QMB Plant Biology Abstracts

P1
Genomic approach in identifying genes and gene networks that regulate strawberry fruit development

Zhongchi Liu

Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA. Email: zliu@umd.edu

Fruits represent a key evolutionary innovation for seed disposal and are derived from successful development and fertilization of flowers. *Fragaria vesca,* the diploid strawberry, is emerging as a better model than the octoploid garden strawberry due to its diploidy and sequenced genome. We employed RNA-seq to investigate the molecular events during different developmental stages of flower and fruit development. The resulting transcriptome data on flower and fruit development (tissue and stage) can be accessed freely (http://bioinformatics.towson.edu/strawberry/). We then focused on comparing gene expression before and after fertilization to identify the earliest signals responsible for fruit initiation. Analysis of phytohormone biosynthesis and signaling genes confirm the critical roles of auxin and GA in fleshy fruit initiation. Further, the endosperm tissue was found to play a more prominent role than embryo in the biosynthesis of auxin and GA for fruit initiation. In addition, we examined genes specifically expressed in the receptacle, a unique fruit tissue of strawberry. Both receptacle-specific protein coding genes and miRNAs were identified and the functions of these genes are being determined. Our studies are beginning to reveal the molecular underpinnings of early stage fruit development. Insights into early stage fruit initiation have laid the foundation for investigations into mechanisms underlying morphological diversity in *Rosaceae* fruits. This new effort is recently funded by the US National Science Foundation and will contribute to the understanding of other *Rosaceae* fruits including apple, peach, and raspberry. Please refer to project website (http://www.clfs.umd.edu/CBMG/faculty/Liu/lab/Rosaceae/index.shtml) for further details.

P2

Evolution of the cycles of life in plants

John Bowman
Monash University, Melbourne.

The life cycles of eukaryotes alternate between haploid and diploid phases, which are initiated by meiosis and gamete fusion, respectively. In both ascomycete and basidiomycete fungi and chlorophyte algae, the haploid-to-diploid transition is regulated by a pair of paralogous homeodomain protein encoding genes. That a common genetic program controls the haploid-to-diploid transition in phylogenetically disparate eukaryotic lineages suggests this may be the ancestral function for homeodomain proteins. Multicellularity has evolved independently in many eukaryotic lineages in either one or both phases of the life cycle. Organisms, such as land plants, exhibiting a life cycle whereby multicellular bodies develop in both the haploid and diploid phases are often referred to as possessing an alternation of generations. We review recent progress on understanding the genetic basis for the land plant alternation of generations and highlight the roles that homeodomain-encoding genes may have played in the evolution of complex multicellularity in this lineage.

P3

Conservation, diversity and surprises in flowering time control in the reference legume *Medicago truncatula*

Putterill, J.; Jaudal, M.; Thomson, G.; Che, C.; Zhang, L., Wen, J., Mysore, K.

Putterill Lab, School of Biological Sciences, University of Auckland, NZ. Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA

Flowering on time to optimise sexual reproduction has major effects on reproductive and vegetative productivity in crop plants. Studies in the model Brassica Arabidopsis and the monocot grass rice initially indicated a high degree of conservation of flowering time genes – such as in the control of seasonal flowering by changing day length. However, subsequent work indicates that although there are universal elements in plants like *FT* genes that promote flowering, there is much diversity in the signalling pathways leading to and from *FTs*, including in legumes.

We focus on genes and epigenetic mechanisms involved in the control of seasonal flowering in the reference legume *Medicago truncatula* (Medicago). Medicago flowering is promoted by the seasonal cues of winter cold (vernalisation) and long day length as is winter annual Arabidopsis. However, the function of key regulatory genes known from Arabidopsis such as *CO* and *FLC* are missing from Medicago.

To complement studies on the genetics of flowering in pea and soybean and to identify genes that regulate Medicago flowering time, we use forward and reverse genetic screens of the *Tnt1* retroelement transposon Medicago mutant collection (about 21, 000 lines with up to ~100 insertions /line) at the Samuel Roberts Noble Foundation. Selected *Tnt1* mutants are characterised genetically and by visual and molecular phenotyping. Our results indicate that some Medicago genes have surprising effects on flowering time. These include a Medicago Polycomb *VRN2-like* gene which represses flowering, an opposite effect than in Arabidopsis. We also are characterising genes that are legume specific. A working model for the control of Medicago flowering time will be presented.

P4

From tomato to kiwifruit, same but different

Schaffer, R.J.[1,2], McAtee, P.A.[1], Gapper, N.[3] Richardson, A.C.[4], Nieuwenhuizen, N.J.[1,2], Gunaseelan, K.[1], Hoong, L.[1,2], Chen, X.[1], Atkinson, R.G.[1], Giovanonni, J.[3], Burdon, J.[1], David, K.M.[2]

[1]The New Zealand Institute for Plant and Food Research Ltd, (PFR) Auckland, NZ, [2]The University of Auckland, Auckland, New Zealand, [3]Boyce Thompson Institute, Cornell University, Ithica, USA, [4]PFR KeriKeri, NZ.

Both *Lycopersicum Solanum* (tomato) and *Actinidia chinensis* (kiwifruit) are ovule based fruit. Both have an outer pericarp surrounding locular jelly containing seeds, and both respond to ethylene during fruit ripening. However, kiwifruit unlike tomato undergoes the majority of ripening related changes in the absence of ethylene. Tomato is a model fleshy fruit and extensively studied. It can be rapidly transformed, and the fruit rapidly tested. A lot less is known about Kiwifruit which has a long juvenile phase and poor fruiting in glasshouse conditions. In tomato there has been a number of global ripening regulators identified through mutation, these include NEVER RIPE (NR), COLOURLESS NON RIPENING (CNR), NON RIPENING (NOR) and RIPENING INHIBITOR (RIN), less is known about these genes in kiwifruit.

To functionally test ripening related genes, a selection of the most similar kiwifruit *NOR* and *RIN* genes were identified and used to complement the *rin* and *nor* mutations in tomato. The most similar *NOR* gene *AcNOR* complemented the tomato *nor* mutation, and a slightly less similar *AcNOR* gene had a partial complementation. The most similar *RIN* gene *AcRIN*[1] has a Ethylene related induction of expression but did not complement the tomato *rin* mutation. Combining these results gives us insights into ripening in both fruit and may explain the differences in ripening behaviour. This example shows how models can be used to rapidly give insights into kiwifruit fruit developmental processes and there is currently planned tests for other fruit related characters such as skin type and texture.

1. McAtee P.A., Richardson A.C., Nieuwenhuizen N.J., Gunaseelan K., Hoong L., Chen X., Atkinson R.G., Burdon J.N., David K.M. and Schaffer R.J. (2016) The hybrid non-ethylene and ethylene ripening response in kiwifruit (*Actinidia chinensis*) is associated with differential regulation of *MADS*-box transcription factors. BMC Plant Biology **15** 1.

P5

Heterochromatin reprogramming and epigenetic inheritance: how to avoid *BadKarma*

Rob Martienssen[1], Filipe Borges[1], Joe Calarco[1], Milos Tanurdzic[1], Kate Creasey[1], Jose Feijo[2], Jorg Becker[2], Bill Thompson[3], Meilina Ong-Abdullah[4], Nathan Lakey[5], Jared Ordway[5], Steve Smith[5], Raviga Sambanthamurthi[4].

[1]Howard Hughes Medical Institute-Gordon and Betty Moore Foundation, Cold Spring Harbor Laboratory, [2] Instituto Gulbenkian de Ciencia, Centro de Biologia de Desenvolvimento, 2780-901 Oeiras, Portugal, [3]North Carolina State University, Raleigh NC, [4]Malyasian Palm Oil Board, Selangor, Kuala Lumpur Malaysia, [5]Orion Genomics LLC, 4041 Forest Park Ave, St Louis MO63108

Epigenetic inheritance is more widespread in plants than in mammals, in part because mammals erase epigenetic information each generation by germline reprogramming. To assess the extent of germline reprogramming in plants, we sequenced the methylome from sperm cells (SC), the vegetative nucleus (VN), and the precursor microspore from developing haploid pollen. We found that asymmetric CHH methylation is lost in microspores and sperm cells, but restored in the VN and in fertilized seed. In the VN symmetric CG methylation is lost from targets of the DNA glycosylases DEMETER (DME) and REPRESSOR OF SILENCING 1 (ROS1) including transposons near imprinted genes, which contributes to imprinting via RNA directed DNA methylation and 24nt siRNA. In contrast, most active transposons give rise to 21nt “epigenetically activated” small RNA in DECREASE IN DNA METHYLATION 1 (DDM1) mutants, in tissue culture and in the VN, which loses heterochromatin. Loss of heterochromatin in the VN is not only due to the loss of DNA methylation but also to histone replacement with variants resistant to modification. Thus genome reprogramming in pollen contributes to epigenetic inheritance, transposon silencing, and imprinting, guided by small RNA. In a real-world example, micropropagation of oil palm clones from somatic cells circumvents germline reprogramming of *Karma* retrotransposons, and results in heritable epigenetic changes reminiscent of paramutation.

P6

The pea CYCLING DOF FACTOR gene LATE2 participates in the flowering response to photoperiod

Ridge S[1], Sussmilch FC[1], Hecht V[1], Vander Schoor JK[1], Lee R[2], Burstin J[3], Aubert G[3], Macknight RC[2], Weller JL[1]

1 School of Biological Sciences, University of Tasmania, Hobart, Tasmania 7001, Australia
2 Department of Biochemistry, University of Otago, Dunedin 9054, New Zealand
3 INRA, UMR1347 Agroécologie, F-21065, Dijon, France.

The molecular mechanisms through which flowering time is controlled by photoperiod have been extensively studied in Arabidopsis and cereals, but remain poorly understood in other major plant groups. We are studying the genetics of flowering in pea and other legumes in order to gain insight into the biology and adaptation in this important crop group. Among numerous flowering time mutants identified from EMS mutagenesis, a new dominant mutant at the *LATE2* locus in pea is late flowering with a reduced response to photoperiod. *LATE2* acts downstream of light signalling and the circadian clock to impair the induction of the main photoperiod-regulated *FT* gene, *FTb2*, implying that it may have a primary role in photoperiod measurement. Mapping identified the *CYCLING DOF FACTOR* gene *CDFc1* as a strong candidate for *LATE2*, and the *late2-1D* mutant was found to carry a missense mutation affecting a C-terminal region of CDFc1 protein implicated in protein interactions. Thismutant form of CDFc1 shows impaired binding to the blue-light photoreceptor FKF1 in yeast two-hybrid assays and delays flowering in Arabidopsis when overexpressed. Arabidopsis, *CDF* genes are important negative regulators of *CONSTANS* transcription, and FKF1-dependent CDF degradation under long days is a key mechanism for long-day specific *CO* and *FT* induction. However, consistent with previous studies on legume *CO*-like genes, we found no effect of *LATE2* on *COL* transcription, indicating that *LATE2* regulation of *FTb2* must occur via a different mechanism. The implications of these findings for evolution of photoperiod response mechanisms will be discussed.

P7

Fungal cell walls are dynamic structures that can enhance cell survival in the presence of antifungal molecules

M. A. Anderson[1], R.G.T.Lowe, J.A.E., Payne, N.L. Van Der Weerden, N.L. And M.R. Bleackley

[1]La Trobe Institute of Molecular Science, La Trobe University, Melbourne, Victoria.

Fungal cell walls are central to the battle between fungal pathogens and innate immunity systems in both plants and animals. Innate immunity molecules work by exploiting differences between the host and the pathogen. There are a variety of differences between prokaryotic bacteria and eukaryotic hosts that can exploited in defence. However, fungi are eukaryotes and as such the differences are minimal. One of the major differences between fungi and host organisms is the fungal cell wall. Over the course of evolution there has been a perpetual tug-of-war between pathogens and hosts. That is, specific recognition of fungal cell walls by the host stimulates the host's innate immunity response including the production of antifungal molecules. On the other hand the fungal cell wall acts as a defence barrier that can physically block entry of the host's innate immunity molecules and senses changes induced by these proteins that in turn stimulates a defence response from the fungus. We have examined the interaction between various AMPs and components of the fungal cell wall to examine how AMPs transit this barrier. We have also discovered that AMPs trigger changes in the cell wall that protect the cell against the deleterious effects of antifungal molecules.

