO research project and contributions from leading researchers from around the world.

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Foreword

Professor Gail Taylor, Coordinator of the WATBIO project

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Developing fast growing non-food trees and grasses as bioenergy feedstocks remains an important challenge for plant science. Climate resilient crops that use resources efficiently and are able to tolerate changing conditions are central to the future bioeconomy, where green plants will deliver food, fuel and a wealth of valuable chemicals. At the same time, stringent climate targets beyond the Paris agreement demand even more from biological systems where several negative emissions technologies (NETs) utilise crop systems in some way, for example for BECCS (Bioenergy with Carbon Capture and Storage). These NETs will be required over coming decades as the world attempts to re-dress the balance between carbon emissions and carbon sequestration.

Against this backdrop, the meeting in Oxford provides a timely opportunity to hear about the latest progress in these new less well-studied crops that are likely to be more widely grown over coming decades. Our international speakers from across the world study different plant species including poplar, willow, arundo, miscanthus and models such as brachypodium. All have a role to ensure we have the right crops for the right place as our climate changes significantly. At the same time, we have invited a number of experts who do not work on bioenergy systems, but have made significant advances in other crops or in studying plant processes of relevance, where we may learn from their approach, to enable rapid 'learning-by-doing' in the deployment of our bioenergy systems.

Our focus over the past five years in the WATBIO project has been to unravel the link between genes and function, often for complex traits such as vigour and tolerance to drought, using all of the latest technologies at our disposal. Our conceptual framework has been to understand biomass resilience and growth maintenance under moderate but frequent drought stress, since this is the most likely 'drought type' that will occur over large tracts of land in Europe that is marginal for food production. Future predictions suggest that much of southern Europe will be subjected to frequent drought and rain-fed agriculture will be even more challenging. Increased biomass productivity is key to a successful future bioeconomy since the yield gap in these systems is large.

In WATBIO, we have considered single gene and multiple gene approaches. For example, we have elucidated very specific mechanisms that may or may not confer drought tolerance through investigating the receptors and signalling for ABA action that determine instantaneous water use efficiency. The relevance of these laboratory-based findings and scalability to the field remains unknown but they offer intriguing targets for future exploitation. Complementing this, we have searched for relevant genes using high throughput sequencing in widely differing germplasm originating from both wet and dry environments. Using these approaches we have been able to understand the importance of plastic, acclimation responses to drought alongside adaptive, genetic differences and to begin to understand how we might use these traits linked to markers in future genomic selection approaches. Modelling probably holds the key to integrating the works on gene expression, genotyping

and functional responses and we have made significant progress in identifying key hubs from our extensive field trials network in WATBIO.

Much remains to be done on the genomics of bioenergy crops, and we hope that this meeting will provide a useful opportunity in which to identify the next steps in moving towards a viable industry.

Professor Gail Taylor

Session 1: Climate change, land availability and biomass potential

Chair: Iain Donnison

Session 1, Presentation 1: Raphael Slade

How policy makers learned to start worrying and fell out of love with bioenergy

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Bioenergy now has a prominent role in national energy strategies in more than 60 countries around the world. The impetus for these policies draws on a range of motivations: improving energy security, diversifying agricultural production stimulating rural development, job creation, and reducing greenhouse gas (GHG) emissions.

Arguably GHG reductions were never the main driver for bioenergy policy, yet controversy over the extent, timing and duration of carbon savings threatens to derail policy initiatives to drive up deployment. This work analyses current controversies around bioenergy in the context of historic developments in the United States, Brazil or European Union. It addresses two key questions: "how did we end up in this policy mess?" And, "how do we get out of it?"

Policy makers have faced three broad challenges to whether policies introduced to support bioenergy can genuinely contribute to GHG mitigation. The first is that carbon accounting frameworks misrepresent the carbon saving benefits of bioenergy, potentially leading policy makers to support policies that have unintended and undesirable consequences. The second is that increasing biomass production on agricultural land can directly, or indirectly, lead to increasing carbon emissions. The third challenge is that increased use of forest biomass does nothing to reduce emissions in the short term but can only reduce carbon emissions in the distant future.

We examine the evidence around each of these challenges and critically evaluate the policy responses. We argue that the greatest risk lies in political loss of confidence and institutional paralysis. Whereas the greatest opportunity lies in the co-evolution of bioenergy production and governance systems, drawing on the collective judgment of stakeholders involved in experiential, interactive and deliberative decision making processes.

Bioenergy: challenges for policy-makers relevant to crop development

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The UK Government has legally binding targets to reduce greenhouse gas (GHG) emissions. The world leading Climate Change Act of 2008 requires an 80% reduction in GHG emissions between 1990 and 2050. The Act delivers certainty to investors and society through a series of five year long carbon budgets set in advance. In summer 2016, the fifth carbon budget covering the period 2028 to 2032 requiring a 57% reduction in average annual emission rates compared to 1990 was put into law.

On 18th November 2016, the UK ratified the Paris Agreement on 18 Novmber 2016. This provides a number of challenges to our society to work towards mitigating the threat of dangerous climate change including seeking to achieve a global balance between sources and sinks of GHG. Enhancing sinks in the landscape plays a key role in this. Better understanding of the ecology and carbon balances of crop and forest management is essential to quantifying change. The Paris Agreement however makes clear that actions that are taken to reduce climate impacts must be sustainable and not threaten the outcomes of poverty alleviation programmes or food security.

In delivering the ambitious mitigation of emissions required to achieve these targets, while achieving our sustainable development goals, there are risks and opportunities from bioenergy deployment and the growth of the bioeconomy. This is heavily contested space on scientific and political grounds with genuine concerns about both action and inaction around carbon stocks, food security, ecological impacts, air quality, and land use change. Many of these concerns are present in UK society as in other countries.

Advances in bioenergy genomics, and deployment of that understanding, offer potential improvements in the efficiency of land use through cultivars that are higher yielding, more disease resistant, and climate resilient. This could reduce some of these tensions leading to better outcomes for all.

Future biomass supply for low carbon European energy provision in a changing world

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European nations have committed to reduce greenhouse gas (GHG) emissions to 20% of the 1990 emissions by 2050. Most have foresworn nuclear energy in favour of energy generated by renewable sources such as tidal, wave, wind, solar, hydro and bioenergy to achieve this goal. Most renewable energy sources are intermittent or cyclic and must be complemented by dispatchable power sources to balance the intermittency of supply to the base load and to supply peak demand. Historically this dispatchable power has been provided by fossil or nuclear power which can, by varying degrees, be increased and decreased to match demand. Pumped hydro storage is also used in this way. Of the renewable technologies bioenergy and to a lesser extend natural flow hydro have the potential provide dispatchable energy as the energy source can be stored and used as required. Bioenergy in all its forms requires biomass feedstock, whose provision requires land and suitable soil and climatic conditions for it to grow. This biomass feedstock is also required to supply other bio-economy chains which will also assist in reducing GHG emissions. The quantity of biomass feedstock is limited by land area and the net primary productivity of this land due to the environmental conditions, which will favour the growth of different types of feedstock species, each of which have their optimum bioclimatic envelope for maximum growth and hence biomass yield. As the climate changes the geographic range of each bioenergy feedstock species will move due to changes in temperature and rainfall. Productive land is the factor limiting the amount of biomass that can be provided as land is also required to provide food for an increasing global population and their dietary aspirations as well as providing forestry for wood products, habitats for wildlife and terrestrial carbon storage. Food crops are used to provide first generation feedstocks for biodiesel, bioethanol and biogas but their use competes with human food provision and causes indirect land use change. This combined with intensive crop management results in little impact on reducing GHG emissions. Bioenergy feedstock can also be provided from woody plants from forest management in the form of thinnings and residues to produce a low GHG feedstock as long as the total quantity of harvested biomass is less than the annual growth so that the carbon stock in the forest remains constant. Second generation lignocellulosic bioenergy crops such short rotation forestry, coppiced woodland and perennial grasses such as miscanthus can be specifically grown for bioenergy using low inputs and land that does not compete with food production to produce a low GHG feedstock.

