

2013

10th Finnish Plant Science Days



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13th-15th May 2013
University of Helsinki
Viikki Campus

10th Finnish Plant Science Days 2013

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10th Finnish Plant Science Days 2013

WELCOMING WORDS FROM THE CHAIR OF THE ORGANISING COMMITTEE

WELCOME TO THE 10th FINNISH PLANT SCIENCE DAYS!

This May (13-15th) the Finnish Plant Science Days, the Kasvitieteen Päivät, is held at the Viikki campus of the University of Helsinki. This congress has traditionally brought together plant scientists from all universities as well as other educational and research institutes in Finland. As this field of science is a heavy user of equipment, we have a considerable number of commercial companies supporting the congress. In addition, also the Finnish Cultural Foundation, Federation of Finnish Learned Societies (TSV) and the Biological Society of Finland, Vanamo, have supported the organisation of the congress.

The previous Plant Science Days were held at the University of Eastern Finland in Joensuu in 2010. After that meeting it was decided that the next meeting would be held in Helsinki to follow the tradition of circulating the organisational duty. The current organising committee includes representatives from the Viikki campus units as well as the Finnish Museum of Natural History.

The Plant Science Days continues the already 25 years old tradition of presenting research topics, sharing research results and future plans in forms of seminars and poster presentations. The aim is again to cover all fields of plant science from fundamental research to applied plant biology. This year the organising committee decided to select the invited speakers among researchers who are at the beginning of their scientific careers. Hence, we can expect interesting presentations spearheading all aspects of plant science in Finland. On the whole, we have 11 invited speakers, 26 selected oral presentations and 70 posters in the program for every one of the 172 registered participants to enjoy.

The scientific sessions, get-together party and poster sessions will be held at the Viikki campus of the University of Helsinki. The Viikki campus is the Life Sciences campus, uniting a multidisciplinary science community, where the main emphasis in plant sciences is in the two faculties of Biological and Environmental sciences and Agriculture and Forestry. The congress dinner will be held in the city centre with a visit to the Botanical gardens in Kaisaniemi.

On behalf of the organising committee I wish all plant scientists warmly welcome to the Finnish Plant Science Days 2013!

Prof. Kurt Fagerstedt

Chair of the organising committee

10th Finnish Plant Science Days 2013

ORGANISING COMMITTEE

The organising committee of the Finnish Plant Science Days 2013 includes representatives from the Viikki campus units as well as the Finnish Museum of Natural History.

- Prof. Kurt Fagerstedt, Chair, Department of Biosciences
- Doc. Anna Kärkönen, Secretary, Department of Agricultural Sciences
- Doc. Pedro Aphalo, Department of Biosciences
- Prof. Paula Elomaa, Department of Agricultural Sciences
- Doc. Johannes Enroth, Department of Biosciences
- Doc. Kristiina Himanen, Department of Agricultural Sciences
- Prof. Marko Hyvärinen, Finnish Museum of Natural History
- Doc. Timo Hytönen, Department of Agricultural Sciences
- Prof. Jaakko Kangasjärvi, Department of Biosciences
- Doc. Timo Saarinen, Department of Biosciences
- Doc. Mervi Seppänen, Department of Agricultural Sciences
- Dr. Karen Sims-Huopaniemi, Department of Biosciences
- Prof. Teemu Teeri, Department of Agricultural Sciences

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PROGRAM

Monday 13th May (Korona Info Centre Auditorium 1 - Viikinkaari 11)

Time	Event
1200	Registration starts in the Korona Info Centre lobby
1250	Opening of the congress: Kurt Fagerstedt (UH)
Session I	Evolution and plant systematics, chair: Marko Hyvärinen (UH)
1300-1330	Invited speaker: Anna-Liisa Laine (UH) Ecology of plant resistance against a fungal pathogen
1330-1350	Helena Korpelainen (UH) DNA barcodes for all plants of Finland: project update and outlook
1350-1410	Minna-Maarit Kytoviita (UJ) Next generation legacy of mycorrhizal symbiosis
1410-1430	Jan Buchmann (UH/MTT) Interspecies sequence comparison of <i>Brachypodium</i> reveals how transposon activity corrodes genome colinearity
1430-1500	Coffee break
Session II	Applied plant sciences, chair: Timo Hytönen (UH)
1500-1530	Invited speaker: Patrik Jones (UT) Photobiological organisms as a catalytic host for direct synthesis of fuel
1530-1550	Mervi Seppänen (UH) Selenium biofortification of the food chain in Finland
1550-1610	Anna Kärkönen (UH) Effect of Lignin Content and Quality on Differential Digestibility in Clones of Timothy
1610-1630	Jorma Vahala (UH) Ethylene Response Factors (ERFs) modify wood properties
1630	Group photograph outside the Info Centre
1630-1700	Refreshment break
Session III	Physiology, chair: Teemu Teeri (UH)
1700-1730	Invited speaker: Saijaliisa Kangasjärvi (UT) Cross-talk in light acclimation and immune reactions in plants
1730-1750	Fred Asiegbu (UH) A genomics approach to understanding interactions between