P8

Learning the Language of the Chloroplast: Retrograde Signals That Regulate Stomatal and ABA responses

Barry Pogson

ARC Centre of Excellence in Plant Energy Biology, Australian National University, Canberra.

The chloroplast is an environmental sensor for the cell, communicating with the nucleus via retrograde signals to change the expression of up to thousands of genes. Recent advances have identified carotenoid derivatives, phosphoadenosines such as PAP, tetropyrroles and heme, together with reactive oxygen species and proteins that build a communication network. To date, retrograde signaling pathways have largely been viewed as a means for bi-lateral communication between organelles and nuclei, ignoring the potential for interaction with hormone signaling regulating physiological processes. Our new findings on how organelle communication is initiated, transmitted and perceived to regulate not just chloroplast processes, but intersect with hormonal signaling will be considered. Specifically, how oxidative and redox stress is perceived by the SAL1-PAP pathway and how the retrograde signal, PAP, enables ABA-mediated stomatal closure. This is likely via a distinct pathway to ABI1/OST1. Thus, SAL1-PAP retrograde signaling regulates stomata closure and germination implicating PAP as a secondary messenger.

P9

A genetic analysis of TOR in plants: Novel facets of an ancient form of growth control

Rexin, D.[1,2,] Larking, A.[2] and Veit, B. [1,2]
[1] Institute of Fundamental Sciences, Massey University, Palamerston North, NZ; [2] Forage Improvement, AgResearch, Palmerston North New Zealand, NZ.

The TOR signalling pathway is pivotal to growth control in all eukaryotic groups, but its specific roles in plants remain obscure. We have used genetic and pharmacological approaches in Arabidopsis to dissect the relative roles of protein subunits that make up TORC1, a protein complex whose kinase activity is central to TOR regulated growth responses. Similar to other eukaryotic groups, the kinase encoding subunit TOR is essential for viability and growth in plants; however, the requirement for LST8 and RAPTOR, two well conserved kinase-associated scaffold proteins, differs. In contrast to animals and fungi, plants that lack intact forms of either of these proteins are viable, but grow extremely slowly. More refined analyses reveal distinctions between LST8 and RAPTOR, as well as plant specific aspects. LST8 deficient plants display increased sensitivity to specific light regimes, suggesting a role in linking photosynthetic processes with growth outputs. Additional plant specific aspects are also evident for RAPTOR, with genetic and expression analyses supporting growth related roles in not only the sporophyte, but also gametophytes. These results offer a useful platform for further studies aimed at understanding how this ancient pathway contributes to regulated patterns of growth in plants and how its activity might be optimised to enhance agricultural productivity.

P10

Comparative analysis of NLR-mediated recognition of a bacterial effector AvrRpt2 in *Malus robusta* and *Arabidopsis thaliana*

Maxim Prokchorchik[1,2] and Kee Hoon Sohn[1,2,3]

[1]Bioprotection Research Centre, Institute of Agriculture and Environment, Massey University, Palmerston north, New Zealand.[2]Department of Life Sciences, Pohang University of Science and Technology, Pohang, South Korea, [3]School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, Pohang, South Korea,

Plant pathogenic bacteria, *Pseudomonas syringae* and *Erwinia amylovora*, translocate a type III secretion-dependent effector protein, AvrRpt2, in host plant cells. AvrRpt2 is a cysteine protease and cleaves its host target protein RIN4. AvrRpt2-directed cleavage of RIN4 is recognized by CC-NB-LRR type immune receptors RPS2 and MR5 in Arabidopsis and Malus, respectively. Interestingly, RPS2 and MR5, although both recognize AvrRpt2, do not share significant sequence homology. We found that RPS2 but not MR5 shows autoactivity as shown by a rapid programmed cell death when transiently overexpressed in *Nicotiana benthamiana* leaf cells. Thus, we hypothesized that the mechanisms by which RPS2 and MR5 are activated by AvrRpt2-directly cleavage of RIN4 differ from each other. Detailed mechanistic details of AvrRpt2-triggered activation of MR5 will be presented.

P11

SymB and SymC, two membrane associated proteins, are required for *Epichloë festucae* hyphal cell-cell fusion and establishment of a mutualistic interaction with *Lolium perenne*

Ms Kimberly Green[1], Dr Yvonne Becker[1], Dr Aiko Tanaka[2], Dr Daigo Takemoto[2], Dr Helen Fitzsimons[1], Dr Herve Lalucque[3], Prof Philippe Silar[3], Prof. Barry Scott[1]
[1]Massey University, Palmerston North, New Zealand, [2]Nagoya University, Nagoya, Japan, [3]Université Paris Diderot, Paris, France

Hyphal cell-cell fusion in fungi is required for colony formation, nutrient transfer and signal transduction. Disruption of genes required for hyphal fusion in *Epichloë festucae*, a mutualistic symbiont of *Lolium* and *Festuca* grasses, severely disrupts the host interaction phenotype. To test if *symB* and *symC,* homologs of genes in *Podospora anserina* (*IDC2* and *IDC3*) that suppress a cell growth defect*,* are required for *E. festucae* hyphal fusion and host symbiosis*,* targeted deletions of each gene were generated. In culture, both mutants were defective in hyphal cell-cell fusion, formed intra-hyphal hyphae, and had enhanced conidiation. Inverted microscopy analysis revealed that SymB-GFP and SymC-mRFP1 localize to plasma membrane, septa and points of hyphall cell-cell fusion associated with septa. SymC also localized to highly dynamic punctate structures. Plants infected with Δ*symB* and Δ*symC* strains were severely stunted. Hyphae of the mutants colonized the vascular bundles, were more abundant than wild type in the intercellular spaces and frequently formed intra-hyphal hyphae. Hyphal branches failed to fuse but instead formed convoluted bundles. Although these culture and plant phenotypes are identical to those previously observed for the cell wall integrity MAP kinase mutants, Δ*mkkA* and Δ*mpkA,* no difference was observed in the basal level of MpkA phosphorylation or its cellular localization in the mutant backgrounds. Collectively these results show that SymB and SymC are key components of a conserved signaling network for *E. festucae* to establish a mutualistic symbiotic interaction within *L. perenne*.

P12

The apoplastic secretome of the tomato leaf mould pathogen *Cladosporium fulvum*

Dr. C.H. Mesarich[1,2,3], Dr. B. Ökmen[2], Miss. H. Rovenich[2], Dr. M. Karimi Jashni[2,4], Dr. C. Wang[2,5], Dr. S.A. Griffiths[2], Mr. L. Hunziker[6], Dr. J. Hunziker[2], Dr. C.H. Deng[7], Prof. R.E. Bradshaw[3,6], Prof. P.J.G.M. de Wit[2,8]

[1] Institute of Agriculture & Environment, Massey University, Palmerston North, New Zealand, [2]Wageningen University, Wageningen, The Netherlands, [3]Bio-Protection Research Centre, New Zealand, [4]Tarbiat Modares University, Tehran, Iran, [5]Zhejiang Normal University, Jinhua, People's Republic of China, [6]Bio-Protection Research Centre, Massey University, Palmerston North, New Zealand, [7]PFR, Auckland, New Zealand, [8]Centre for BioSystems Genomics, Wageningen, The Netherlands

Leaf mould disease of tomato is caused by the non-obligate biotrophic fungal pathogen *Cladosporium fulvum*. Following entry through open stomata, the fungus exclusively colonizes the apoplastic space between mesophyll cells, where it secretes a collection of proteins. These include effectors that function to modulate host immune responses, as well as other proteins, including, for example, carbohydrate-active enzymes (CAZys), that facilitate nutrient acquisition. The same proteins, however, can also trigger immune responses in plants carrying cognate Cf immune receptors, rendering the pathogen avirulent. Characterization of the *C. fulvum* apoplastic secretome is required to further understand the abovementioned processes, and to identify novel sources of resistance against this pathogen.

We used liquid chromatography–tandem mass spectrometry (LC–MS/MS) and RNA-Seq to identify 105 *in planta*-induced secreted and surface-associated fungal proteins present in apoplastic fluid samples of compatible *C. fulvum*–tomato interactions. In addition to previously characterized effectors, this protein set was found to contain approximately 60 new *C. fulvum* candidate effectors (CfCEs). Furthermore, several proteins likely related to nutrition, including, for example, a relatively large number of CAZys belonging to glycoside hydrolase family 43 (GH43), were also identified.

As most of the identified effectors and CfCEs lack homology to proteins of known function in public sequence databases, we used structural modelling, based on ten different prediction servers, to glean more information about their possible functions *in planta*. Based on this analysis, several effectors/CfCEs were predicted to have structural homology to various antimicrobial proteins, suggesting that they may play a role in mediating antagonistic microbe–microbe interactions *in planta*. These results will be presented, together with an overview of the *C. fulvum* apoplastic secretome, and a comparison with the predicted effector repertoire of the closely related pine pathogen *Dothistroma septosporum*.

P13

Is it greener on the other side? Predicting disease resistance in radiata pine using genomics

Graham, N.[1], Klapste, J.[1], Gardner, J.[2], Williams, N.[2], Suontama, M.[1], Telfer, E.[1], Dungey, H.[1]

[1] Forest Genetics, Scion, 49 Sala Street, Rotorua, NZ, [2] Forest Protection, Scion, 49 Sala Street, Rotorua, NZ.

Long-term health and survival are critical for long rotation forestry species to attain their genetic potential in terms of growth and wood quality. As such, resistance to diseases, such as Dothistroma needle blight and Cyclaneusma needle cast, have been extensively assessed and incorporated as selection criteria for several decades in NZ's radiata pine breeding programmes. Red needle cast (RNC), however, has a relatively short history in NZ, and information to guide selection programmes is still being collected.

One of the challenges in estimating traditional breeding values (EBVs) for RNC resistance has been the scarcity of outbreaks in genetic trials, as the majority of NZ's planted forest estate originates from open pollinated or bulked seed lots. The development of a laboratory-based artificial screening method has offered an alternative for assessing resistance. Using this approach, we have generated EBVs for RNC resistance in an elite cloned population of radiata pine, for which no resistance data was previously available. This method has enabled the identification of phenotypic extremes in this population, with histopathology analyses confirming differences in pathogen behaviour post-inoculation in these individuals. However, screening remains labour-intensive and expensive and relies on the availability of clonal material to ensure statistical robustness.

Genomic selection (GS) is proving particularly useful for traits that are difficult to measure, require individuals to reach a certain age, or require exposure to a certain set of conditions or pathogens. We have already shown the ability of GS to predict breeding values with high accuracies for certain form and wood quality traits in other radiata pine populations. Recently, we have genotyped the RNC-screened elite cloned population using our 44K exome capture panel. We report on the development of prediction models for RNC resistance, and generation of the first RNC resistance genomic estimated breeding values.

P14

Genomics assisted yield improvement in alfalfa - Are we making more hay?

Charlie Brummer University of California Davis, USA

P15

Mānuka genome assembly using chromosome conformation capture (Hi-C) analysis

David Chagné*[1], Amali Thrimawithana[1], Ross Crowhurst[1], Ivan Liachko[2], Shawn Sullivan[2], David Lewis[1], Julie Ryan[1], Munazza Saeed[1], Tracey Van Stijn[3], Jeanne Jacobs[3], Rudiger Brauning[3], Shannon Clarke[3], Cecilia Deng[1], Helge Dzierzon[1], Ella Grierson[1], Elena Hilario[1], Kathy Schwinn[1]

1 The New Zealand Institute for Plant & Food Research Ltd
2 Phase Genomics, Inc. 4000 Mason Road, Seattle, WA 98195, USA
3 AgResearch Ltd

We have evaluated a novel strategy combining capture of chromatin interaction within the nucleus (Hi-C), next-generation sequencing and new bioinformatics methods (Proximity-Guided Assembly, PGA) for developing a near-complete pseudo-chromosome assembly of the mānuka (*Lepstospermum scoparium* 'Crimson Glory') genome. The method relies on capturing the folded conformation of chromosomes inside the nucleus and on the fact that loci that are genetically close on the same chromosome are more likely to interact in 3D space. Chromatin proximity interactions are captured through *in vivo* crosslinking and the resulting DNA junctions are sequenced using NGS. The PGA bioinformatics approach scaffolds the genome by arranging contigs into chromosomes in way most congruent with Hi-C data, thus maximizing the likelihood that contigs are arranged in correct order. Using PGA analysis, we inferred the location of previously unanchored contigs and created chromosome length ultra-scaffolds, enabling a ~100 times increase in the N50 scaffold length compared with assembly in the absence of this analysis. Furthermore, the Hi-C technique allowed the separation of the mānuka genome from contaminant contigs assembled from associated endophytic fungal and bacterial species. The newly assembled mānuka genome was compared to the genome of *Eucalyptus grandis* and high density genetic maps of mānuka constructed using genotyping-by-sequencing. The mānuka genome sequence will help shed new light on the genetic control of unique characters such as nectar and foliage biochemical composition, flowering time and disease resistance.