Session 2: Generating, capturing and utilising genetic variation

Chair: Michele Morgante

Session 2, Presentation 1: Jerry Tuskan

Inter-kingdom signaling – a *Populus* case study

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Experimental evidence pointing to inter-kingdom signaling between *Populus* and its endophytic community will be presented specifically related to *Laccaria* colonization, small secreted protein signaling and quorum sensing. These data initially emerged from the sequenced, assembled and annotated *Populus* genome, where over 35 archaeal, bacterial and fungal genomes were detected and assembled. Since then, over 500 bacterial endophytic and 35 fungal associates of *Populus* have been sequenced, assembled and annotated. As a result of these efforts, *Populus* lectin receptor-like kinase has been identified to control *Laccaria* colonization. The *Populus* transgenes has been transformed into *Arabidopsis*, which resulted in the formation of a Hartig-net, the first report of a mycorrhizal association in *Arabidopsis*. The PtRLK gene induces metabolic changes in *Arabidopsis* that mimic fungal challenge but in the presence of *Laccaria* these responses subside. From the *Populus* genome assembly, over 200 small secreted proteins have been identified and characterised. Several of these are actively taken up by fungal associates and are then subsequently localised to the nucleus of *Laccaria*. The presence of the *Populus* secreted proteins in the *Laccaria* nuclei cause changes in *Laccaria* hyphal branching. From the *Populus*-based, endophytic bacterial collection, several genera, i.e., *Rhizobium*, *Rahnella*, *Albidiferax* and *Pseudomonas*, were found to contain quorum sensing genes that respond to *Populus* leaf macerate. The plant signal is actively transported and is most likely a dipeptide, resembling a D-Ala-D-Leu dimer. It appears that *Populus* has metabolic signals that attract favourable bacteria via co-option of their quorum sensing machinery. Strategies for leveraging this information indicate we may be able to intentionally and specifically manage the *Populus* microbiome in an environmentally relevant manner. Inter-kingdom signaling between a plant host and its microbiome, through exchange of metabolites and proteins, appears to be pervasive.

Brachypodium functional genomics: pan-genomics, polyploidy and a cast of mutants

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Brachypodium distachyon has been developed as a model grass to accelerate the functional characterisation of grass genes. *B. distachyon's* small size, rapid generation time and simple growth requirements facilitate functional studies under controlled conditions. In addition, numerous genomic resources including an essentially 'finished' genome sequence, a collection of >600 000 sequence-indexed mutations, several recombinant inbred line populations, a large collection of natural accessions (many sequenced) and a highly efficient transformation method greatly facilitate the identification and characterisation of genes of interest. An overview of *Brachypodium* genomics will be presented with an emphasis on three topics:

1) the creation of a of *B. distachyon* pan-genome based on *de-novo* assembly and annotation of the genomes from 54 natural accessions. The analysis of this pan-genome indicates that a large portion of the genetic diversity of this species was not captured by the reference genome, a result with obvious implications for crop breeding;

2) the use of two diploid species (*B. distachyon* and *B. stacei*) and their derived allotetraploid (*B. hybridum*) as a model for polyploid genome evolution and regulation; and

3) the cataloging of hundreds of thousands of mutations to create a new resource for functional genomic studies.

Mutagenesis and genomic analysis in *Arundo donax*

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Arundo donax L. (giant reed) is a perennial C_3 *Poaceae* species of Mediterranean-subtropical origin characterized by high carbon accumulation efficiency which makes it a promising bioenergy crop. *A. donax* is highly polyploid, completely lacks sexual reproduction and propagates vegetatively. Very little genetic variation is found between ecotypes, which makes *A. donax* domestication and genetic improvement a challenge. We utilised physical mutagenesis with gamma and fast-neutron irradiation treatments of *in-vitro* cultures in order to increase *A. donax* genetic variability. For the gamma-ray treatments, LD50 was reached with irradiation doses between 40 and 60 Gy. A total of approximately 1 100 independently regenerated plants are currently available. Variation in morphological traits such as plant height or habitus, and chemical traits such as cellulose or lignin content have been identified. To our knowledge, these are the first results of artificially induced mutagenesis in *A. donax*. At the same time, cytogenetics and genomics-based investigations are being applied in order to reveal the molecular nature of induced mutations and to shed light on the polyploid genome structure and the gene content of this important species.

Using genotyping-by-sequencing with single primer enrichment technology to identify the genetic basis of drought tolerance in *Populus nigra*

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Populus holds significant potential as a second generation feedstock for the production of renewable bioenergy and biofuels. Understanding the genetic basis of key traits such as biomass yield, wood quality and, due to global climate change, water use efficiency and drought tolerance are research priorities in this species. Here we report on the genotyping-by-sequencing (GBS) of more than 500 accessions from a natural, wide population of the native European tree *P. nigra* (black poplar). We decided to target the entire gene space using a novel enrichment strategy, single primer enrichment technology (SPET), to be able to assay genetic diversity within all predicted protein coding genes and associate it with phenotypic diversity in a genome-wide association study. A panel of ~90 000 target single nucleotide polymorphisms (SNPs) was developed from previous low-pass sequencing of 51 accessions: 1–4 target SNPs were selected in every annotated gene with priority given to SNPs within exonic regions and the 5' UTR where regulatory regions impacting expression may reside. Probes were designed so that their 3' end, being an extension initiator, was close enough to the target SNP to ensure read-through capability of the reverse read in a 2x130 bp sequencing configuration. Sequencing proceeded in 48-plex using an Illumina Hi-Seq 2500. This approach was successful in reliably genotyping the targeted SNP from the reverse read but also permitted significant *de novo* SNP discovery from the forward read providing additional, untargeted markers. In total this provided more than 100 000 genotyped SNPs available for this population. This novel methodology showed promising features which are applicable to other contexts where cost-efficiency and scalability need to be coupled with knowledge-driven marker selection. The population has been cultivated under short rotation coppice (SRC) at a site in Savigliano, Italy and subjected to extensive phenotypic analysis for many of the traits outlined above. These phenotypic and genotypic data are a powerful resource for genome-wide association studies (GWAS) to identify candidate genes and assist the molecular breeding and sustainable intensification of this important bioenergy crop.

Expanding the bio-economy: developing the Asian C4 perennial grass miscanthus

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Abundant and affordable biomass feedstock is key to expanding the European bioeconomy. Over three decades the EU has supported projects across a wide range of annual and perennial species that have potential to produce high volume and/or high value lignocellulosic biomass yields. The expression 'horses for different courses' is often used in relation to biomass because there are so many feedstock types. It can be argued that this has reduced the focus of research and that practical progress by policy makers and industry in upscaling biomass supply has been slower than expected. Developing new crops for biomass requires longterm and sustained investment to collect wild germplasm, select parents with key traits, hybridise and evaluate the progeny. *Miscanthus*, a C$_4$ grass from Asia, is one of the few candidate species to have received sufficient attention to initiate genetic improvement through breeding. Work on *Miscanthus* in the UK has focused on recreating variants of the highly productive and nutrient efficient hybrid *M. x giganteus* (BSBEC project, 2011-2015) to reduce establishment costs (GIANT LINK project, 2011-16) and improve drought (FP7-WATBIO project, 2012-17), flooding (NERC Nexus project, 2016-18) and cold resilience (UK-BBSRC CSP project, 2017-22). In glasshouse experiments and multi-location

plot trials, a wide range of genotype x environment responses show how combinations of physiological traits confer resilience to abiotic stresses in both wild germplasm and novel hybrids (e.g. FP7-OPTIMISC and OPTIMA 2012-16). For speed of delivery of new hybrids combining desirable traits to trials, breeders have relied on cloning. However, as clonal propagation is both costly and relatively slow to upscale, our programme with strong industrial involvement prioritised the development of seed-based *Miscanthus* hybrids. Ongoing pre-commercial scale seed production and agronomic trials (MUST-Innovate UK, 2016-2018) will be upscaled to ~40 hectares across ten European locations in 2018, providing both sufficient feedstock for industrial scale process tests by 2020 (in the BBI-GRACE project, 2017-2022) and the land areas needed to better quantify environmental impacts of land use change. Breeding and agronomy are keys to biomass yield and qualities needed for specific value chains. Sustained public-private investment in translation of underpinning breeding science into practical breeding is essential to overcome the barriers to deliver the large quantities of multi-purpose *Miscanthus* biomass needed to reduce dependence on fossil feedstocks for energy and materials.