	forest trees and phytopathogenic fungi
1750-1810	Paula Elomaa (UH) Evolution and functional diversification of CYC/TB1-like transcription factor genes in regulation of Asteraceae inflorescence architecture
1810-1900	Transfer to A-building, poster hanging in the lobbies of A- and B-buildings
1900-2100	Get-together and poster party in 'Ladonlukko' and the lobbies of the A- and B-buildings

Tuesday 14th May (Viikki B building lecture room 1 - Latokartanonkaari 7)

Time	Event
0830	Registration continues in the lobby of B-building
Session IV	Physiology, chair: Anja Hohtola (UO)
0900-0930	Invited speaker: Laura Jaakola (U Tromsø) Developmental and environmental regulation of anthocyanin biosynthesis in bilberry (<i>Vaccinium myrtillus</i> L.)
0930-1000	Invited speaker: Michael Wrzaczek (UH) Getting information from the outside - signal perception from the extracellular space in plants
1000-1020	Kristiina Mäkinen (UH) Tracking potyvirus infection in four cellular compartments
1020-1040	Katriina Mouhu (UH) FvSOC1 regulates both flowering and vegetative growth in woodland strawberry, <i>Fragaria vesca</i> (L.)
1040-1100	Paula Mulo (UT) Chloroplast-targeted ferredoxin-NADP+ oxidoreductase isoforms in Arabidopsis
1100-1230	Lunch & poster session
SPECIAL TALKS	Industry, chair: Kristiina Himanen (UH)
1100-1120	Industry speaker: Joerg Vandenhirtz (LemnaTec) High-throughput plant phenotyping - a boost for genomics in the 21st century
1120-1200	Break
1200-1220	Industry speaker: Hailu Sun (BGI-Shenzhen) New Trends of Vast Specimens Genetic Variation Analyzing base on NGS

Session V	Ecology and ecophysiology, chair: Riitta Julkunen-Tiitto (UEF)
1230-1300	Invited speaker: Piippa Wäli (UOulu) Effects of large scale moth outbreaks on vegetation and fungal communities in mountain birch forest ecosystem
1300-1320	Tao Li (UEF) Variation in endophyte-mediated effects on constitutive and induced volatile emissions among fescue species
1320-1340	Tapio Linkosalo (Finnish Forest Research Institute) An improved heat-dissipation method for measuring sap flow in woody plants
1340-1400	Virpi Virjamo (UEF) Phenological change of <i>Picea abies</i> (L.) H. Karst. piperidine alkaloids and condensed tannins
1400-1500	Coffee & poster break
Session VI	Environment, chair: Pedro Aphalo (UH)
1500-1530	Invited speaker: Francoise Martz (Finnish Forest Research Institute) The effects of the changing climate on plant metabolism
1530-1550	Pär Davidsson (UH) Oligogalacturonide signalling in plant innate immunity
1550-1610	Johanna Riikonen (Finnish Forest Research Institute) Carbohydrate concentrations and freezing stress resistance of silver birch buds grown under elevated temperature and ozone
1610-1630	Mikko Anttonen (Finnish Forest Research Institute) Adaptability of silver birch (<i>Betula pendula</i> Roth) to elevated temperature and changing water regimes
1730-1900	Visit to the Kaisaniemi Botanical Garden
1900-2300	Congress dinner in Ravintola Kaisaniemi (Kaisaniementie 6)

Wednesday 15th May (Viikki B building lecture room 1 - Latokartanonkaari 7)