P16

“Genotyping-by-sequencing” platform to recover genetic relatedness

Klápště, J.[1], Telfer, E.[1], Dungey, H.[1]

[1]Forest Genetics, Scion, 49 Sala Street, Rotorua, New Zealand.

Current developments in next generation sequencing technologies have enabled the implementation of genomics in organisms with no reference genomes, as is the case for most forest trees. The availability of genomic resources and success of genomic selection achieved in animal and crop breeding has now turned the attention of breeders towards the implementation of genomic selection in forest tree breeding programs. Genomic predictions capitalize on capturing genetic relatedness, co-segregation and linkage disequilibrium (LD) between markers and causal variants (quantitative trait loci - QTL). However, common forest tree genome properties such as large size, fast LD decay and large effective population size hinder the ability of genomic prediction models to capture LD between markers and QTLs, which is usually the most stable part of genomic prediction. This means that genomic prediction models in forest trees rather depend on the quality of relatedness recovery, and should be used to predict only related genetic material.

Our study is focused on the investigation of a “Genotyping-by-sequencing” (GBS) platform to recover genetic relatedness. This platform is cost efficient to genotype large training populations but usually suffers a large amount of missing data and low sequence depth. Under such conditions, it can be problematic to perform reliable missing data imputation and relatedness estimations in species with a large effective population size. We deployed tools specifically proposed for GBS data[1] to construct a genomic relationship matrix. Our analysis found there was benefit in avoiding missing data imputation and taking read depth into account for the reliable recovery of genetic relatedness. This strategy can efficiently identify genomic outliers and remove them from the training population, since the informativeness and ability to predict such individuals would be very limited.

1. Dodds, KG., McEwans, JC., Brauning, R., Anderson, RM., van Stijn, TC., Kristjánsson, T, Clarke, SM. (2015). *Construction of relatedness matrices using genotyping-by-sequencing data.* BMC Genomics 16:1047. Doi: 10.1186/s12864-015-2252-3

P17

Predicting the future of C3 plants using a modified small structural protein

N. Roberts, S. Winichayakul, K. Richardson, M. Andrews, S. Rizvi, G. Bryan
AgResearch, Palmerston North

Our laboratory is predominately focussed on metabolic engineering of forage plants for the benefit of the NZ pastoral industry. Sometimes seemingly minor modifications to one pathway result in substantial changes to others; the work we present here is an example of this. Through the substitution of 6 residues within an oleosin protein (a seed oil body protein) we have increased CO_2 fixation by 20-25% and increased biomass production of over 50% in Arabidopsis and ryegrass. In both species we measured a number of well documented physiological changes associated with C3 plants grown in elevated CO_2 environments; interestingly, we also observed some relatively controversial changes. This presentation discusses our findings and the ramifications of C3 plant growth with an increase in atmospheric CO_2.

P18
Identification of plant cell wall genes using seed mucilage

Burton, R.A.[1]

[1] ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, Waite Campus, University of Adelaide, Urrbrae, SA 5064, Australia

Seed mucilage is gaining popularity as an accessible system to study polysaccharide biosynthesis and interactions. The ratios of different polysaccharides in mucilage vary according to species; pectin is dominant in Arabidopsis, pectins and xylans are found in equivalent amounts in flax whilst for *Plantago* species (commonly called psyllium) it is predominantly xylan. Xylan is a ubiquitous component of the cell wall throughout the plant kingdom comprised of a linear backbone decorated with substituent groups which vary in identity, spacing and amount between species and tissue. Thus xylans are variably soluble and fermentable, particularly in a human health context and their structure influences their downstream utility. This is particularly relevant to wheat, which contains predominantly xylan in grain cell walls. Using *Plantago* we are uncovering gene expression patterns governing polysaccharide structural diversity in two ways. Firstly we have used RNAseq and quantitative PCR on *Plantago* species with mucilage heterogeneity to track families of glycosyl transferase (GT) genes. Examples of differential transcript patterns will be presented and linked to variations in mucilage chemistry. Secondly we have made a mutant *Plantago ovata* population using gamma irradiation and have screened many M2 lines for visible and chemical changes using stains, antibodies and monosaccharide profiles and NIR techniques respectively. We have found some exciting mutant phenotypes and using RNAseq and our own *Plantago* genome assembly will present progress on the identification of the causative lesions that are affecting mucilage composition and behaviour. Study of these mutations will aid in the identification of novel genes involved in polysaccharide biosynthesis and allow functional analysis in other economically important plant species.

P19

Spatially resolved systems biology to identify novel salinity tolerance mechanisms in barley roots

W.H. Ho[1], C.B. Hill [1,3], S. Natera[2], D.L.S. Lopez[1], D. Yu[1], B.A. Boughton[2], U. Roessner[1,2]

[1]School of BioSciences, The University of Melbourne, 3010 Victoria, Australia, [2]Metabolomics Australia, School of BioSciences, The University of Melbourne, 3010 Victoria, Australia, [3]Murdoch University, Perth, Australia

Barley (*Hordeum vulgare* L.) is an essential food and brewing crop and suffers substantial yield loss under saline conditions. Little is currently understood of salinity perception and responses in plant roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level. We develop new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signalling is an integral part of complex regulatory networks involved salinity responses through modifications of membrane lipids, which occur through the activity of phospholipases, lipid kinases and phosphatases that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and for an appropriate response of the plant to environmental stimuli. Initial analyses indicate that different tissue types within the root respond differently to salt stress in tolerant and sensitive cultivars. Here we study the root responses to salinity using a combination of next generation RNA-sequencing, cell wall composition analysis and targeted metabolite and lipid analyses of three key sections of barley roots. We are also using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. We also use MALDI-FT-MS based imaging technologies to monitor spatial distributions of metabolites and lipids across root sections of salt-treated tolerant and sensitive barley genotypes. Transcriptomics results are now being integrated with spatial biochemical data, enhancing our understanding of system-wide and tissue-specific responses of roots to salinity stress. Given the lack of fundamental knowledge of the genes and proteins involved in signalling, cell wall and lipid metabolism under salinity stress, and the enormous potential for biotechnological application in this area, our results provide insight into novel mechanisms responsible for salt tolerance of barley.

P20

Evolution and function of betalain pigments

Kathy E. Schwinn[1], Hsiao-Hang Chung[2], Gagandeep Jain[3], Hanh M. Ngo[1], David H. Lewis[1], Baxter Massey[2], Kate E. Calcott [1,3], Ross Crowhurst[4], Daryl C. Joyce[2], Dion K. Harrison[2], Kevin M. Davies[1], Kevin S. Gould[3]

[1]The New Zealand Institute for Plant & Food Research Limited, Palmerston North, NZ, [2] The University of Queensland, Gatton, QLD, Australia, [3]Victoria University of Wellington, Wellington, NZ, [4]The New Zealand Institute for Plant & Food Research Limited, Auckland, NZ

In an evolutionary event that has long intrigued, a lineage within the core Caryophyllales has replaced anthocyanins, the near ubiquitous red to blue plant pigments, with those of the betalain type. In recent years, research has been building to address how the pathway may have evolved and what functions the pigments serve (and the underlying mechanisms), which will help address why the pathway evolved. Betalains (yellow or red) are formed in a pathway unrelated to that of the anthocyanins, having a tyrosine-derived central chromophore, betalamic acid (BA). BA is formed through the activity of DOPA 4,5-dioxygenase, which has homology to the LigB domain of bacterial extradiol 4,5-dioxygenases. We have found a conserved expansion of LigB homologues in betalain taxa, suggesting *LigB* gene duplication contributed to the evolution of the betalain pathway. We are also interested in the function of betalain pigments. Betalain taxa include species that survive in harsh environments such as sand dunes and salt marshes. The New Zealand native iceplant *Disphyma australe* has coastal sympatric red- and green-leaved morphs. The red morph accumulates red betalains (betacyanins) in response to salt stress, and it was observed to be more abundant than the green morph in areas with higher salinity levels. The green morph is unable to synthesise betacyanins unless it is fed a biosynthetic precursor. By studying both morphs, we have found that the betacyanins confer salt tolerance, and one of the mechanisms by which they do this appears to be by altering Na+ localization.

P21
Ancient origins of UV tolerance in land plants

Nick W. Albert[1], William Clayton[2], Brian R. Jordan[2], Simon C. Deroles[1], Kathy E. Schwinn[1] and Kevin M. Davies[1]
[1]The New Zealand Institute for Plant & Food Research Limited, Private Bag 11-600, Palmerston North, New Zealand. [2]Lincoln University, PO Box 85084, Lincoln 7647, Christchurch, New Zealand

Approximately 500 million years ago, plants left the relative safety of their aquatic habitats to colonise land. In doing so, these pioneers required ways of coping with the new stresses and challenges that terrestrial life presented. The increased exposure to intense UV-B radiation appears to have been one of these major challenges. We hypothesise that evolving responsive UV-B protection mechanisms would be a key adaptation to allow plants to colonise and flourish on land. In higher plants, flavonoids (flavonols/flavones) and polyphenolics (sinapate esters) act as sunscreens to prevent cellular damage. They are produced in response to UV-exposure, mediated through the UVR8 photoreceptor, and are regulated by various R2R3-MYB transcription factors. In lower plants, UV-B protection mechanisms are poorly understood, although flavonoids are present in liverworts – one of the earliest lineages of land plants. This suggests that the ability to produce these compounds may have been an important adaptation for UV tolerance that has been retained in other groups of land plants. We are using the liverwort model *Marchantia polymorpha* to investigate how UV-responsive flavonoid and phenylpropanoid biosynthesis occurs in bryophytes and comparing this to Angiosperms. *Marchantia* produces flavones in response to UV-B exposure and we have used RNAseq to analyse UV-treated plants to identify putative flavonoid biosynthetic and regulatory genes. Candidate regulatory genes have been identified, which we are characterising using Crispr/Cas9 mutagenesis and transgenic overexpression. We have identified a transcription factor that is induced with UV-B that regulates the synthesis of flavonoid compounds. Functional characterisation of this transcription factor is ongoing and we continue to investigate whether UV-B perception and signalling through UVR8 is conserved between liverworts and higher plants.

P22

Establishment of Chromatin Modification: How the cell recognizes and triggers transposable elements for trans-generational silencing

R. Keith Slotkin
Department of Molecular Genetics and the Center for RNA Biology, The Ohio State University

Transposable elements (TEs) are fragments of DNA that have the ability to duplicate and mobilize themselves from one region of a genome to another. TEs are ubiquitous and ancient inhabitants of prokaryotic and eukaryotic genomes subject to dynamic boom and bust cycles of proliferation, resulting in their contribution of up to 85% of a genome. On the generation-to-generation short-term timescale TEs act as parasitic mutagens that the cell tries to repress, while on the evolutionary timescale TEs drive gene diversification and the production of novel traits. From the time of their discovery by McClintock, investigation of TE regulation has led to critical discoveries of heterochromatin, epigenetic inheritance and small RNA-directed chromatin modification. The Slotkin laboratory investigates the epigenetic regulation of TEs in the reference plant Arabidopsis, with emphasis on determining how the cell recognizes a new or active TE, how it deciphers the TE from an active gene, and how epigenetic TE silencing is initiated and established. Research in the Slotkin lab has focused on two core projects: the small RNA-directed chromatin modification mechanisms responsible for the initiation of epigenetic silencing (the so-called 'non-canonical' RNA-directed DNA methylation pathways), and second how germ cells and their neighboring nurse cells communicate to ensure that TEs are epigenetically marked and silenced from the very first cell of the next generation. In this presentation, recent unpublished data will be presented demonstrating the mechanisms responsible for the initiation of TE silencing and the establishment of epigenetic transcriptional repression.