De novo transcriptome assembly and comprehensive expression profiling in *Arundo donax* to gain insights into responses to drought stress

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Arundo donax has attracted renewed interest as a potential candidate energy crop for use in biomass-to-liquid fuel conversion processes and biorefineries. This is due to its high productivity, adaptability to marginal land conditions, and suitability for biofuel and biomaterial production. Despite its importance, the genomic resources currently available for supporting the improvement of this species are still limited. *De novo* assembly is needed. Here we report high-quality *de novo* transcriptome assembly and functional annotation, and provide molecular tools for the development of this biofuel feedstock. We then leverage the transcriptome to decipher the response of three contrasting ecotypes to drought based on molecular and physio-morphological characterisations. Phenotypic analyses confirm the contrasting responses of the *A. donax* ecotypes to drought. Response patterns of differentially expressed genes (DEG) reveal variable transcriptional responses under well-watered (WW) and moderate drought (mDr) conditions and at different time points. Notably, ecotype-specific early responses to drought highlight a tight coordination between the nucleus and organelles. Genes that are involved in primary metabolic process show a time-dependent expression in response to mDr. Furthermore, a multifactorial analysis was performed to detect the ecotype response to drought across time points. DEGs captured by multifactorial analysis common to either the ecotype, treatment and time point (G+T+P) components were analysed by differential cluster analysis, and clusters showing the most interesting features are reported. In addition, gene co-expression analysis coupled with enrichment analysis of 1 118 drought-responsive genes reveal four modules of highly correlated genes. Module-trait relationship establishes a clear correlation between drought and regulation of ABA signaling and biosynthesis and identifies transcription factors possibly involved in the regulation of both routes. Overall, these candidate genes hold the potential to improve drought stress tolerance and enhance *A. donax* productivity under suboptimal field conditions.

Session 3: Ideotypes for yield in a changing climate – drought stress and beyond

Chair: Bill Davies

Session 3, Presentation 1: Greg Rebetzke

The Annals of Botany Lecture

Linking improved genetic and physiological understanding to deliver greater water productivity to breeding programmes

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Climate change threatens to reduce precipitation and increase rainfall variability in many of the world's wheat-growing regions. Together with increasing population growth and a reduction in available water for irrigation, more efficient use of water (i.e. greater water-use efficiency) is being targeted in the development of new wheat varieties. Breeding and selection for water-limited environments have been successful but at rates of genetic gain below that of favourable environments.

Many factors contribute to slow breeding progress in rainfed drought-stressed environments: 1) selection for strict requirements of improved grain quality and disease resistance reduce genetic variance; 2) water stress is a dynamic entity changing in timing and severity from one year/site to the next; and 3) both the above contributing to large genotype × environment interaction that reduces repeatability of genotype performance and confidence in selection. The opportunity exists to complement existing selection for biomass and yield in populations containing appropriate water productivity trait variation to improve performance.

A range of tools that complement empirical selection are available to increase genetic gain in breeding programs. These include the use of improved physiological understanding and rapid, high-throughput phenotyping, and molecular techniques/platforms aimed at more cost-efficient screening for greater water-use efficiency. Managed environment facilities (MEFs) are also being used to reliably measure and validate complex traits and genes within targeted populations alongside the best wheat varieties under targeted water-limited scenarios in the field. Significant potential lies in high-throughput phenotyping of early- and advanced-generations for enrichment of genetically correlated, physiological traits underpinning performance in environments managed to represent specific, high frequency environment types. Together, improved repeatability and confidence should produce correlated increases in genetic gain at reduced cost or shorter cycle intervals to breeding programmes. Examples highlighting this integration of genetics and physiology will be given in delivering greater early vigour, biomass and weed competitiveness to commercial breeding programmes.

Transcriptomic analysis of interspecific diploid and triploid willow hybrids

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While there are long-standing models to describe the genetic basis for heterosis (hybrid vigour) in inbred crops, experimental evidence supports different and often complex mechanisms in different species. None of these models can be directly applied to explain heterosis in undomesticated, outcrossing, highly heterozygous, and polyploid species, such as in *Salix* bioenergy crops. While significant improvements in willow biomass yield have been realised through interspecific hybridisation, we consistently observe greatest heterosis in triploid progeny produced from the hybridisation of diploid and tetraploid species. We produced a series of reciprocal crosses to test for heterosis in intra-specific F_1 and F_2 families of *Salix purpurea*, inter-specific diploid F_1 hybrids between *S. purpurea* and *S. viminalis*, and inter-specific F_1 hybrids between the diploids *S. purpurea* or *S. viminalis* and the tetraploid *S. miyabeana*. Progeny from these families have been evaluated in a replicated field trial and a subset grown under controlled environment conditions for precise phenotyping and RNA-seq analysis to assess the extent to which differential and non-additive gene expression contributes to heterosis for yield and wood composition traits. Allele-specific expression is principally conserved in intraspecific F_1 and F_2 families, but is highly divergent in interspecific F_1 families. Further, non-additive inheritance patterns (principally dominant inheritance) account for a majority of the differential expression observed in all families assayed. Sex-biased gene expression also contributes to non-additive inheritance of expression, primarily represented by genes localised to the sex determination region on chromosome 15. The results of RNA-seq analysis will be used to identify non-additively expressed candidate genes, develop models to describe the genetic basis for heterosis in triploid hybrids of willow, and advance the breeding of this important bioenergy crop.

Water stress from phenomics to field: towards identifying a suitable ideotype for resilent yield in miscanthus

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A diverse collection of miscanthus genotypes were screened under control and water stressed conditions at different experimental scales: from controlled environment to field scale. The aim was to identify exemplar genotypes and suitable ideotypes for improved yield performance under water stress conditions, to determine how experiments at controlled experiment scales predict performance at field scale and identify ideotypes as breeding targets.

High-throughput phenomics was used to screen a diverse collection of miscanthus genotypes to identify genotypic variation associated with moderate and extreme drought. Genotypes were screened to determine if it is possible to identify combinations of desirable traits such as high yield and water use efficiency. Selected genotypes were identified from either experimental or natural drought studies to examine drought associated traits in detail. Yield in miscanthus growing under well-watered (control) conditions was correlated with stem height and number as well as phenological traits that determine canopy duration. In short term controlled glasshouse experiments, some trait correlations with yield decreased under water stress and genotypic differences in yield declined. However, yield correlated with leaf growth in water stress treatments. Genotypic variation has also been identified in photophysiological traits. Genotypes that produce superior yield under natural drought included genotypes that primarily responded to water stress via stomatal mechanisms or at the level of electron flow through photosystems. Modelling of yield was used to determine the empirical association of the mechanistic determinants of yield under water stress conditions. We discuss how modelling and multi-scale experiments, including at the molecular level through RNA-seq, can inform potential ideotype selection to assist in breeding resilient miscanthus for growth in diverse environments.