Time	Event
Session VII	Ecology and ecophysiology, chair: Kurt Fagerstedt (UH)
0900-0930	Invited speaker: James Blande (UEF) Plant volatile-mediated interactions in a polluted atmosphere: Communication breakdown
0930-1000	Invited speaker: Teemu Hölttä (UH) Ecophysiological aspects of xylem and phloem transport
1000-1020	Jenna Lihavainen (UEF) Molecular responses of silver birch (<i>Betula pendula</i> Roth) and

	hybrid aspen (<i>Populus tremula</i> x <i>P. tremuloides</i> Michx.) to increased air humidity
1020-1040	Boy Possen (Finnish Forest Research Institute) Genetic variation in leaf traits and growth within a <i>Betula pendula</i> population
1040-1100	Hanna Tuomisto (UT) Environmental, floristic and species diversity gradients across Amazonia
1100-1200	Lunch break
Session VIII	Evolution and plant systematics, chair: Johannes Enroth (UH)
1200-1230	Invited speaker: Neil Bell (UH) Macromorphology in mosses - architectural convergence and phylogeny in simple land plants
1230-1250	Sanna Laaka-Lindberg (UH) From strategy to action – the first steps in the ESCAPE project
1250-1310	Samuli Lehtonen (UT) Phylogenetic structure of rain forest fern communities
1310-1330	Gabriela Zuquim (UT) Field guide and online key - improving identification tools to the ferns in Amazonia
1330-1400	Coffee & poster break
Session IX	Physiology, chair: Jaakko Kangasjärvi (UH)
1400-1420	Mikko Tikkanen (UT) Regulation of energy flows in the photosynthetic machinery
1420-1440	Alexey Shapiguzov (UH) Immune reactions are intertwined with photosynthetic electron transport in <i>Arabidopsis</i>
1440-1500	Kaisa Nieminen (Finnish Forest Research Institute/UH) Silver birch (<i>Betula pendula</i>): a novel model tree for molecular genetics
1500-1530	Invited speaker: Ari-Pekka Mähönen (UH) Growth dynamics of vascular cambium
1530-1550	Best poster presentation and prizes
1550-1600	Closing of the Finnish Plant Science Days 2013

SPEAKER ABSTRACTS

Session I Evolution & plant systematics, chair: Marko Hyvärinen (UH)

Ecology of plant resistance against a fungal pathogen

Hanna Susi, Anna-Liisa Laine

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Variation in disease resistance is a widespread phenomenon in wild plant–pathogen associations. Here, we study the ecology of resistance in *Plantago lanceolata* against its obligate fungal pathogen, *Podosphaera plantaginis*. First, we quantify how stable resistance phenotypes are over variable biotic and abiotic gradients, and show that the ecological context strongly affects how resistance is expressed. Second, we investigate how different resistance strategies impact on disease dynamics during the course of epidemics. We find that host resistance strategy is one key determinant of epidemiological dynamics. Finally, we measure potential costs associated with resistance in *P. lanceolata* to understand how diversity is maintained in local plant populations. In long-term experimental populations, no costs of resistance emerge during the first year. Ongoing studies of these populations will demonstrate whether costs of resistance appear with age in this perennial plant.

DNA barcodes for all plants of Finland: project update and outlook

Helena Korpelainen, Maria Pietiläinen

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The Finnish Barcode of Life (FinBOL) is a national project with the goal of creating DNA barcodes for all species of plants, animals and fungi occurring in Finland. FinBOL is part of the International Barcode of Life (iBOL) consortium, which has the long-term aim of producing DNA barcodes for all species worldwide. FinBOL coordinates DNA barcoding within Finland, the target being to establish a barcode library for species found in the country and to provide a solution to the "taxonomic impediment" by enabling the rapid and accurate identification of any species using standard, relatively short genomic sequences, which are parts of two chloroplast gene regions, *rbcL* and *matK*, in the case of plants. DNA barcodes also allow identification from a small sample from an individual, independent of life stage or gender. FinBOL creates a unique national resource for biological research, allowing DNA barcodes to be applied in many fields. The information produced by FinBOL will be made public for species identification.

This presentation will introduce the strategy, methods, the present state and outlook of the project, and application potential of plant barcoding, particularly in Finland. Potential applications include, e.g., biodiversity investigations, monitoring of alien species, identification and monitoring of endangered species, and food control. A prerequisite for effective use of DNA barcodes is to establish a comprehensive DNA barcode reference library, which is well in the way in Finland.

Next generation legacy of mycorrhizal symbiosis

Minna-Maarit Kytöviita

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Parents determine the genotype of their offspring, but can also influence them beyond direct genetic contributions in response to environmental heterogeneity. Transgenerational phenotypic plasticity or parental effects occur when the phenotype or performance of the offspring is affected by the phenotype or the environment of its parents. Parental effects may be divided into maternal and paternal effects.