P23

Where are all the small peptides, and what might they do?

Hellens, R.P.[1,2]

[1]Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Australia
[2]Department of Biochemistry, University of Otago, Dunedin, NZ

While genome sequencing is now commonplace, genome annotation and functional characterisation of components of the genome remains an enormous challenge. Like small regulatory RNAs, short open reading frames (sORFs) often reside in 'non-coding' regions of the genome that have long been considered to be 'junk DNA [1].
Translatable sORFs of less than 100 amino acids are extremely difficult to predict from genome sequences as the number of potential ORFs increases exponentially as the potential peptide lengths get smaller [2]. This challenge is made more complicated by mounting evidence that these short proteins do not always comply with genetic convention, and are frequently encoded by short ORFs that use a translation start codon other than AUG [3].

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P24

CRISPR and Crops for crisp crops

Curtin, S.J.[1], Cermak, T.[2], Tiffin, P. [3], Voytas, D.F. [2], Stupar R.M. [4], Young, N.D.[1,5], Rouse, M.[1]

[1]USDA-ARS Ceral Disease Laboratory, USA, [2]Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, USA, [3]Department of Plant Biology, University of Minnesota, USA, [4]Department of Agronomy and Plant Genetics, University of Minnesota, USA and [5]Department of Plant Pathology, University of Minnesota USA

Several techniques and methodologies have been recently developed to target specific DNA modifications within a genome. The most recent of these is the CRISPR/Cas9 reagent which has been an important break-through in the field of genome engineering. Researchers in a wide range of crop species are now able to develop knockout or in-frame mutations at specific loci for desirable trait outcomes. The ease of construction of the CRISPR/Cas9 reagent has allowed the researcher with minimal molecular biology specialization to perform targeted mutagenesis and gene editing on any crop host amenable to genetic transformation[1,2]. This talk will address recent applications of these reagents in crop species with a focus on soybean functional genomics, validation of genome wide association (GWAS) candidates in Medicago and the uses of CRISPR to compliment wheat plant breeding efforts against stem rust.

1. Curtin, S.J., Tiffin, P., Guhlin, J., Atkins, P., Baltes, N.J., Denny, R., Voytas, D.F., Stupar, R.M., Young, N.D (2016). *Validating genome-wide association candidates: selecting, testing, and characterizing genes that control quantitative variation in rhizobial nodulation* (*submitted to Plant Physiology)*

2. Čermák, T., Curtin, S.J., Gil-Humanes, J., Čegan, R., Starker, C., Kono, T., Konečná, E., Greenstein, R., and Voytas, D.F. (2016) . *Optimizing gene editing using a multi-purpose cloning system for plant genome engineering (in preparation)*

P25
Genome editing *Nicotiana benthamiana*

Niam, F.[1], Bally, J.[1], Jung, H.[1], Hellens, R. P.[1] Waterhouse, P.M. [1,2]
[1]Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Australia. University of Sydney, NSW, Australia

A single lineage of *Nicotiana benthamiana* is widely used as a model plant and has been instrumental in making revolutionary discoveries about RNA interference (RNAi), viral defence and vaccine production. It is peerless in its susceptibility to viruses and in its amenability in transiently expressing transgene. We show by comparison with wild accessions that the laboratory strain of *N. benthamiana* is an extremophile originating from a population, in the Northern Territory of Australia, that has retained a mutation in Rdr1 for 750,000 years and thereby traded its defence capacity for early vigour[1]. We have been engineering the nuclear and chloroplast genomes of *N.benthamiana* by conventional breeding, RNAi, CRISPR and homologous recombination. Using these techniques and technologies we have conferred wide spectrum virus resistance, enhanced insect resistance, altered fatty acid metabolism and expressed sentinel reporter genes that measure recombination frequencies. The successes and challenges of these technologies will be discussed.

1. Bally, J., et al. *The extremophile Nicotiana benthamiana has traded viral defence for early vigour.* Nature Plants **1** : 15165 (2015) doi:10.1038/nplants.2015. 165

P26

Kauri: forest giants in a changing climate

Cate Macinnis-Ng, School of Biological Sciences, University of Auckland

New Zealand kauri (*Agathis australis*) are amongst the largest and longest-lived trees in the world. They are ecologically and culturally significant for their dominant role the forest and their position as a toanga species. They also produce highly-prized wood, a quality that has resulted in more than 95% of kauri forest being harvested. Currently, the main threat to kauri is *Phytophthora agathidicida* (the water mould formally known as PTA or kauri dieback) which causes eventual death of all infected trees. My research involves exploring plant-climate interactions and I am particularly interested in the impacts of climate on cycles of water and carbon. We know that kauri are responsive to climate because of the tree-ring record that is used as a proxy of past climate but the nature of their responses are unclear. Research elsewhere in the world tells us that trees are highly vulnerable to extreme weather events such as heatwaves, high winds and droughts. While New Zealand's climate is reasonably mild, parts of the country will experience more frequent and severe extreme events, including drought, particularly in Northland, the natural distribution of kauri. In this presentation, I will present results from our ongoing research on kauri physiology. I will explain the evidence we found for drought avoidance strategies in kauri during the 2013 drought and I will describe plans for the establishment of a controlled field-based drought experiment in the kauri forest.

P27

Functions of Kunitz proteinase inhibitors in white clover (Trifolium repens L.): development and defense

Islam, A.[12], Dijkwel, P.P.[1] & McManus, M.T.[1]

[1]Institute of Fundamental Sciences, Private Bag 11-222, Massey University, Palmerston North, New Zealand. [2]Current address: Department of Molecular and Cellular Physiology, Ehime University, 454 Shitsukawa, Toon, Ehime 7910204, Japan.

Plant Kunitz proteinase inhibitors (KPIs) belong to the serine proteinase inhibitor group and numerous KPIs have now been isolated from different plant species. These proteins were originally proposed as storage protein on the basis of their occurrence in storage tissue, as regulator of proteinases during germination and as potent defensive factor against insect herbivory. In most plant species, the KPIs comprise a multi-gene family and some members of the family inhibit a range of proteinases *in vitro*-suggesting that as a group they have extremely diverse proteinase targets and functions. To address the multiple functions of KPIs more directly, we have examined four phylogenetically distinct members of a KPI gene family from the perennial legume white clover (*Trifolium repens* L.). The gene family displayed differential transcription during seed germination and also in different tissues of the mature plant. Over-expression of some selected members conferred some degree of tolerance against the generalist herbivore, *Spodoptera litura.* Selective RNAi knock-down lines displayed significantly altered vegetative growth phenotypes with inhibition of shoot growth and a stimulation of root growth and retarded larval growth of *S. litura*. Further examination of these RNAi lines revealed constitutive stress-associated phenotypes as well as altered transcription of some cellular signaling genes. Though hormonal control and possible triggering mechanism for *Tr-KPIs* is unknown, our findings clearly implies that the transcription of the *Tr-KPI* gene family is a part of an intrinsic mechanism by which critical cell functions are controlled: if this is disrupted then changes to cellular homeostasis occurs and major stress response changes ensue.

POSTERS

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P42	Apple *MADS8* controls fruit flesh development and ripening	Ireland, H	PFR, Auckland
P43	Functional analysis of *Epichloë festucae* genes encoding proteins with LysM domains in the symbiotic interaction with Lolium perenne.	Nazanin Noorifar	Massey University
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P53	Molecular genetics of resin production in *Pinus radiata*	Wajid Waheed Bhat	Scion, Rotorua
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P56	Putative Xylanase Activity of Pseudomonas syringae pv. actinidiae (Psa)	Rasika Subasinghe	University of Waikato

P28

Identification of promoter elements that determine male germline fate through activation of *DUO1* in eudicots

Peter, B.A., Casey, J., Zohrab, S., Brownfield, L.

Department of Biochemistry, University of Otago, Dunedin, New Zealand

The development of the male germline within pollen relies upon the transcription factor DUO POLLEN1 (DUO1) activating numerous target genes enriched in sperm cells. DUO1 expression is restricted to the male germline and is first detected shortly after the asymmetric division that segregates the germline. Transcriptional regulation appears to be critical in controlling DUO1 expression since transcriptional and translational fusions show similar expression patterns. Here we report on our investigation into the DUO1 promoter region and our identification of key regions that regulate DUO1 transcription. Phylogenetic footprinting in eudicots and in Arabidopsis accessions led to the identification of a small promoter region, Regulator of DUO1 (ROD1), that replicates the DUO1 expression pattern in developing pollen of *Arabidopsis thaliana*. Further, the ROD1 region from the legume *Medicago truncatula* directs male germline-specific expression in *A. thaliana*, showing conservation of DUO1 regulation in the eudicots. The ROD1 region contains several short conserved cis-regulatory elements. Three copies of the motif DNGTGGV are essential for expression and two YAACYGY repeats are required for positive feedback whereby DUO1 enhances its own expression. Thus, expression of DUO1 is controlled by a conserved regulatory domain in eudicots, highlighting the importance of the strict male germline-specific expression of DUO1.

P29

Multiple differentially expressed *TPS1* genes and biochemically active TPS1 proteins may contribute to sugar signalling and regulation of kiwifruit growth and development

Voogd, C.[1], Brian, L.A.[1], Varkonyi-Gasic, E.[1]

[1]The New Zealand Institute for Plant & Food Research Limited, Mt Albert, Auckland, NZ

Trehalose metabolism and its intermediate trehalose-6-phosphate (T6P) are implicated in sensing and signalling sucrose availability. Alteration of the trehalose pathway and modification of T6P content affects growth, stress responses, flowering time, inflorescence architecture, and starch metabolism. We characterized the kiwifruit class I *TREHALOSE-6-PHOSPHATE SYNTHASE* (*TPS*) genes. The kiwifruit genome has four class I *TPS* genes, with homology to *Arabidopsis thaliana TPS1*, three of which have both the TPS and trehalose-6-phosphate phosphatase (TPP) domain, while the fourth gene gives rise to a truncated transcript lacking part of the TPS and the entire TPP domain. The transcript with highest sequence homology to *Arabidopsis TPS1*, designated *TPS1.1a* was the most highly abundant *TPS1* transcript in all examined kiwifruit tissues. An additional exon giving rise to a potential N-terminal extension was found for two of the *TPS1* transcripts, designated *TPS1.2a* and *TPS1.2b*. Expression of full-length and potential splice variants of these two kiwifruit *TPS1.2* transcripts was sufficient to substitute for the lack of functional TPS1 in the yeast *tps1Δ tps2Δ* mutant, but only weak complementation was detected in the less sensitive yeast *tps1Δ* mutant and no or very weak complementation was obtained with the *TPS1.1a* construct. Transgenic *Arabidopsis* lines expressing kiwifruit *TPS1.2* under the control of 35S promoter exhibited growth and morphological defects. We investigated the responses of plants to elevated kiwifruit *TPS1* activity at the transcriptional level, using transient expression of *TPS1.2a* in *Nicotiana benthamiana* leaves, followed by RNA-seq. Differentially expressed genes were identified as candidates for future functional analyses.

P30

'Silver apples'; a novel phenotype created by overexpression of a fruit specific cell wall enzyme in apples.

Gunaseelan Kularajathevan.[1], Ria Rebstock.[1], Ratnasiri Maddumage.[1], Ian C. Hallett.[1], Roswitha Schröder.[1], Robert J Schaffer.[1] and Ross G. Atkinson.[1]

[1] New Zealand Institute of Plant and Food Research, Auckland 1142, New Zealand.

POLYGALACTURONASEs (PGs) are plant cell wall enzymes which cleave pectin chains causing a loss of cell-to-cell adhesion. They have been shown to play important roles in plant growth, abscission and fruit softening. PGs are encoded by large gene families, however in apple, only one gene (*PG1*) is highly expressed during fruit ripening. Suppression of *PG1* (*PG1as*) gave a range of fruit phenotypes including slower softening and reduced water loss[1]. In earlier studies, transgenic apple plants overexpressing *PG1* (*PG1ox*) showed silver coloured leaves and premature leaf shedding[2]. Unfortunately these trees did not produce fruit. Recently 20 new independent apple lines have been produced with *PG1ox* overexpression construct which have moderate to high *PG1* gene expression patterns. The new transgenic lines produced fruit and consistent with the leaf phenotype the apples developed a silvery appearance and lost more water than the untransformed controls. These phenotypes were associated with a loss of adhesion of the epidermal layers. In addition, the apples were softer at fruit maturity compared to untransformed lines and showed little browning when sliced and exposed to air.