The nature of the progression of drought stress drives differential metabolomics responses in *Populus deltoides*

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Use of woody crops for quad-level (10^{15} BTU) energy production will require the use of marginal agricultural lands, where water stress is common. *Populus* species have the capacity to increase dehydration tolerance by lowering the osmotic potential via osmotic adjustment, the active accumulation of solutes under stress, and allowing turgor and growth maintenance under mild to moderate stress. The inherent genetic potential of a given clone to respond to drought interacts with the nature of the drought exposure to determine the degree of the biochemical response. A drought stress study on *Populus deltoides* 'WV94' was conducted in a greenhouse and the resulting metabolomic profiles of leaves were determined for plants subjected to cyclic mild (-0.5 MPa predawn leaf water potential) drought vs cyclic severe (-1.30 MPa) drought in contrast to well-watered controls (-0.1 MPa) after two or four drought cycles, and in contrast with plants subjected to acute drought, where plants were not rewatered, but were allowed to dessicate for up to eight days. Aqueous ethanol (80%) extracts of rapidly-sampled leaves were analysed for metabolites by gas chromatography-mass spectrometry (GCMS) with electron impact ionisation (70 eV) following trimethylsilylation. The nature of drought onset (cyclic vs acute), frequency of drought (number of cycles), and the severity of drought (mild vs severe), all interacted to influence the degree of osmotic adjustment and the nature of the organic solutes that accumulated. Acute onset of prolonged, severe drought induced the greatest osmotic adjustment after withholding water for seven days (1.42x) with the greatest accumulations in large, complex higher-order salicylate conjugates. Organic solute accumulation under cyclic stress relative to well-watered controls was moderate (1.20x) and was largely constituted by soluble sugars, organic acids, and amino acids. The metabolite responses are discussed in the context of stress acclimation versus metabolic perturbation.

Plastic or adaptive? Linking genotype to phenotype to define an ideotype for drought tolerance in *Populus nigra*

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Populus nigra is a fast-growing, perennial tree with significant potential for exploitation as a bioenergy feedstock. However, high productivity is often coupled with large water requirements and the species can be sensitive to drought. An important step in breeding resilient trees for future climates is to identify a drought tolerant ideotype, the heritability of traits that contribute to a successful ideotype and how this may be exploited in future. Through complementary association genetics, transcriptomics and detailed phenotyping approaches in the laboratory and field, we have identified the morphological, physiological and genomic components of yield resilience in *P. nigra*.

Using a diverse germplasm collection with genotypes selected from extremely dry to wet habitats across Europe, we have explored the importance of trees adapted to droughted environments and compared their responses to drought with those from wetter, cooler climates that nevertheless show an extreme plastic acclimatory response to drought. At a physiological level, we found that genotypes with high hydraulic capacity, and large leaves made up of many cells to be best suited to the multiple drought and non-stressed environments across Europe. Thus we conclude (i) that yield penalty may be acceptable for moderate drought environments across Europe, as a consequence (ii) understanding the genetic basis of vigour, captured in the Yield Stability Index (YSI) may be of greater value than studying the Drought Resistance Index (DRI). If we accept that yield is a key target for our understanding then development of large leaves with many cells provides an important target for selection and breeding.

Extending these ideas further, we took more than 500 genotypes from a natural, *P. nigra* mapping population, drawn from across the species' western European range. They were subjected to drought under short rotation coppice (SRC) in Savigliano, Italy and phenotyped for biomass yield and leaf size traits under irrigated and moderate drought treatments. Sequence capture genotyping-by-sequencing (GBS) targeting the gene space yielded 132 000 single nucleotide polymorphisms (SNPs) representing 29 400 genes. Genome wide association genetics identified candidate genes for biomass, leaf size and drought tolerance (DRI, YSI) while genetic outlier analyses identified additional signatures of adaptive selection to important precipitation and temperature variables. RNA-seq was then employed to elucidate the transcriptomic response of three diverse genotypes to drought stress with over 2 700 differentially expressed genes found to be involved in the drought response. When gene-phenotype networks were re-constructed for leaves of distinct developmental stages using the top 100 most differentially expressed genes, five key candidates were identified through gene ontology as being central to the drought response. These microtubule-related genes act as gene hubs in young leaves, playing a central role in leaf development and resilience to drought. Subsequently, as leaves mature, these genes are reduced in their connectivity and are situated in the peripheral parts of the networks. Genes of interest overlapping between the GWAS, outlier and RNA-seq analyses were identified and these are strong candidates for application in advanced molecular breeding or genome editing for the sustainable intensification of this important lignocellulosic crop. Moreover, although water use efficiency and saccharification potential are less heritable breeding targets, we identified genotypes which combine yield, water use efficiency and saccharification potential and these are an important resource for future breeding programmes.

Dissecting complex traits in *Salix*: progress in gene discovery

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As part of the UK biomass willow breeding programme, we have been using molecular genetic and genomic approaches to further our understanding of several complex traits and to provide markers linked to quantitative trait loci (QTL) for use in molecular breeding.

Although not a prerequisite for selection and breeding *per se*, knowledge of the genes and causal variants underlying QTL can be extremely valuable in that it can offer new insights into fundamental biological processes and deliver genes of relevance to industrial biotechnology. However, moving from QTL intervals to the underlying genomic determinants of observed phenotypic variation remains a challenge.

By applying integrated genetics and genomics approaches, we have made progress in the identification of some key candidate genes underlying variation in several target traits, namely biomass yield, architecture, phenology, and secondary metabolic traits. A review of progress and discoveries in this area will be presented in addition to details of more recent experiments where we are using similar approaches to look at drought responses in large, field-based experiments

Drought adaptation characteristics of a giant reed mutant

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Giant reed (*Arundo donax* L.) is a spontaneous C_3 perennial grass, which propagates only vegetatively due to seed sterility. This prevents the development and establishment of targeted improving breeding programmes. The development of mutants could improve the performance and/or suitability of the species for bioenergy production purposes, especially in stressful environments. Even though giant reed is considered a drought tolerant species, nothing is known about the effects of mutagenesis on its morpho-productive and physiological traits relevant to yield responses to drought. The objective of this study was to characterise some phenotypic traits of mutagenised giant reed under drought conditions. The trial was carried out under semi-controlled environmental conditions in a total of 16 rhizotrons of one cubic meter capacity. Calibrated soil moisture probes were installed at different depths in order to adjust the soil moisture content to 25% (field capacity) and 10% (*v/v*) in the well-watered and dry treatments, respectively. A mutant (UniBO3) developed by UniBO and Genetic Lab through gamma-irradiation was selected from a pool of 100 mutants and compared with the non-mutagenised precursor of local origin. Biometric, production, and physiological parameters were evaluated at young and mature growth stages. Under well-watered conditions at both growth stages the tiller density of UniBO3 was significantly higher than in the local genotype, but both genotypes showed similar biomass yield. The sustained biomass yield of the droughted local genotype compared with the well-watered conditions was related to unchanged number of tillers. On the other hand the reduced biomass yield of the droughted mutant (UniBO3) was significantly correlated with reductions in photosynthetic capacity (Pn), maximum carboxylation rate (Vcmax), potential electron transport rate (Jmax), quantum yield (Fv/Fm), and leaf water potential (LWP) at young stages, while at mature stages with Fv/Fm and LWP. In summary, the UniBO3 mutant showed a reduced tolerance to drought at productive and physiological levels.

Morphological and physiological comparison of *Arundo donax* ecotypes to drought stress in the field

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The giant reed (*Arundo donax*) is currently being developed as a biomass crop in regions with warm to hot climates. The rapid growth of *A. donax* is due to high rates of photosynthesis that are accompanied by high levels of transpirative water-loss. The large water requirements of *A. donax* may impact upon its successful cultivation in rain-fed marginal lands not currently utilised for food production. We conducted a common garden study in Catania (Sicily, Italy) to explore the impact of growth under rain-fed and irrigated conditions on three ecotypes of *A. donax* from contrasting habitats: a warm sub-humid region of Central Italy (800 mm precipitation per annum and a mean summer temperature of 24 ˚C); a Mediterranean climate in Sicily (400 mm p.a.; 28 ˚C); and an arid pre-desert area of Marrakesh, Morocco (200 mm p.a.; 30 ˚C). Any differences in phenotype may be used in the development of more drought resistant varieties for commercial exploitation. All varieties of *A. donax* performed well under rain-fed conditions with irrigation inducing a comparatively minor 14% increase in biomass yield. This may be due to the high degree of stomatal control and modification of stomatal behaviour under drought stress exhibited by *A. donax*. Biomass production under rain-fed conditions was slightly greater in the Moroccan ecotype. The contrast in yield might be associated with differences between the ecotypes in the methionine and methyl-erythriol pathways that play a role in the response to abiotic stress.