Arbuscular mycorrhizal symbiosis is known to improve plant fitness in terms of frequency of flowering, number of flowers and seed production. However, it is largely unknown how the symbiotic status of the parental generation affects the performance of the offspring. Our laboratory and field experiments show that the symbiotic status of the parental generation has long-lasting effects on the performance of the offspring including parameters such as relative growth rate, mortality, flowering frequency and seed production. Next generation symbiotic effects were fungal species-specific, occurred through both paternal and maternal pathway and were not related to respective effects on parental generation.

Interspecies sequence comparison of *Brachypodium* reveals how transposon activity corrodes genome colinearity

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Intergenic sequences evolve rapidly in plant genomes through a process known as genomic turnover. To investigate the influence of DNA transposons on genomic turnover, we compared 1 Megabase pair (Mbp) of orthologous genomic sequences from *Brachypodium distachyon* and *Brachypodium sylvaticum*. We found that *B. distachyon* and *B. sylvaticum* diverged approximately 1.7--2.0 million years ago. Out of a total of 219 genes identified on the analyzed sequences, 211 were colinear. In contrast, only 24 transposable elements (TEs) out of a total of 451 were orthologous (i.e. inserted in the common ancestor). We could characterize in detail 59 insertions and 60 excisions of DNA transposons in one or the other species which exchanged 17 % of the intergenic space. DNA transposon excision sites showed complex and highly diagnostic sequence motifs of double strand break (DSB) repair. This indicates that excisions can lead to extensive deletions of hundreds of basepairs (bp) of flanking sequence if the DSB is repaired by "Single Strand Annealing", or to insertions of up to several hundred bp of "filler DNA" if the DSB is repaired by "Synthesis Dependent Strand Annealing". In some cases, DSBs were repaired by a combination of both. We present a model for the evolution of intergenic sequences in which repair of DSBs upon DNA transposons excision is a major factor in the rapid turnover and erosion of intergenic sequences.

Session II Applied Plant Sciences, chair: Timo Hytönen (UH)

Photobiological organisms as a catalytic host for direct synthesis of fuel

M. Kalim Akhtar¹, Matteo Cossu¹, Filip Ginter², Fernando Guerrero¹, Patrik R. Jones¹, Suwisa Kaewphan², Pauli Kallio¹, Sanna Kreula¹, Jari Kämäräinen¹, Andras Pasztor¹

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Cyanobacteria are important global contributors to carbon and nitrogen fixation. Given the speed and efficiency at which cyanobacteria can convert solar energy into chemical energy, they are also potentially an important biotechnological host for sustainable applications, including the conversion of solar energy, CO₂ and H₂O into infrastructure-compatible fuels. The industrial use of cyanobacteria for biotechnological applications, however, is still immature and yet to be implemented on a large-scale, at least for products with a low unit value (e.g. fertilizer, fuel).

In our research, we aim to contribute towards evaluating and enabling the use of cyanobacteria as a catalytic host for the direct conversion of solar into chemical energy, with a particular focus on low unit-value fuel. The talk will include some concepts and challenges and then describe the development of tools and materials for both understanding and engineering cyanobacteria fuel production.

Selenium biofortification of the food chain in Finland

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Selenium (Se), an essential micronutrient for humans and animals, is circulated to food chain via plants. In Finland soils are low in Se and thus, the Se content of locally produced food and feed is inadequate without annual Se fertilization. Low Se intake of humans and animals causes health problems and therefore Se biofortification, e.g. enrichment of NPK fertilizers with Se, was started in 1984. Selenium utilization efficiency (SeUE = Se exported from field crops/applied Se) is low, as only 10 % is translocated to seeds and removed from fields during harvest. The long fertilization practice has not, however, increased the Se content in soils significantly. The fate of fertilizer Se is unknown and the current 2- to 3 year field and supplementary greenhouse experiments were conducted to reveal the Se cycle in field ecosystem. The results show that Se uptake efficiency is high; up to 60 % is taken up by plants at tillering or rosette stage, but the translocation to seeds is low. Majority of Se is left in plant debris but the bioavailability of Se which is bound in organic compounds is low. Our results further suggest that soil microbes may have an active role in either binding Se compounds or transforming Se in volatilized compounds as 10 % of Se is lost from the cycle. Our results also indicate that there is a positive interaction with SeUE and plant N-status.