References.

[1] Atkinson *et al.* (2012). *Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (Malus x domestica) fruit.* BMC Plant Biology (2012) **12**:129

[2] Atkinson *et al.* (2002). O*verexpression of Polygalacturonase in Transgenic Apple Trees Leads to a Range of Novel Phenotypes Involving Changes in Cell Adhesion.* Plant Physiology (2002) **129** 122-133

P31

An improved *Actinidia chinensis* genome assembly enhances our understanding of the sex determination region in kiwifruit

Pilkington, S.M.[1], Crowhurst, R.N., Hilario, E., Datson, P.M., Chagné, D., Deng, C., Schaffer, R.J. and the Kiwifruit Genome Consortium[2]

[1]Breeding and Genomics, The New Zealand Institute for Plant & Food Research, Auckland, NZ, [2]http://www.actinidia.nz/wiki/index.php/Main_Page

Actinidia chinensis (Kiwifruit) is a dioecious plant. During floral development, pollen and pistil abortion occurs in female and male vines, respectively. Sex is determined by a region at the end of chromosome 25. To identify genes associated with this region, the draft genome of *A. chinensis* 'HongYang' was examined. With nearly 30% of the genome unassigned to chromosomes, and many gene models appearing sub-optimal quality or missing from the published gene set, it was clear that a higher quality genome was needed.

Another *A. chinensis* cultivar Red5, which is 37% homozygous, was selected for a new genome sequencing effort. Paired end sequence combinations were assembled using a pseudo Sanger approach. The resulting 3880 contigs were grouped into chromosomal groups using 25,000 combined mapping markers from the Kiwifruit Genome Consortium and low coverage sequencing of ~12,000 BAC clones. It allocated 230Mb of Red5 scaffolds, which were homologous to unassigned 'HongYang' fragments, to chromosomal locations, leaving only 15Mb of sequence unassigned. The WebApollo annotation resource was used to manually annotate the kiwifruit genome, including the sex chromosome (CHR25). The sex region is located in an area of low recombination with a low density of genes.

This improved version of the kiwifruit genome and high quality manual annotation are pivotal for the understanding of sex determination in kiwifruit. These resources can now be used for accurate mapping, gene identification and transcriptomics studies, which ultimately will allow us to identify and functionally test genes associated with kiwifruit sex determination.

P32

Temperature effects on the accumulation and regulation of anthocyanin production in Pinot noir grape berries

Kirk, R.A.[2], Bennett, J.[1], Espley, R.[2], Plunket, B.[2], Friend, A.[3], Albright, A.[1], Haycock, S.[1], Horner, R.[1] and Bishell, R.[1]

[1] New Zealand Institute of Plant and Food Research, PO Box 845, Blenheim 7201, NZ
[2] New Zealand Institute of Plant and Food Research, Private Bag 92169, Auckland 1142, NZ
[3] New Zealand Institute of Plant and Food Research, 55 Old Mill Road, RD 3, Motueka 7198, NZ

Pinot noir grapes and wines produced in cooler and/or higher altitude areas of New Zealand are said to have more intense colour and structure. Industry observations suggest this is the result of wide diurnal temperature ranges (warm-hot summer days followed by cool nights) leading to increased phenolic (flavonoid) production during berry development and ripening. Three classes of flavonoids are commonly detected in grapes and wine: anthocyanins, flavonols, and proanthocyanins and are largely responsible for the colour, structure and the quality of red wine. While both temperature and light have significant influences on anthocyanin production and grape colour (Spayd *et al.* 2002; Zheng *et al.* 2013), some studies suggest that temperature has a greater direct influence on anthocyanin biosynthesis in grapes than light (Boss & Davies 2009; Mori *et al.* 2007).
To determine the extent of the temperature effect on New Zealand cool climate Pinot noir grapes individual bunches were heated for 7 days to a temperature range of 22-40 °C, while unheated control bunches experienced an ambient temperature range of 11-31 °C. Berry sampling over the first two days (24 hour cycles) took place to study the natural diurnal cycles of berry physiology and gene expression activity. The elevated temperature range and doubling of accumulated heat (GDD) during treatment resulted in array of short to long term changes in berry pulp and skin chemistry, including a 25% reduction on skin anthocyanin concentration. Gene expression results suggested that parts of anthocyanin synthesis pathway were heat sensitive and may have been the mechanism(s) by which anthocyanin synthesis based accumulation was reduced.

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P33

The *Constans-like* gene family is involved in the environmental regulation of anthocyanin development in apple peel

Plunkett, B.[1], Lin-Wang, K.[1], Kirk, R.[1], Friend, A.[1], Wicket, S.[1], Mouhu, K.[2], Espley, R.[1], Putterill, J.[3], Allan, A.[1]

[1]New Zealand Institute of Plant and Food Research, Private Bag 92169, Auckland 1142, NZ, [2]Faculty of Agriculture and Forestry, University of Helsinki, Finland, [3] School of Biological Sciences, University of Auckland, Private Bag 92019, NZ

The development and regulation of colour in apple has received considerable research attention and the metabolic pathway as well as major controlling transcription factors have been identified (Espley et al., 2007; Allan et al., 2008). It has been established that light related genes affect apple fruit colour via activation by MYB transcription factors (Takos et al., 2006; Peng et al., 2013; Bai et al., 2014). It is not yet known how many environmentally regulated genes are involved in tailoring apple colour response to varying light conditions. We identified the apple COL gene family using homology to known COL genes from *Arabidopsis*. We used qPCR to assess the transcription of the COLs and other light related genes both in a diurnal series and in a fruit development series. Strong diurnal rhythms for COLs were observed in apple peel. Transient assays were used to assess the ability of COLs to activate the promoter of the anthocyanin regulating transcription factor MYB10. Changes to gene expression patterns, in response to light by bagging fruit during development, was also examined using differential gene expression analysis. As varying light levels changed the temperature, the effect of increased temperature on the diurnal expression pattern of MYB10 was also assessed. The data generated from these experiments allowed us to identify strong activators of the MYB10 promoter. These activators also displayed diurnal expression rhythms and were transcriptionally present in the fruit throughout development. Coupled with fruit bagging data, these results suggest that COL genes are likely to be working in concert to provide a link between environmental light and temperature cues and the corresponding change to the colour of apple peel.

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P34
Redesigning softwood lignin – a 'get hard' approach

Phillips, L.[1], Flint, H.[1], Wagner, A.[2], Ralph, J.[3, 4], Thorlby, G.[1]

[1]Biotransformation, Scion, Rotorua, NZ, [2]Formerly: Biotransformation, Scion, Rotorua, NZ, [3]Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA, [4]US Department of Energy's Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, Madison, WI 53726, USA

Conifer (softwood) forests are of major economic importance worldwide, providing a vast resource for timber, wood-based composites, pulp fibre, extractives and solid biofuel. Within New Zealand they represent 98% of the 1.72 million hectare planted forest estate, which in total accounted for $4.8 billion of New Zealand's annual export earnings in 2014 (3% of the NZ GDP).

Softwoods are integral to the wood processing industries. The length and strength of their fibres make them an essential component in the manufacture of high performance paper, containerboard and fibre-composite products. Another advantage is their hexose rich hemicellulose composition which make them a potentially superior source of fermentable sugars for the manufacture of industrial biorefinery products.

A disadvantage of softwoods is their highly condensed guaiacyl (G) lignin composition, which make them more complicated and expensive to extract during the pulping and saccharification processes than the 'more digestible' syringyl (S)/guaiacyl (G) based hardwoods.

Research at Scion aims to use genetic modification to alter the lignin composition of radiata pine and produce improved lignocellulosic feedstocks. Using a transformable pine tracheary element (TE) system we have been able to functionally characterise many of the genes involved in the biosynthesis of softwood lignin. We have also introduced key genes involved in the synthesis of S-monomers and successfully demonstrated their production and incorporation into the lignin polymer. Our results show that it is possible to produce a 'hardwood-like' lignin polymer in conifers.

The ability to improve the processing and saccharification properties of conifer-derived biomass through the manipulation of lignin composition would lead to significant economic and environmental benefits for the global forest and wood processing industries.

P35
Chasing ryegrass fescue hybrids

Arnet, M.J.[1], Jameson, P.E.[1], Eady, C. [2]

[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ, [2]New Zealand Agriseeds Ltd.

Within plant breeding allopolyploids have proved to be important tools in commercial cultivar development e.g. *Triticale* and *Brassica* species. Potentially an interspecific hybrid between tall fescue and ryegrass could be such a useful allopolyploid, but to date those created have not proved to be very useful. In part, this may be due to the frequency with which they are produced and the speed with which they can be analysed. A method similar to Kindiger (2012)[1], whereby *Lolium perenne* and *Lolium multiflorum* were crossed with *Festuca arundinacea* has been used to develop an allopolyploid. The resulting F_1 hybrids are essentially sterile but occasionally produced seed of various ploidy levels. Ploidy of the F_2 hybrids was tested with flow cytometry using parental tall fescue and ryegrass as controls. A range of ploidy level offspring have been produced (with 7 = n) and these will be used in conjunction with FISH staining to develop a rapid q-PCR technique to determine degrees of 'hybridness'. Initial data indicates that some of the F_2 hybrids were diploid and suspected doubled haploids. Analysis using seven simple sequence repeats (SSRs), one for each *Lolium* chromosome, gave a mean heterozygosity of the 12 plants assayed of 0.5 to 0.83 indicating that the plants were not homozygous and therefore not doubled haploids.

1. Kindiger, B. (2012). *Sampling the genetic diversity of tall fescue and ultilizing gamete selection*. In Mahmut Çalişkan (Eds.) *Genetic diversity in plants* (pp. 271-284). INTECH Open Access Publishers.

P36

Investigating the role of *FLOWERING LOCUS T (FTs)* in mast flowering

Lehri, S.U.[1], Lee, R.[2], Song, J.[1], Poole, A.M.[1], Kelly, D.[1], Turnbull, H.M.[1], Macknight, R.[2], Jameson, P.E.[1]

[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ, [2]Department of Biochemistry, University of Otago, Dunedin, NZ.

Floral initiation is a major physiological change that sets the switch to initiate reproductive development in plants species. The flowering plasticity of perennial plants in response to environmental cues (e.g. seasonal temperatures, winters and hot summers) can occur at multi-year timescales that are too long to respond via known physiological processes such as photoperiodic flowering in annual plants. For example, flowering cycles vary among years in response to environmental cues, most dramatically in perennial 'mast-seeding' plants-snow-tussock. Mast seeding is the synchronous production of seeds among years in a population of perennial plants. However, there is no known mechanism for how plants could 'measure and remember' the difference between current and previous year temperatures to produce synchronous flowering in masting years. To maximise reproductive success, orchestrated changes in multiple flowering genes (e.g. floral timing genes) occur in response to environmental cues. To be specific, these floral integrators perceive signals from light, cold temperature and ambient temperature and subsequently induce the expression of *FLOWERING LOCUS T* (*FT*). *FT* encodes the floral signaling hormone, florigen. This proposed research is set to investigate the hypothesis that epigenetic modifications to *FT* and *FLC-like* genes in response to ambient temperature are critical to the multi-year masting response in snow-tussock (*Chionochloa sp.)*. Preliminary deep RNA-seq data analysis has identified candidate *FT* and *TFL1* genes *de novo*. Characterising putative PEBP family members in snow-tussock may be a start towards understanding the molecular mechanisms involved in mast flowering.

P37

Conformational changes of DAD2 are an important mechanism in the signal reception of strigolactone

Lee, H.W.[1,2], Newcomb, R.D.[1,2], Snowden, K.C.[1]

[1]New Zealand Institute for Plant and Food Research, Auckland, New Zealand, [2]School of Biological Sciences, University of Auckland, Auckland, New Zealand.