Session 4: Genetic and genomic approaches to biomass crop improvement

Chair: Rishi Bhalerao

The SweTree Technologies Lecture

Session 4, Presentation 1: Steve Long

Bioengineering improved photosynthetic efficiency to sustainably increase productivity in C_3 and C_4 bioenergy crops

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Given small profit margins, sustainable productivity improvement is a key target in achieving economic and environmental viability in bioenergy cropping systems. Analysis shows that the photosynthetic efficiency of our most productive bioenergy crops still falls far short of the theoretical maximum, suggesting an opportunity to gain higher yields. Multiple field experiments in which photosynthesis has been artificially elevated by increasing CO_2 concentration, show that if efficiency could be increased genetically, significant productivity gains would likely result. Issues of allocation and nutrition complicate increasing yield in food crops through improved photosynthetic efficiency. For most bioenergy crops, where the harvested product is the entire shoot, these are not issues. Improving photosynthetic efficiency is therefore particularly pertinent to the genomic improvement of bioenergy crops. Because of the depth of knowledge of the photosynthetic process, a forward genetic approach to improving efficiency is viable. This allows construction of detailed mechanistic models integrating full biochemical pathways into crop canopies. Application of optimisation routines to mechanistic models of both C_3 and C_4 crops has suggested seven major routes for substantially increasing photosynthetic efficiency. Two of these have been proven in replicated field trials: 1) Accelerating the rate at which photosynthetic efficiency adjusts to natural light fluctuations in tobacco; and 2) Increasing the rate at which the primary CO_2 acceptor of C_4 photosynthesis is produced in sugarcane. Although these demonstrations are limited to two species, the universality of the mechanisms modified suggests that these technologies could be applied across all bioenergy crops.

Designing Future Bioenergy crops: a key role for the hidden half?

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When designing future bioenergy crops and varieties, researchers often focus on the shoot form and function without giving attention to the critical roles that root traits play. Given that bioenergy crops should not occupy prime agricultural land, and that if fertilised their bioenergy benefits quickly diminish, optimising adaptations to marginal land by improving water, N, P capture via their roots represent an important goal for bioenergy crop development.

Plants also transfer a significant proportion of fixed carbon to the soil through their roots. Soil organic matter, which is primarily composed of carbon, is a key determinant of a soil's overall quality. Even though crop productivity has increased significantly over the past century, soil quality and levels of topsoil have declined during this period. Low levels of soil organic matter affect a plant's productivity leading to increased fertiliser and water use.

Automated tools and methods to accelerate the process of measuring root and soil characteristics and the creation of advanced algorithms for analyzing data can accelerate the development of field crops with deeper and more extensive root systems. Crops with these root systems could increase the amount of carbon stored in soils, leading to improved soil structure, fertiliser use efficiency, water productivity, and crop yield, as well as reduced topsoil erosion. If deployed at scale, these improved crops could passively sequester significant quantities of CO_2 from the atmosphere that otherwise cannot be economically captured.

This presentation describes results from several projects and UK-US collaborations to develop deeper rooting crop varieties.

Genomics and ecophysiology of poplar drought acclimation

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Woody biomass can substitute many oil-based products because of its multiple usages, for example, as construction material, as raw material for added-value wood products, as feedstock for the chemical industry and as a resource for energy generation. The demand for sustainable production of renewable resources is increasing but climate change with increasing periods of drought limits productivity and enhances the risk of mortality for tree species with high water demand. Drought acclimation of trees involves structural changes in wood anatomy with thicker cell walls and smaller vessel lumina to avoid cavitation. Consequently, wood of drought-stressed trees exhibits higher densities than that of well-watered trees. While the ecophysiological responses to drought have been well characterised, the time course of the molecular responses to gradually increasing stress across different tissues that eventually lead to changes in wood anatomy have barely been studied. Here, we show results of poplar ecophysiological, anatomical and molecular acclimation to moderate, gradually increasing drought stress. We uncovered divergent, tissue-specific networks that were correlated with hormonal changes in roots, stem and leaves. Apart from structural changes in the xylem, drought also affected the saccharification potential in drought-tolerant and -sensitive genotypes of poplar. Our results provide insights into the genomics and genetics of wood formation, which are an essential basis securing sustainable wood production in a future climate.

Engineering biomass crops for the biorefinery

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Lignin is an aromatic heteropolymer that is mostly present in secondary-thickened cell walls where it provides rigidity and imperviousness. However, for the production of fermentable sugars from the biomass, this polymer needs to be extracted by chemical treatments, and this costly pretreatment is one of the main hurdles that hampers the transition from a fossil-based to a bio-based economy. Engineering lower amounts of lignin in biomass crops is therefore an attractive strategy to improve biomass quality. From a co-expression analyses with known genes involved in lignin biosynthesis, we discovered a gene encoding a new enzyme in the biosynthesis pathway to the monolignols, CAFFEOYL SHIKIMATE ESTERASE (CSE). The corresponding *Arabidopsis* mutant had a reduced lignin amount and a 4-fold increase in cellulose-to-glucose conversion without biomass pretreatment. The lignin modification was associated with a yield penalty. However, the yield penalty could be overcome by restoring *CSE* expression only in the vessels, while maintaining the reduced lignin amount in the fibers and the improvement in saccharification efficiency. Given these promising results, the *CSE* gene was downregulated in poplar and investigated for improvements in wood composition for saccharification. On the other hand, higher lignin amounts in biomass crops can be beneficial as well in scenarios where lignin can be valorized through catalytic breakdown. By downregulation of the *CHALCONE SYNTHASE* (*CHS*) gene in maize, plants were obtained with significantly higher lignin content.

Vanholme et al., Science 341, 1103 (2013); Vargas et al., Biotech for Biofuels, 9, 139 (2016); Eloy et al., Plant Physiol 173, 998 (2017).

Engineering of trees for improved yield including yield under drought conditions

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SweTree Technologies is a plant and forest biotechnology company providing products and technologies to improve the productivity and performance properties of plants, wood and fiber for forestry and related industries. SweTree works in a broad array of biotechnology including gene-based breeding (including GM forestry and site directed mutagenesis), automated clonal propagation using somatic embryogenesis, nutritional solutions, and tree regeneration systems. One of the key strengths of SweTree is the ability to successfully work with academia benefiting all involved parties.

In the area of biotechnology, SweTree Technologies has been testing and developing genes and 'biotech' trees since 2002. For trait/gene identification we have successfully screened and tested over 1 500 genes. SweTree's current gene portfolio consists of a combination of internally identified genes, genes identified by Swedish academics and other third parties such as Mendel Biotechnologies, and genes identified in EU research projects such as WATBIO. SweTree is constantly acquiring and identifying new genes. We also actively field test and further develop our most promising trait gene technologies including product development in elite germplasm in collaboration with end customers.

Our primary gene development programmes are performed in hybrid aspen for which we have a very efficient transformation protocol enabling efficient testing. However, for practical tree biotechnology the genes and DNA constructs have to be transformed into the best commercial clones.

Results from our gene portfolio, concentrating on results from technologies related to yield and drought responses will be presented.

Elucidating ABA signalling and its role in drought tolerance in perennial trees

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Abscissic acid (ABA) is a major plant hormone known to mediate in a variety of abiotic stresses such as cold and drought. Our knowledge of ABA signalling and its downstream targets is primarily derived from work in model systems such as *Arabidopsis* and rice. In contrast, little is known about ABA signalling and its role in drought in perennial trees. Using genetic and biochemical and genomic approaches we describe the components of ABA signalling in short rotation bioenergy crop poplar. We show that the key components of ABA signalling are conserved in poplar. Our data from transgenic hybrid aspen trees shows that compromising ABA response leads to severe sensitivity to drought. We further identify potential downstream targets of ABA and evaluate their role in improving drought tolerance in bioenergy trees in the future.

Session 5: Predictive modelling for yield – bringing genotype and phenotype together

Chair: Joost Keurentjes

Session 5, Presentation 1: Carl J. Bernacchi

Scaling growth, yield, and water use of miscanthus from the plot to the mid-western United States

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Bioenergy crops hold tremendous potential to meet growing energy demands while offsetting carbon emissions from fossil fuels. Furthermore, strategic establishment of renewable bioenergy crops can potentially mitigate climate change, increase food production, and provide additional ecosystem services. Strategies, however, will rely on focused measurement campaigns coupled with scaling from plots to ecosystems and landscapes, necessitating large-scale modelling efforts. Experiments were designed to apply ecosystem-scale flux measurements, from establishment through maturity, for the perennial grass bioenergy feedstocks miscanthus (*Miscanthus × giganteus*) and switchgrass (*Panicum virgatum*). These data were then used to incorporate these feedstocks into a land-surface model, Agro-IBIS, to scale the responses from the plot to the Midwestern U.S. Establishment scenarios, which were generated based on percent land cover change from traditional row crop to perennial grass feedstocks to assess the impact on the carbon and hydrologic cycles. Throughout most of the study domain, miscanthus showed greater productivity and soil carbon accumulation but also high water use. However, the increase in carbon uptake was greater than the increases in water use, resulting in greater water use efficiency (WUE) where yields were high. Except for the drier portions of the study domain, switchgrass did not show greater yields than maize, but did show greater soil carbon accumulation. Yet, the slightly lower yields for switchgrass compared with maize coupled with the increased water use led to lower WUE. The impact of decreased water use on stream flow and water quality for various establishment scenarios was investigated using a coupled ecosystem-hydrology model. Establising perennial grasses led to reductions in dissolved inorganic nitrate in streams much greater than reductions in water runoff, with the highest replacement scenarios decreasing long-term mean dissolved inorganic nitrogen export by ca. 15% and 20% for switchgrass and miscanthus, respectively.

The added value of natural variation in the elucidation of quantitative trait regulation

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Over the last decade, genome wide association (GWA) mapping has rapidly become a major tool for the identification of natural polymorphisms explaining observed population variation. Especially in *Arabidopsis*, the field has rapidly developed and contributed to many aspects of modern biology. Here, we will highlight some of the advancements made and illustrate this with recent case studies. GWA mapping has been instrumental in the discovery of regulators of complex traits. As such, it has contributed to our further understanding of physiological traits, plant metabolism and adaptation and evolution. Now that many studies have appeared it is becoming increasingly clear that natural variation at specific loci can have strong pleiotropic effects on multiple traits. This is especially noticeable in the coregulation of plant metabolism, where often hotspots of trait associations are found. In addition, many examples of epistatic genetic regulation can be seen, further adding to the complexity of quantitative trait regulation. Nonetheless, GWA mapping has allowed the discovery of players in the pathway topology of many traits. Some of these show strong signatures of evolution, such as selective sweeps or balancing selection. This is often reflected in the geographical distribution of allelic variation and thus provides indications for ecological adaptation. We will provide examples to demonstrate the contribution that GWA mapping has made to our understanding of the evolution and regulation of quantitative trait variation.

Integrated genomic prediction in bioenergy crops

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In addition to its intended application in breeding, genomic prediction (i.e., phenotypic trait prediction from a genome-wide set of markers) provides a conceptual framework for bringing together biometry, population/quantitative genetics, physiology and molecular biology. The integrative potential of this framework has recently been illustrated by several studies that successfully combine genomic prediction and crop growth modelling to begin unravelling G×E×M interactions. We will present empirical examples of this approach and outline ongoing and future projects aimed at developing pragmatic models for the needs of breeding programmes, as well as bringing novel insights about the genomic architectures of adaptive traits.

Exploring variation in water relations responses to soil water deficits within a *Populus nigra* wide genome association population

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Genotypic variation in the capacity of plants to maintain growth under water deficit is sometimes determined by differences in the regulation of the interrelationships between plant water status, stomatal conductance, and growth, as has been shown in other woody species like grapevine. In this study, we explored this variability in 15 *Populus nigra* genotypes representing the whole geographical range of a wide genome association population from western Europe, both in a field experiment on trees and using a gravimetric phenotypic platform on young cuttings. Both experiments confirmed the large morphological differences (leaf size, shape, branching pattern, and growth) observed in previous assessments of the population. In the field trial located in northern Italy, variation in predawn and midday water potential either between genotypes and between irrigated and mildly droughted trees was small, while stomatal conductance, osmotic potential at full turgor and abscisic acid (ABA) concentration in leaves was reduced under drought but with little differences between genotypes. This suggests a strong isohydric behaviour that is consistent across genotypes and that is achieved through high sensitivity of stomatal conductance to changes in cell turgor. In the gravimetric phenotyping platform, cuttings were subjected to a more pronounced decrease in soil moisture, resulting in a decrease in leaf water potential, with no differences between genotypes. Sensitivity of transpiration to soil drying showed only moderate variation across genotypes. Taken together, both experiments suggest that the large morphological variation within this population does not result in marked differences in water relations in response to soil drying. The search for traits related to enhanced drought tolerance should focus on other aspects such as the differences in sensitivity of growth to changes in cell turgor or in root traits conferring drought avoidance capacity at early stages.

Multi-trait genotype-to-phenotype models for complex traits

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Prediction of phenotype is central to genetics, breeding and evolutionary biology. Complex traits, like yield and biomass, are defined as traits driven by many genes with mainly small effects. Complex traits show strong context dependencies. This means genotype by environment interactions and epistatic interactions are important. Therefore, prediction of complex traits is challenging. Suitable genotype-to-phenotype (G2P) models for prediction of complex traits are multi-trait models. Various classes of multi-trait models will be discussed, where the differences between the models follows from the way in which relations between traits are defined. Examples will be given of the application of multi-trait mixed models, network models and crop growth models.

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In silico exploration of genotype x environment x management effects of short rotation willow

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Predictions of future climate are especially uncertain in terms of water availability as affected by rainfall distribution. The conflict with food crops regarding land availability and quality increases the need for bioenergy crops with low water demand and high water use efficiency (WUE). Here, we illustrate 1) how canopy structure and size of willow genotypes (*Salix ssp.*) affect the resource use efficiency (light, water), and 2) how phenotypic differences compensate for negative environment effects (soil, climate).

Our model explicitly optimises traits determining light interception, parameters of vertical and horizontal leaf distribution [1] and simulates the evapotranspiration as a function of leaf area, root distribution and water stress sensitivity [2]. The model and its parameter sensitivity was evaluated against a long time series of biomass and water dynamics in two contrasting UK sites. In a long-term scenario analysis across a wide range of climate-soil combinations we identified the respective best performers and calculated the probability of exceeding an economic threshold yield (9 t ha^{-1}).

The sensitivity analysis revealed that parameters relating to canopy structure ranked highly (11th, 15th) under conditions of ample water supply. Their rank dropped under water stress (24th, 30th), when parameters of root growth and water stress became dominant (9th), with differences between phenotypes.

The scenario analysis showed that apart from the southwest of Britain, the production on marginal soils (water availability <100 mm) was unlikely to be economically sustainable. Under high evaporation demand (southern Britain), the effects of phenotypic differences (canopy size) on biomass production were smaller than in the north. Under the highest water stress (south-east), small canopy had a significantly higher WUE, if soil water can be exploited.

In conclusion, modelling identified consistently traits of smaller canopies paired with strong root development for selection of drought resistant phenotypes. Trade-offs with water savings are being analysed.