Strigolactones (SLs) are a class of plant hormones that regulate developmental growth through the modulation of shoot branching, root development, stem secondary growth, leaf senescence and flower development. DAD2, the petunia receptor for SLs, is an α/β-hydrolase fold protein with a canonical catalytic triad in the cavity active site. DAD2 hydrolyses SLs, undergoes a conformational change and interacts with its signal transduction partner PhMAX2A, a central component of SL signalling to inhibit branching in plants. There is little understanding on the exact role of DAD2 in this mechanism and how this mechanism leads to changes in axillary branching.

Therefore, a mutagenesis approach was used to uncouple the hydrolytic activity of DAD2 from its ability to interact with PhMAX2A and to determine the role of DAD2 conformational change and enzyme activity in SL signal transduction. Random mutagenesis was used to identify DAD2 mutants that are able to interact with PhMAX2A in the absence of SL. Site-specific mutations were generated for various conserved amino acid residues predicted to be important for conformational shifts and/or interaction with PhMAX2A, and/or catalysis. A core hydrolase fold surface mutant of DAD2 showed increased interaction with PhMAX2A regardless of the presence or absence of the hormone ligand SL. This mutant can still interact and hydrolyses SL. Subsequent knockout of the catalytic residue of this core surface mutant abolished its enzyme activity but did not completely diminish its interaction with PhMAX2A. It is likely that the core surface mutant of DAD2 has attained a certain conformation to interact with PhMAX2A. Thus, it is hypothesized that the conformational changes of DAD2 are crucial for interaction with PhMAX2A, which is possibly induced by the binding and/or hydrolysis of SL.

P38

To life on land: How plants developed UV sunscreens

Clayton W.[1], Jordan B.[1], Davies K.[2], Albert N.[2], Deroles S.[2] and Schwinn K.[2]

[1]Lincoln University, Lincoln, New Zealand. [2]Plant and Food Research, Palmerston North, New Zealand.

Terrestrial plant life evolved from an aquatic ancestor 400-600 million years ago. This significant step from water to land involved a suite of genetic adaptations allowing for colonisation of new environments. One such adaption was for tolerance of incident UV-B radiation. UV-B radiation is particularly damaging to plant life because of its absorption by molecules such as DNA and the production of reactive oxygen species. Therefore the development of UV-B tolerance by early land plants would have been particularly important. Flavonoid synthesis contributes significantly to UV-B tolerance in higher plants, yet it is not known whether the acquisition of flavonoid production allowed the first land plants to tolerate UV-B and colonise the land environment. Here we examine the model bryophyte *Marchantia polymorpha*, from one of the oldest extant land plant lineages, to determine the effect of flavonoid production in response to UV-B. Under set environmental conditions and UV-B irradiance *M. polymorpha* is shown to respond to UV-B with an increased production of flavonoid compounds (predominantly flavones). We show through RNA-seq and qPCR this induction corresponds to gene activation of the specific flavonoid pathway, as well as non-specific stress pathways, in order to ameliorate the effects of incident UV-B. These results may lead into an understanding of the importance of flavonoid production in enabling the successful colonisation of land by plant life.

P39

Small RNA and epigenetic silencing of transposable elements in grapevine embryogenic cell cultures

Lizamore, D.K.[1], Winefield, C.S.[1]

[1]Department of Wine, Food and Molecular Biosciences, Lincoln University, Christchurch, NZ, [2]Plant and Food Research Ltd., Lincoln, Christchurch, NZ

Transposable elements (TEs) are a ubiquitous mutagenic force within the genomes of eukaryotes. Their mobilisation can rearrange gene exon sequences and regulatory motifs, leading to altered phenotypes. To prevent this potentially deleterious activity, transposon activity is usually highly repressed, particularly in the gametes, by localised epigenetic modification of the DNA. Grapevine is a unique and interesting model in which to study the activity of endogenous TEs since it has been vegetatively propagated for thousands of years. As a result, an accumulation of somatic mutations defines the genotypes of elite clones currently farmed.

In order to study the transposition rates, insertion bias an impacts of endogenous TEs in grapevine, we have been regenerating vines from embryogenic callus cells. We have found that through this process, transposon activity is increased, allowing the recovery of a population of new clonal diversity. To characterise the biology of this process, we have completed the first whole-methylome analysis of grapevine tissue. Our results show that much of the conserved epigenetic silencing of TEs seen in vegetative cells is lost in totipotent embryogenic callus cultures, particularly for those TEs that lie within genes. Using massively parallel sequencing of small RNAs, which guide the enzyme complexes that methylate DNA, we observed an increased targeting of TEs for repression in embryogenic callus. This was accompanied by an abundance of new methylation around transposable elements independent of their context, indicative of *trans*-silencing.

Although the consequences of historical TE activity is well-studied, the data presented here reveal the real-time process by which dormant transposons become active, create new mutations, and are once again silenced by their host cells in a wild-type genome. This provides insight into the process of somatic mutation and consequent clonal diversification.

P40

A case of mistaken identity - fine endophyte (*Glomus tenue*) are phylogenetically aligned with the Mucoromycotina, not the Glomeromycota.

Orchard, S.[1], Hilton, S.[2], Bending, G.D.[2], Dickie, I.A. [3], Standish, R.J.[4], Gleeson, D.[5], Jeffery, R.P.[1], Powell, J.R.[6], Walker, C.[1,7], Bass, D.[8], Monk, J.[3], Simonin, A.[6], Ryan, M.H.[1]

[1]School of Plant Biology and Institute of Agriculture, The University of Western Australia, Perth, Western Australia, [2]School of Life Sciences, University of Warwick, Coventry, UK, [3]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [4]School of Veterinary & Life Sciences, Murdoch University, Murdoch, Western Australia, [5]Soil Biology & Molecular Ecology Group, School of Earth and Environment, The University of Western Australia, Perth, Western Australia, [6]Hawkesbury Institute for the Environment, Western Sydney University, Australia, [7]Royal Botanic Garden, Edinburgh, UK, [8]Life Sciences, Natural History Museum, London, UK.

Fine endophytes (FE) are morphologically distinct mycorrhizal fungi observed in stained plant roots. While FE may colonise numerous plant hosts with a global distribution, they are prolific within New Zealand, especially in pastures colonising the roots of grasses and clovers. The presence of arbuscules led to FE being classified as *Glomus tenue*, an arbuscular mycorrhizal (AM) fungus, in the phylum Glomeromycota. However, support for this classification was weak and lacking molecular evidence.

We designed a novel method to maximise FE colonisation within roots of *Trifolium subterraneum* by sieving and dilution of a field soil to remove other AM fungi (Enrichment experiment). Roots were visually assessed for FE and AM fungal colonisation, and community composition was determined by 18S rRNA gene sequencing. Roots from another experiment containing mixed AM fungal species, including FE, were also examined (Contrast experiment).

The percentage of the colonised root length which comprised FE was 90.2 ±2.5 and 28.9 ±6.3 in the Enrichment and Contrast experiments, respectively; other AM fungi were <10% in the former and 41.8 ±8.9% in the latter. The percentage of sequences that matched Mucoromycotina was 88.4 ±1.8% and 24.3 ±4.7% in the Enrichment and Contrast experiments, respectively. The percentage of Mucoromycotina sequences in the Enrichment experiment was correlated to FE colonisation (R^2=0.91).

Our results demonstrate that FE are not glomeromycotan fungi, but belong in the sub-phylum Mucoromycotina. These results are significant as they demonstrate that arbuscules are produced by fungi belonging to the Mucoromycotina and is the first report of arbuscules outside the Glomeromycota. Further, molecular studies of AM fungal communities using primers which target glomeromycotan fungi are overlooking a significant component of the mycorrhizal community.

P41

Investigating microRNA 156 and 172 in temperature-mediated episodic mast flowering in *Celmisia lyalli*

Samarth[1], Kelly, D. [1], Turnbull, M.H. [1], Song, J.[1], Poole, A. [1], Macknight, R. [2], Jameson, P.E. [1]
[1] School of Biological Sciences, University of Canterbury, Christchurch, NZ, [2] Department of Biochemistry, University of Otago, Dunedin, NZ

Masting is a phenomenon of synchronous flowering by populations of plants. These have been recorded in several New Zealand alpine plants and are characterized by high flowering years known as mast years. Various hypotheses have been put forward as a possible mechanism(s) controlling masting, the most recent being temperature (a weather cue). The weather cue hypothesis predicts mast years by calculating the temperature difference (δT) between the two previous summers. However, the molecular basis of this phenomenon is not understood. Plants somehow are able to sense this difference in temperatures leading to the periodic mast flowering events. The current study deals with the identification of a possible pathway controlling the temperature-mediated masting syndrome in plants. Numerous studies have already established the role of microRNA 156 and 172 as key regulators of flowering time in response to ambient temperatures in plants. Initially, in this study, expression analysis using RT-qPCR is being performed to assess the expression levels of microRNA 156 and 172 using stem-loop specific designed primers. Expression profiling of micro RNAs in conjunction with floral integrator-like genes including *FT, FLOWERING LOCUS C, CONSTANTS, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*, *SVP* and *TRIOSE PHOSPHATE SYNTHASE 1*, may reveal information about the complex molecular operation of flowering in *C. lyalli*.

P42
Apple *MADS8* controls fruit flesh development and ripening

Hoong, L.[1,2], Ireland, H.[1], Tomes, S.[1], Gerneke, D.[3], Sutherland, P.[1], Gunaseelan, K.[1], Yao, J-L.[1], David, K.M.[2], Schaffer, R.J.[1,2].

[1]New Zealand Institute of Plant & Food Research Ltd, Auckland, NZ, [2]School of Biological Sciences, University of Auckland, Auckland, NZ, [3]Auckland Bioengineering Institute, University of Auckland, Auckland, NZ.

Suppression of apple *SEPALLATA1/2*-like genes (*MdMADS8/9*) uncovered their role in the control of fruit flesh development and ripening[1]. Apples were fleshless, and during ripening had low ethylene production and little ripening-related starch clearance, colour change, and aroma production. Building on our understanding of these key genes, overexpression of *MADS8* (*MADS8ox*) was investigated. Ectopic expression in independent *MADS8ox* lines led to modifications in tree architecture due to elongated internodes, acropetal stem thinning, reduction in lateral branching, and high incidence of vegetative to floral meristem transitions. Physiological measurements of fruits from *MADS8ox* lines identified both an increase in fruit flesh area as well as enhanced ripening. Specifically, during fruit ripening there was a more rapid rate of background skin colour change, a tendency towards earlier starch clearance, and accelerated softening. Interestingly, while ethylene biosynthesis genes exhibited higher expression in *MADS8ox* lines compared to WT, overall ethylene production was not enhanced. This research helps to designate specific roles for the apple *SEPALLATA* clade member, *MADS8*, in both fruit flesh development and ripening control, and more generally the function of *SEP*-like genes in plants.

1. Ireland, H.S., et al., *Apple SEPALLATA1/2-like genes control fruit flesh development and ripening.* Plant Journal, 2013. **73**(6): p. 1044-1056.

P43

Functional analysis of *Epichloë festucae* genes encoding proteins with LysM domains in the symbiotic interaction with Lolium perenne.

Nazanin Noorifar, Matthew S. Savoian, Barry Scott

Institute of Fundamental Science, Massey University, Palmerston North, Manawatu,
New Zealand.

Epichloë festucae is a filamentous fungus, which forms symbiotic associations with aerial tissues of *Lolium* and *Festuca* grass species. Scanning confocal microscopic analysis of epiphyllous hyphae labelled with the chitin probe WGA conjugated with Alexa Fluor 488 are characterised by robust and homogeneous cell wall and septa staining. Conversely, only septa are labelled on fungi found within leaves. This suggests that hyphal cell wall chitin is masked or remodeled in endophytic hyphae. The aim of this project is to test whether *E. festucae* LysM-containing proteins have a role in masking chitin and thereby preventing PAMP (pathogen-associated molecular pattern)-triggered immunity as demonstrated in *Cladosporium fulvum*-tomato pathogen interactions.
Analysis of the *E. festucae* genome identified seven genes encoding LysM domain containing proteins. Two of these, *lymA* and *lymB*, were down-regulated in the fungal transcriptome of three different *E. festucae* symbiosis mutants suggesting they are functionally important in host-fungal interactions. Interestingly, both are divergently transcribed from chitinase encoding genes, which are themselves down regulated in the symbiosis mutants. To functionally analyse the role of these genes in symbiosis, we have generated Δ*lymA*, Δ*lymB,* Δ*chiA*, Δ*chiB* single deletion mutants and a Δ*lymA*/B double deletion mutant. Plants infected with single Δ*lymA*, Δ*lymB* and Δ*chiA* fungal mutants have the same plant-interaction phenotypes as wild-type controls. Confocal and transmission electron microscopy analyses of leaf tissues indicate the cell wall staining and morphology remain unaltered. Examination of the Δ*chiB* and Δ*lymA*/*B* mutants is currently in progress. Future work will employ eGFP-derived biosensors towards characterising the compositional and structural differences of E. *festucae* endophytic and epiphytic hyphae.

P44

Functional analysis of *Epichloë festucae* small secreted proteins in the symbiotic interaction with *Lolium perenne*

Hassing, B.[1], Mesarich, C.H.[2], Eaton, C. J.[1], Scott, B.[1]

[1]Institute of Fundamental Sciences, Massey University, Palmerston North, NZ, [2]Institute of Agriculture and Environment, Massey University, Palmerston North, NZ.

Epichloë endophytes form stable and mainly mutually beneficial relationships with a wide range of cool-season grasses. In a transcriptome analysis, 14 genes encoding small secreted proteins (SspB–SspO) were identified as differentially expressed in four different symbiosis-deficient strains of *E. festucae* during *in planta* colonization of *Lolium perenne*[1]. Many Ssps, also called effectors, have been demonstrated to interfere with the host defence response in pathogenic and symbiotic interactions. All 14 putative effectors were analysed with bioinformatic tools, and from these, four, specifically SspL, SspM, SspN and SspO, were chosen for further functional analysis. Their secretion was verified using a secretion signal-trap assay[2] and Western analysis. The deletion of *sspL*, *sspM*, *sspN* and *sspO* did not result in an obvious whole-plant interaction phenotype distinguishing WT- and deletion mutant-infected plants, except for a slight reduction in anthocyanin levels. Furthermore, the analysis of infected plant tissue with confocal microscopy and TEM did not identify any obvious differences in cellular phenotype. In future experiments, the effect of Ssp overexpression on whole-plant interaction and cellular phenotypes will be explored. This will be combined with Ssp localization studies to gain further information about the function of these proteins *in planta*. To this end *ssp*-mCherry translational fusion-expressing strains have been generated and will be inoculated into *L. perenne* seedlings.

[1]Eaton CJ., Dupont P.Y., Solomon P., Clayton W., Scott B., Cox M.P. (2015). *A core gene set describes the molecular basis of mutualism and antagonism in* Epichloë spp. Mol. Plant Microbe Interact. 28:69-85.
[2]Jacobs, K.A., Collins-Racie, L.A., Colbert, M., Duckett, M., Golden-Fleet, M., Kelleher, K., Kriz, R., LaVallie, E.R., Merberg, D., Spaulding, V. (1997). *A genetic selection for isolating cDNAs encoding secreted proteins*. Gene. 198: 289-296.

P45

Photosynthetic microbial fuel cells : algae and cyanobacteria in bioelectrochemical devices, why, how and what?

Veerle Luimstra, Sophie Jean Kennedy, Johanna Güttler, Josie Völker, Julie Labro, Timothy Craig, Thibaud le Guen, Susanna A Wood, Jonathan Puddick, Michael A Packer

[1]Cawthron Institute, Private Bag 2, Nelson 7010, New Zealand

Microbial fuel cells (MFC's) are bioelectrochemical systems (BES's) that exploit biological catalytic processes for the generation of electrical power or the accumulation of useful chemicals (such as hydrogen gas or hydrogen peroxide). This normally occurs by electrons being released from organic substrates through oxidation via cellular electron transfer pathways. Liberated electrons then pass through an external circuit as electric current and combine to with cathodic electron acceptors through electrocatalytic or biocatalytic reductions. These BES's can be 'mediated', using mobile electron shuttles as redox mediators, or 'mediator-less', where electrons are transferred directly from the microorganism to the electrode. This is referred to as direct electron transfer (DET) where electrons may be delivered by electron transport enzymes or, modified pilus-like structures ('nanowires') directly to the anode. Photosynthetic MFC's (pMFC's) are those that utilise photosynthetic microorganisms such as algae and cyanobacteria to provide reducing power at the anode. Here, a reproducible light-dependent electrogenic effect occurs as the algae and cyanobacteria convert light energy to electrical in the BES.

Investigating how and why some microorganisms donate electrons to their environment gives insights on how to better utilise this effect, for instance, to improve performance of MFC. In addition to the generation of electricity, the phenomenon may be useful in niche circumstances such as for bioelectrosynthesis or for use in environmental biosensors.

P46

Effects of Gibberellin and Nitrate Treatment on Nitrogen Uptake Efficiency, Regrowth and Root System Architecture

Brennan, M.P.[1], Turnbull, M.H.[1], Love J.[1], Guo, Q.[1], Roche, J.[1], Jameson, P.E[.1]
[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ.

The timing of the application of fertiliser to pasture post defoliation is critical to regrowth in *Lolium perenne*. After defoliation, *L. perenne* has reduced carbohydrate stores and limited photosynthetic capacity as well as an increased demand for carbohydrate resources for regrowth. The application of fertiliser further magnifies this demand. We show that the shift from carbon sink to carbon source happens between days 3 and 7 post defoliation.

A deep pot system is being used to replicate field conditions to determine the optimal time to apply fertiliser post defoliation to achieve the most efficient nitrogen uptake and regrowth. Gibberellic acid is being applied to pastures to stimulate leaf growth. There is some evidence that application of GA_3 could exacerbate the carbohydrate imbalance in future rotations, and that the effects are dependent on the season. We are investigating whether the timing of fertiliser and GA_3 application post-defoliation affects the shoot/root ratio during regrowth. The nitrogen and GA_3 applications were spread over 28 days post defoliation. We hypothesise that GA_3 application will cause a shift of carbon storage from roots to shoots explaining the difference in the effect of GA_3 application between seasons and over multiple rotations.

In vitro experiments are also being used to examine root system architecture whilst plants are exposed to high and low concentrations of organic N (arginine) and nitrate, as well as a concentration gradient of GA_3. Results indicate that 5 mM arginine was inhibitory to root growth compared with 5 mM nitrate. There was little or no effect of GA_3 when applied with 5 mM nitrate.

P47

Understanding the protein complexes involved in the reception of plant hormone strigolactone

Sharma, P.[1, 2], Newcomb, R.D.[1, 2], Hamiaux, C.[2] Snowden, K.C.[2]
[1] School of Biological Sciences, University of Auckland, Auckland, NZ, [2] The New Zealand Institute for Plant and Food Research Ltd, Auckland, NZ.

Strigolactones (SLs) are a recently discovered class of plant hormones that regulate axillary shoot branching. In the current model of SL signalling in petunia, two proteins, an α/β hydrolase, DAD2 and a member of the SCF complex, an F-box protein, PhMAX2A, have been associated with the initial reception of the hormone signal. Based on the current literature, the SL signalling pathway is thought to begin once the SL molecule is perceived by DAD2. DAD2 hydrolyses the SL molecule and undergoes a conformational change that likely favours its interaction with PhMAX2A, possibly leading to the formation of a complex consisting of the hydrolysed SL, DAD2 and the SCF complex. This complex then associates with target proteins that are likely recruited to the complex by DAD2. The SCF complex then polyubiquitinates the target proteins which are then degraded by the 26S proteasome. Although the sequence of events is well understood, it still remains unclear how SL facilitates the interaction between PhMAX2A and DAD2 as there is no structural evidence to show that SL is bound to DAD2 when it interacts with the F-box protein. Further the structure of PhMAX2A is yet to be determined and there is still no structural evidence to support the existence of a SL-DAD2-SCF complex. Therefore, the current study aims to use a structural approach to determine the structure of PhMAX2A and the SL-DAD2-PhMAX2A complex. This will help to increase our understanding about the SL signalling pathway and to identify how the various proteins involved in the pathway interact with each other to initiate the signal transduction for SLs. Progress towards fulfilment of these goals will be presented.

P48
An Electronic Fluorescent Pictograph Browser for Kiwifruit (*Actinidia spp.)*

Brian, L.A.[1], Warren, B.A.W. [1], McAtee, P. [1], Richardson, A. [2], Kashuba, P. [2], Corbett, T. [3], Schaffer, R. [1], Varkonyi-Gasic, E. [1]

[1] The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research) Mt Albert, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand
[2] The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research) Kerikeri, 121 Keri Downs Road, RD1, Kerikeri 0294, New Zealand
[3] The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research) Palmerston North, Private Bag 11600, Palmerston North 4442, New Zealand

The rise of high-throughput sequencing technologies such as RNA-seq has revolutionised our ability to investigate and understand gene function, providing insight into the spatial, temporal, and level of expression of a particular gene of interest. However, these large scale datasets are often difficult to visualise and easily interrogate with a gene of interest and tools such as heatmaps do not intuitively render biological context. The electronic Fluorescent Pictograph (eFP) browser[1] allows the collation of many large datasets that can be easily and quickly examined with pictographs illustrating different tissues, developmental stages and treatments. The eFP browser has been developed as a resource for many model species such as *Arabidopsis,* tomato, rice, maize, barley, poplar, *Medicago* and soybean. To improve the understanding of the woody perennial vine, kiwifruit (*Actinidia spp.*) and increase the accessibility and re-usability of RNA-seq datasets, we have developed the kiwifruit eFP browser. In total, 26 *Actinidia chinensis* 'Hort16A' tissue samples derived from two RNA-seq datasets representing vegetative, developmental and postharvest conditions are available for users to visualise and query with their gene of interest. The kiwifruit eFP browser can be adapted to include new graphical representations and datasets.

1. Winter, D., Vinegar, B., Nahal, D., Ammar, R., Wilson, G.V., & Provart, N.J (2007) *An "Electronic Fluorescent Pictograph" Browser for Exploring and Analyzing Large-Scale Biological Data Sets*. PLoS ONE 2(8): e718. doi: 10.1371/journal.pone.0000718

P49

Differential gene expression in apple cells layers during fruit development and ripening

Collins, P.[1,2], Hallett, I.[2], Schaffer, R.J.[1,2], Brummell, D.[3]

[1]School of Biological Sciences, The University of Auckland, Auckland, NZ, [2]The New Zealand Institute for Plant & Food Research Limited, Mount Albert Research Centre, Auckland, NZ, [3]The New Zealand Institute for Plant & Food Research Limited, Food Industry Science Centre, Palmerston North, NZ

The fruit skin is a key component in many aspects of fruit development and quality. It is the first line of defence against pathogens, controls water loss, contains pigments and phytochemicals, and controls fruit growth through its ability to grow and stretch. *Malus domestica* (apple) skin has two distinct cell layers, the epidermis and hypodermis which cover the cortex cells (flesh). During fruit ripening the skin becomes more porous to water as the apple softens, and this porosity has been linked in part to activity of the cell wall hydrolase enzyme encoded by the *POLYGALACTURONASE 1 (PG1)* gene. Due to the complex nature of the skin and the difficulty of studying individual layers of cells that comprise it, how the skin changes during development and ripening is poorly understood. With advances in laser microdissection technology and the increased sensitivity in molecular analysis it is now possible to investigate this in more detail.

Malus x domestica fruit from wild type and transgenic PG1 antisense and over expression lines in cultivar 'Royal Gala' were sampled over five developmental stages of growth and ripening. Laser capture microdissection methodology was developed for isolating the two specific cell-types of the skin: hypodermis and epidermis, along with the cortex cells underneath the skin. This methodology was optimized to retain mRNA quality in each specific cell-type. RNA-seq will be used to determine the expression of genes involved in cell wall synthesis and modification during fruit expansion, and identify changes in the skin in the presence and absence of the cell wall enzyme PG1. Uncovering the tissue-specific regulation of cell wall related enzymes involved in fruit expansion and quality after harvest can enhance our understanding of the mechanisms that govern fruit quality.