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Bioenergy with Carbon Capture and Storage (BECCS) in the UK: contrasting land-use scenarios and implications for natural capital

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Emerging scenarios that explore energy pathways designed to meet ambitious 1.5 °C and 2 °C targets emerging from the Paris Agreement emphasise the importance that Bioenergy with Carbon Capture and Storage (BECCS) could provide through significant "negative emissions" for the UK and globally. The feedstock demand of BECCS would require substantial land-use change to bioenergy crops. Existing research has shed some light on the environmental implications of land-use change associated with dedicated bioenergy crops. Such research needs to be incorporated into any BECCS policy in the UK, where the demand for negative emissions and energy must be balanced with other societal and environmental needs such as food and water security, economic development, and enhancing natural capital. We consider contrasting land-use scenarios associated with a BECCS policy in the UK, and the key environmental and societal implications associated with these scenarios. Analysis highlights the trade-offs and synergies that are associated with a BECCS policy, and the resulting implications for natural capital and government policy.

Session 6: Integrated poster session

Chair: Donal Murphy-Bokern

Session 6, Poster 1: Kirsten Kørup

Drought tolerance and biomass production in nine C_3 or C_4 perennial grasses

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Biomass production in crops grown on marginal land can be reduced by drought. Cultivars with high drought tolerance or water use efficiency (WUE) are therefore interesting. An evaluation of these traits was performed in four C_3 species, *Dactylis glomerata* cvs. Sevenop and Amba, *Festuca arundinacea* cvs. Jordane and Kora, *Phalaris arundinacea* cvs. Bamse and Chieftain and *Festulolium pabulare* cv. Hykor, and two C_4 species *Miscanthus × giganteus* and *M. lutarioriparius.* Drought treated and control (irrigated) grasses were grown on coarse and loamy sand in 1 m^2 lysimeter plots protected against rain by a mobile roof. Three cuts were made during the growing season adding up to total biomass production (DM_{total}). The drought period was between the first and second harvest, which was performed when 80% of available soil water had been used (Kørup et al. 2017). Dry matter yield (DM) was measured.

At the termination of the drought period there was a significant difference in DM between cultivars concerning ($p<0.001$), N content ($p<0.001$), C:N ratio ($p<0.001$) and WUE ($p<0.01$). The N content was higher in drought stressed than control plants ($p<0.05$) across soil types. Even though drought caused a decrease in DM ($p<0.001$) for all cultivars during the drought period, the drought treatment did not affect the DM_{total}. Compared to control, drought treated plots showed increased growth in the period after drought stress. Amongst the evaluated grasses *M. lutarioriparius* had the highest DM_{total} (15.0 t ha^{-1}) and WUE_{total} (3.6 g l^{-1}). The results indicate that some grasses have a high level of drought tolerance while maintaining a high yield potential. Furthermore, one or two drought events did not affect total biomass production, so drought at the investigated level raises water use efficiency.

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A roadmap for drought effects on leaf development in *Populus*

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Water scarcity limits plant productivity and consequently threatens food, fuel and fibre production. Although food production remains high on the research agenda, ensuring energy security is becoming increasingly important. This research focuses on characterising the drought response in a drought sensitive genotype or *Populus nigra*, with the overarching goal of delivering new germplasm for sustainable biomass production for bioenergy. Plant productivity is fundamentally influenced by leaf growth, due to the nature of leaves as the photosynthetic organs of the plant. The development of leaves, both in terms of overall surface area and the underlying cell patterning enables plants to maximise light capture while minimising water loss. Crucially, leaf growth is responsive to water availability and is a sensitive indicator of drought tolerance.

Leaf growth segregated into two distinct phases in this experiment, which consisted of a six week drought experiment in a fully automated glasshouse: 1) rapid leaf growth and declining soil moisture and 2) steady soil moisture and stable leaf area. Leaf cell density dropped over time, as cells expanded and this was more prominent when plants were not exposed to drought. Further, cell expansion was greatest in the first phase of growth but this was sensitive to drought. Underlying these cell patterning changes, ~2 700 genes were identified by RNA-seq as being differentially expressed in response to drought. Gene network reconstruction elucidated distinctive shifts in the transcriptomic basis of the two phases of the drought response, with the early networks showing relatively low numbers of genes interacting compared to the drought response network reconstructed for mature leaves. Using these networks we were able to identify key hub genes such as those involved in abiotic stress responses, cell division, cell cycle and microtubule function. These genes represent candidates for future breeding programmes.

Estimating miscanthus canopy precipitation interception and a comparison of evapotranspiration models

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Flooding events have highlighted the potential role of crop cover in flood mitigation. The bioenergy crop miscanthus has a high water demand to quickly increase biomass with rapid canopy closure and effective rainfall interception, these traits may be of benefit in reducing flooding. Evapotranspiration (ET, the combination of plant and ground surface transpiration and evaporation) forms an important part of the water cycle and can be estimated from a number of models. This study aimed to establish the interception of precipitation by the canopy in a commercial scale miscanthus plantation and to determine the most accurate ET model.

In a 4 year old 6 ha plantation canopy precipitation interception was measured from June 2016 to March 2017 using stem-flow and through-flow gauges within the crop and rain gauges outside of the crop. Daily ET estimates from 2012 to 2016 were calculated using weather data from an in-field weather station and the Hargreaves-Samani, Priestley-Taylor, Granger-Gray and Penman-Monteith (short grass) models. Results were compared to data from on-site eddy covariance (EC) instrumentation to determine accuracy and calculate the crop coefficient (K_c) model parameter.

Canopy precipitation interception was 24% and remained high during the autumn and winter suggesting benefits for flood mitigation. The closest estimated ET to the EC data was the Penman-Monteith (short grass) model. The use of seasonally calculated K_c values with the Penman-Monteith model improved accuracy.

Accurate K_c values will enable better ET estimates with the use of the Penman-Monteith (short grass) model. This information will be of importance in models used to estimate potential yields and investigate hydrological impacts of land use change.

Yield response of 82 *Salix* genotypes grown under two contrasting water regimes

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A *Salix* spp. research programme has been ongoing at Rothamsted Research since 2002. Willows are of interest as they provide a source of renewable carbon for bioenergy and biofuels. Willows are particularly suitable to this application when grown as short rotation coppice (SRC). SRC willows are easily propagated and have a much shorter duration from planting to harvestable product compared with conventional forestry systems.

Drought is a potential constraint on yield in commercial SRC willow plantations. Future climate change predictions forecast that drought is likely to become more prevalent, raising questions about the future sustainability of yield from current SRC willow varieties. The aim of this study was to assess the degree of genotypic diversity for drought tolerance and identify useful populations for further study. Ultimately the output of this work will be infused into the Rothamsted Research willow breeding programme.

The effect of drought on 82 willow genotypes, representing key germplasm, was studied in a split plot experimental design planted in a rain out shelter at Rothamsted Research in 2014 and 2015. Within the experiment genotypes received two water treatments, drought stressed and the control, which was irrigated to field capacity. Water stress was imposed twice during each experiment. Yield results show there is genotypic diversity for drought tolerance. Dry matter yield analysis revealed interaction between genotype and drought treatment in 2014 ($p=0.040$, F-test), no interaction in 2015 ($p=0.195$, F-test). There were significant differences between genotypes in 2014 & 2015 ($p<0.001$, F-test).

Abscisic acid-related effects on poplar wood formation

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Drought, one of the main causes for the reduction of yield in poplar plantations, is predicted to increase in many areas. Meanwhile, the demand for wood as working material or for energy is increasing, resulting in a shortage of wood in the future. Therefore, it is crucial to understand how woody plants can cope with drought by elucidating the physiological and molecular bases of drought adaptation. It is known that plant growth is inhibited under drought stress. The intracellular abscisic acid (ABA) concentration increases rapidly and leads to stomatal closure and reduced CO_2 assimilation. In stem tissues, higher vessel frequency is found and smaller vessel lumen areas, as well as thicker fibre cell walls are formed. We hypothesise that these drought induced wood anatomical changes are triggered by ABA.