P50

Selenium treatment differentially affects sulfur metabolism in high and low glucosinolate producing broccoli cultivars

McKenzie, M.J.[1], Chen, R.K-Y.[1], Leung S.[2], Joshi, S.[2,3], Rippon, P.E.[4], Joyce, N.I.[4] McManus, M.T.[2]

[1] The New Zealand Institute for Plant & Food Research Limited, Palmerston North, NZ, [2] Institute of Fundamental Sciences, Massey University, Palmerston North, NZ, [3] Present address; Department of Life Sciences, Korea University, Seoul, Korea, [4] The New Zealand Institute for Plant & Food Research Limited, Lincoln, NZ.

Broccoli (*Brassica oleracea* L.) is a selenium (Se) accumulating plant that produces both Se- and S-containing compounds (e.g. methylselenocysteine and the glucosinolates), which have demonstrated health properties in humans. As Se and S are chemically similar elements, most S metabolic enzymes do not distinguish between the two, meaning the metabolism of both elements is closely entwined in plants.

With a view to producing broccoli with increased content of health beneficial Se- and S-compounds we investigated the effect of Se application on Se, S and glucosinolate content in two cultivars that differed in glucosinolate and methylselenocysteine content. Both cultivars were fertilised for 4 weeks during head development with increasing rates of sodium selenate. Increased Se fertilisation resulted in an increase in Se uptake *in planta*, but no change in total S or glucosinolate content in either cultivar. Furthermore, no significant change was observed in the activity of the S metabolic enzymes ATP sulfurylase (ATPS) or O-acetylserine(thiol) lyase (OASTL). However, in the first investigation of APS kinase (APSK) expression in response to Se fertilisation, an increase in transcript abundance of one variant of APS Kinase 1 (*BoAPSK1A*) was observed in both cultivars, and *BoAPSK2* in one cultivar.

Based on these results, we propose a mechanism by which increased APSK mRNA abundance provides a means of maintaining the content of S-containing compounds, including glucosinolates, following Se uptake by the plant.

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The regulation of ascorbate (vitamin C) levels in plants

Chisnall, M.A.W.[1,a], Ritchie, E.S.[1,a], Laing, W.I.[2], Hellens, R.P.[1,3], Macknight, R.C.[1,2]

[a]Joint first authors, [1]Department of Biochemistry, University of Otago, Dunedin, NZ, [2]The New Zealand Institute for Plant and Food Research, Auckland, NZ [3]Science and Engineering Faculty, Queensland University of Technology, Brisbane, Australia

Ascorbate (vitamin C) is an essential vitamin that humans are unable to synthesise. In higher plants, ascorbate is synthesised via the Smirnoff-Wheeler pathway. The enzyme catalysing the rate-limiting step is GDP-$_L$-galactose phosphorylase (*GGP*)[1]. Within the 5' UTR of *GGP* is an upstream open reading frame (uORF), which is highly conserved across plant species and starts with an ACG (rather than AUG) codon. The uORF regulates *GGP* translation in response to cellular ascorbate levels via a negative feedback mechanism[1], possibly through ribosomal stalling. We are investigating the uORF mediated feedback mechanism and how a few plants species have very high ascorbate levels in their fruit.

The translational regulation of the *GGP* gene is being investigated using dual luciferase transient assays. uORF translation initiation from its ACG start codon was found to occur with low efficiency and was not affected by ascorbate levels. Removal of stop codons between the uORF and *GGP* did not affect translation from the downstream *GGP* start codon. These results suggest that ribosomes mostly fail to initiate at the uORF and likely scan past to initiate *GGP* translation. However in the presence of high ascorbate, we hypothesise that the when translation of the uORF does occur, the uORF encoded peptide binds ascorbate, causes ribosome stalling and thereby preventing other ribosomes scanning past and translating the *GGP* coding region.

To discovery why some plants have high ascorbate, transcriptomes have been generated for high ascorbate fruits. Significant expression differences in ascorbate synthesis genes were observed. The uORFs of these plants have been isolated, tested, and show feedback is still occurring but potentially in a relaxed manner. We hypothesise that increased transcription, in combination with subtle sequence changes, contributes to increased ascorbate levels. Furthering our knowledge of *GGP* regulation, should provide new ways to increased ascorbate levels in crop plants.

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P52
Wanted: Dead or Alive. A study of exocarp formation in kiwifruit

Macnee, N.C.[1,2], Hallett, I.C[1], Schaffer, R.J.[1,2],Bulley, S.M[3]

[1]The New Zealand Institute for Plant & Food Research Limited (PFR), Mt Albert, Auckland, NZ. [2]School of Biological Sciences, The University of Auckland, Auckland, NZ. [3]PFR, TePuke Research Centre, Te Puke, NZ

The berry fruit of *Actinidia species* (kiwifruit) is covered by an exocarp or 'skin'. There are two distinct exocarp phenotypes found within *Actinidia* those that have epidermal exocarp 'live skin' and those that have a peridermal exocarp 'dead skin'. The morphological development of the two exocarp types have been previously reported[1], however little is known about the molecular factors involved in exocarp development. This project aims to characterise the exocarp phenotypes at the genetic and transcriptomic level. An RNA sequencing study is being undertaken to identify patterns of gene expression involved in periderm formation by comparing kiwifruit with an epidermal exocarp to those with a peridermal exocarp aligned to exocarp developmental changes. Gene changes will be compared to other plants that produce periderms such as potato tuber skin and russet pipfruit. In order to identify the control points of exocarp formation a genotyping by sequencing (GBS) study on a population that segregates for exocarp type is planned. Prelimary results suggest periderm formation in kiwifruit is a natural component of development with a cork meristem forming beneath the epidermis. This meristem produces thin walled cells that are suberized and compressed cells as the fruit matures. The difference between periderms formed by wounding or development will be further characterised in kiwifruit, with analysis of intermediate phenotyped russeted fruit found in the segregating population.

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Molecular genetics of resin production in *Pinus radiata*

Wajid Waheed Bhat*, Elspeth MacRae, Glenn James Thorlby*
Scion, 49 Sala Street, Rotorua 3010, Private Bag 3020, New Zealand
*wajid.waheed@scionresearch.com, *Glenn.thorlby@scionresearch.com

Pinus radiata D. Don is New Zealand's premier tree species covering 1.6 Million hectares of land and forming the basis of a multi-billion-dollar-a-year export industry. Although the most valuable product is timber, *P. radiata* also produces and stores vast quantities of a complex resin-based mixture composed chiefly of mono- and diterpenes complex terpene mixture, known as oleoresin. The major diterpenes contained in *P. radiata* oleoresin are levopimaric, palaustric and neoabietic acids. These are currently used in many industrial applications, principally in the form of the low value bulk commodity product Rosin. Pine oleoresin is produced in specialised epithelial cells and subsequently sequestered into tube-shaped intercellular spaces known as resin canals or resin ducts. When sequestered into the resin duct system the toxicity of oleoresin does not compromise the health of the tree allowing large volumes to be synthesised and stored.

This features of the Pine resin duct system makes it an ideal platform for the production of large quantities of diterpenes. Therefore, with an ultimate aim to use these resin canals as "plant bio factories", we identified a suite of terpene synthase genes from *P. radiata* using our in house transcriptome data. We envision that a thorough understanding of the endogenous diterpene genes, their regulation and the contribution each makes to oleoresin composition is essential to maximise the production of desirable diterpenes in *P radiata*.

P54
Mobile Herbicide Resistance in the NZ Pseudomonad Pangenome

Taiaroa, G [1,2] Zhao, M [1] Macknight, R [1] Cook, G [2] Butler, M [1] Poulter, R [1]

[1]Department of Biochemistry, University of Otago, Dunedin, NZ

[2]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ

Horizontal gene transfer is a major driving force in the evolution of plant pathogens. Such transfers can impart a pathogen with resistance determinants and novel virulence factors[1]. This research explores horizontal gene transfer in PSA (Pseudomonas syringae pv. actinidiae) – a bacterial pathogen that has decimated the New Zealand kiwifruit industry since arriving in 2010[2].

PSA has expanded in New Zealand as a clonal outbreak, and an array of new genetic elements are now found in the New Zealand PSA pangenome. Obtained through horizontal gene transfer, these elements include megaplasmids, ICE elements and transposons imparting antibiotic resistance. Of our initial sample, at least 10 horizontal gene transfer events have been identified, each acting to diversify the genome of this plant pathogen.

Of significance, one of the horizontal gene transfers in New Zealand PSA involves genes imparting herbicide resistance – specifically to the herbicide phosphinothricin (also known as 'Basta' or 'Challenge'). This herbicide has a secondary antibacterial activity that can be overcome by bacteria through the activity of phosphinothricin-acetyl-transferases. A gene encoding one such transferase is shown to be located on an ICE element in PSA strains isolated in Te Puke – found alongside copper resistance operons and genes involved in alginate biosynthesis. These elements of the ICE present co-selection mechanisms, with herbicide use in kiwifruit orchards selecting for copper resistance and vice versa[3].

This ICE element has now been shown to transfer efficiently between PSA strains, and there is the potential for a significant environmental reservoir as copper can accumulate and remain stable in the soil for a long period of time[4].

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P55
Adaptation of bulb onion to different latitudes

Khosa JS[1], McCallum J [1, 2] and R Macknight [1, 2]
1. Biochemistry Dept, University of Otago, Dunedin, New Zealand
2. New Zealand Institute for Plant and Food Research, Christchurch, New Zealand

Bulb development in onion is primarily regulated by day length. Bulb onion is grown in a wide range of latitudes since its domestication and cultivars vary greatly in their response to day length. Cultivars grown at lower latitudes form bulbs under short days (SD) (daylight period of greater than about 8 hrs is required) while, at higher latitudes bulbing occur under long days (LD) (daylight period of greater than 12 hrs is required). We are using genetic and molecular approaches to identify genetic changes responsible for bulbing differences in SD and LD onions. We have found that the *FLOWERING LOCUS T* (*FT*) gene family regulates bulbing in SD and LD onions. Differences in the sequence and expression patterns of various circadian clock genes were observed which might be responsible for the bulbing differences in SD and LD populations. This information will help us to understand how onion was able to adapt to different regions during domestication and provide tools to breed new onion cultivars tailored to grow in specific latitudes.

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Putative Xylanase Activity of Pseudomonas syringae pv. actinidiae (Psa)

Mrs. Rasika Subasinghe[1], Dr Michael Clearwater[1], Dr Linda Peters[1], Prof Roberta Farrell[1]
[1]*University of Waikato, Hamilton , New Zealand*

Actual invasion strategies and mobility of the *Pseudomonas syringae* pv. *actinidiae (Psa)* pathogen in the kiwi fruit plant remain inconclusive.
Psa , indicated a significant level of xylanase activity especially when the pathogen was cultured on minimal media supplemented with 5% ground kiwifruit tissue.

Further studies of in-planta activity of *Psa* xylanse were conducted. Twenty mature Hort 16 A kiwifruit plants were used; ten plants uninfected and ten plants infected with *Psa.* When disease symptoms appeared in the inoculated plants, both infected and non infected shoots were harvested.

Psa was re-isolated from infected plants. Duplex PCRs were conducted to confirm that symptoms were due to *Psa* infection. For detection of Xylanase activity, using ground plant stem pieces, Remazol Brilliant Blue (RBB) and 3,5-Dinitrosalicylic acid (DNSA) assays were conducted. Using the RBB assay, xylanase activity was detected in *Psa* infected kiwi fruit pieces and no xylanase activity was detected in non-infected kiwifruit pieces. In DNSA assays, there was no detectable xylanase activity in the infected tissues. Therefore, further RBB assays were conducted to ascertain whether the difference between infected and non-infected tissues was due to enzymatic activity.
Strength tests were conducted to determine the strength of the kiwi fruit shoots post 4 weeks infection with *Psa* versus non-infected stems. The average strength per mm thickness of kiwifruit xylem was demonstrated to be less in infected stems.

RNA were extracted from infected tissues and PCRs were conducted with primers specific to annotations for xylanase in the *Psa* genome. One xylanase gene was expressed during infection.

Experimental results using *Psa*-infected kiwi fruit plants are consistent that a gene annotated for xylanase was expressed during infection and infected stems contain xylanase activity, which may cause the observed reduction of strength.