ABA was supplied to poplars grown in hydroponic culture. Additionally, a drought experiment with ABA-insensitive poplar lines was conducted. Height, stem diameter and stomatal conductance were measured while plants were growing. Anatomical analysis of stem cross sections was conducted after harvests. Here, we report the results of plant growth and anatomical characteristics affected by ABA application as well as in the ABA-insensitive lines exposed to drought. Transcriptomic analysis is currently being conducted to get further insight into the underlying molecular mechanisms.

Changes in wood formation in poplar trees affected by drought stress under different daylengths

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The demand for fast growing wood to be used as construction materials and as an energy source has increased rapidly over the last years and will increase even more in the future. However, the production of woody biomass in forest ecosystems is expected to be negatively affected by climate change leading to hotter and drier living conditions for plants. Thus, it is important to investigate the resilience and adaptability of wood producing plants in regard to environmental constraints. To obtain mechanistic information on fundamental processes of wood formation the tree model plant poplar was used.

It is already well known that drought stress has an effect on wood formation and leads to smaller but increased number of vessels. Fibers are affected by developing smaller lumen with thicker double cell walls under drought. Interestingly, similar changes in the wood anatomy are also observed at the end of the growing season when the days become shorter. The aim of this study was to compare changes in anatomy and molecular pathways of wood formation in *Populus* in response to drought stress in the presence of long-days or short-days.

In a growth chamber experiment, *Populus × canescens* trees were exposed to two levels of water availability (control and moderate drought) and two levels of photoperiod (long- and short-day) in a full-factorial design. Physiological parameters were monitored regularly, samples for wood anatomical studies were taken and RNA-sequencing was conducted. Here, we report about our results on anatomical and molecular changes of wood formation in the presence or absence of drought under different day-lengths.

A possible role for abscisic acid in restoring water transport in embolised vessels

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Populus is a model tree species that offers the opportunity to study water stress responses in a perennial deciduous tree. It is also extensively cultivated for biomass production. Water transport through non-living xylem conduits and photosynthetic productivity are closely interlinked in vascular plants. Under drought conditions, increased xylem tensions make plants more vulnerable to the formation of embolism within the xylem conduits. Limits to the capacity to replace transpired vapour with water transported from the soil may lead to extensive xylem dysfunction that causes desiccation and death. Short-term responses to withstand the imbalance between transport capacity and transpiration demand are dependent upon stomatal movement and on the ability of the plant to refill embolised xylem conduits. It has been suggested that abscisic acid (ABA) is involved in embolism avoidance via control of xylem tension, and also embolism repair. We explored: 1) water status and xylem hydraulics during drought and subsequent re-watering to investigate the vulnerability to embolism and eventual refilling processes in a French genotype of *Populus nigra*; and 2) the role of ABA in starch mobilisation and availability of soluble sugars for osmoregulation and/or embolism repair processes. All measurements were performed on well-watered (WW), drought stressed (WS) plants and re-watered plants (RW) to monitor post stress recovery. We conducted physiological (leaf conductance to water vapour (gL), maximum photochemical efficiency (F_v/F_m), leaf water potential(Ψw_{leaf}), leaf osmotic potential ($\Psi \pi$), stem water potential (Ψw_{stem}), loss of stem hydraulic conductance (PLC)) and biochemical measurements (ABA and its metabolites/catabolites, starch and carbohydrates). Our results show a relatively high vulnerability to embolism in this genotype of *Populus nigra*. Under drought (WS), the decline in Ψw_{stem} and Ψw_{leaf} led to a significant reduction in gL (stomatal control) and a marked increase of PLC (>60 %). Embolism, alongside Ψw_{stem} and Ψw_{leaf}, recovered fully 24 hours after re-watering (RW). However, gL values remained low, suggesting efficient recovery mechanisms in the genotype. Indeed, during RW our analysis showed correlations between changes in ABA concentration and variation in the levels of hexoses regulated through starch degradation. These observations suggest a role for ABA in the restoration of xylem transport capacity via regulation of stem carbohydrate metabolism during drought relief.

Photosynthetic and antioxidant capacities as potential indicator of water stress in some tolerant and sensitive tomato genotypes

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A greenhouse experiment was conducted to assess water stress induced modulation in gas exchange and some key enzymes of oxidative defense system in eleven local/exotic tomato genotypes. The treatments were 80% (optimum watered), 60% and 40% (water stress) of field capacity. There were differences between genotypes' responses to these water regimes. A significant decrease was recorded for net CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) in all tomato genotypes but this decline was less in tolerant genotypes. Concentrations of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased in water stress tolerant genotypes, whereas activities of these enzymes in water stress sensitive and moderately water stress tolerant genotypes decreased, or remained unchanged. Thus, degrees of stress tolerance in tomato genotypes cannot be related with greater activities of these antioxidant enzymes. Although wild type tomatoes were water stress tolerant, they are not preferred from a market view point. Water stress tolerant genotypes Lyallpur-1 and CLN1767 were the most tolerant tomato genotypes characterised by higher antioxidant and photosynthetic capacity. Overall, it was found that some tomato genotypes maintained their water stress tolerance during growth but the mechanism of water stress tolerance varied in different tomato genotypes. It can be concluded that selection based on photosynthetic activity and antioxidant capacity under appropriate water stress conditions similar to target environments are critically important for improving both drought tolerance and tomato yield potential which is of great commercial importance hence these could be used as potential selection criteria for screening germplasm for drought tolerance.

Analysis of model-based networks of a time-course experiment in *P. Nigra*

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We present a model-based network analysis of a time-course experiment conducted in 2013 to study drought tolerance in *Populus nigra*. In the time-course experiment an Italian genotype was grown and measured over the course of five weeks under two irrigation treatments (control=80% REW, drought=20% REW). Gene expression was measured in several tissues, and leaf and physiological phenotypes were measured in the same plants. In a first step the RNA-seq data was analysed for differential expression in leaf tissues, after which a set of differentially expressed genes was selected.

In the following step regression models were fitted to each trait as a function of genes, and to each regressor gene as a function of time and condition. The constructed hierachical network assumes three layers, in the top layer we have time and drought modelling differential expression in genes, in the second layer the genes explain a bottom layer of leaf traits. Obtained models explain a large proportion of the variation observed in traits.

Co-expression network analysis identifies drought-specific gene signatures in the bioenergy crop *Arundo donax*

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Giant reed (*Arundo donax*) is an economically important crop, essential for the biofuel and biomaterial industries. Yet, its productivity on existing farmland is likely to be affected by climate-change-driven drought stress. Despite its relevance, expression atlases or co-expression networks of drought-responsive modules is still lacking. Here, the recently available transcriptomic data on *A. donax*, including data on multiple developmental stages and drought stress treatment samples were applied to construct co-expression networks, uncovering relationships between genes, based on their correlation patterns, and identifying core functional modules that are conserved under drought stress condition. We applied the WGCNA (weighted gene correlation network analysis) algorithm to mine pooled RNA-seq datasets. We constructed two arundo gene co-expression networks (AGCN) that were subdivided into 20 and 16 gene modules for each dataset (moderate drought (mDr) and well-watered (WW)), respectively. The different responses to two irrigation treatments (mDr and WW) were explained based on these modules. Remarkably, we note that two modules of co-expressed genes in the mDr network are weakly preserved in the WW network. The functional profile of these two modules was inferred by gene ontology (GO) enrichment analysis. Brown module exhibited the most significantly enriched GO terms, including 'protein folding', 'photosynthesis', 'tetrapyrrole binding', 'stress response' and other important biological processes. While the brown module was more variegated in terms of functional diversity, the green module was exclusively characterised by genes involved in 'RNA and non-coding (ncRNA) metabolic processes' as well as 'RNA methylation', suggesting that post-transcriptional regulation of gene expression is, to some extent, affected under the moderate drought condition. Our results provide insights into AGCN gene modules and offers important references for gene functions and the discovery of candidate genes at the module level.

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