Effect of Lignin Content and Quality on Differential Digestibility in Clones of Timothy

Anna Kärkönen¹, Tarja Tapanila², Tapio Laakso², Mervi Seppänen¹, Perttu Virkajärvi³, Maarit Hyrkäs³, Mika Isolahti⁴, Pekka Saranpää²

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Lignin content in grasses increases with maturity, as the need for structural strength increases during stem elongation. This reduces the digestibility of cell wall polysaccharides as the lignin makes them inaccessible to rumenal enzymes. We have studied lignin amounts and quality in field-grown timothy (*Phleum pratense* L.) clones that have difference in digestibility in relation to their stem proportion. The clones were collected at three developmental stages. Lignin amount in clonal material was quantified with the acetyl bromide method (AcBr) using Klason lignin as a standard. Lignin quality analysis was conducted by copper oxidation method that also gave information about hydroxycinnamic acid content in the cell wall. FTIR spectra were run from cell wall residues and lignins prepared with different methods to give additional information. In vitro organic matter digestibility (cellulase) and neutral detergent fibre (NDF) was assayed from the same clonal material for the corresponding developmental stages and correlation with lignification was analysed.

Results show that clones of better digestibility have less AcBr-lignin in cell walls of stems, leaf sheaths and stems containing leaf sheaths than those of lower digestibility. Lignin concentration in cell wall residue of leaf sheaths was higher in tillers at full heading stage than in tillers at earlier stages. However, no difference in stem lignin concentration at different developmental stages was observed. Lignin qualitative results will be completed by the time of this presentation. These data will be discussed.

Ethylene Response Factors (ERFs) modify wood properties

Jorma Vahala, Judith Felten, Jonathan Love, Andrés Gorzsás, Lorenz Gerber, Airi Lamminmäki, Björn Sundberg, Jaakko Kangasjärvi

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The plant hormone ethylene can affect different aspects of wood formation such as cambial growth and development of xylem cells, and is strongly involved in tension wood formation. Ethylene responses are mediated by Ethylene Response Factors (ERFs), a large family of transcription factors. We have identified all genes encoding ERFs in the *Populus trichocarpa* genome assembly, and determined their transcriptional responses to ethylene treatments in stem tissues. Some of the ERFs were induced also during tension wood formation. Twenty-four *Populus* ERFs were selected for functional characterization, and overexpressed in wood forming tissues of *Populus tremula* × *tremuloides* hybrid. Under greenhouse conditions, overexpression of several ERFs resulted in enhanced growth rate, and one ERF dwarfed trees with altered wood development. Additionally, five ERFs modified wood chemotypes that indicate changes in amounts of lignin and polysaccharides. Currently, we have studied the performance of ERF overexpressor trees during normal growing season under semi-natural conditions, where the trees are exposed to ambient temperature, light and wind. Under these environmental conditions, two ERFs resulted in enhanced growth rate consistently. Our future goal is to identify target genes of the selected ERFs. Another goal is to study interaction between ethylene and cytokinin, which has been shown to also mediate wood formation. Taken together, overexpression of ERFs may provide a novel generic tool to enhance growth rate of trees and to modify wood properties.

Session III Physiology, chair: Teemu Teeri (UH)

Cross-talk in light acclimation and immune reactions in plants

Andrea Trotta¹, Moona Rahikainen¹, Grzegorz Konert¹, Konstantin Denessiouk², Erich Glawischnig³, Sajjaliisa Kangasjärvi¹

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Plants have evolved versatile machineries to optimize their growth and survival upon environmental challenges. Among regulatory networks, extracellular perception systems and light-dependent organellar pathways carry out vital functions in determining appropriate stress reactions in plants. A particularly important role has been assigned to chloroplasts, which not only fuel the cells with energy but also provide metabolic intermediates that may have antioxidant, antimicrobial or insecticidal effects in plant cells. Pathways governing light acclimation and plant immunity share a level of commonalities, but the molecular mechanisms have remained poorly understood. Reversible protein phosphorylation by counteracting kinase and phosphatase activities is an ancient mechanism of cellular regulation. We have identified key roles for protein phosphatase 2A (PP2A) in the cross-talk between light acclimation and disease resistance in *Arabidopsis thaliana*. PP2A phosphatases are structurally complex, consisting of catalytic subunit C, scaffold subunit A and variable regulatory subunit B, which determines the substrate specificity of PP2A holoenzymes. By tracking structural and functional characteristics of Arabidopsis PP2A, we aim to decipher how plants integrate environmental signals to adjust metabolic activities in cruciferous plants. By now, genetic, phosphoproteomic and metabolomic approaches have shown that PP2A controls the biosynthesis of sulphur-rich secondary metabolites in response to light-dependent organellar signals. Analysis of protein interactions further revealed that PP2A physically interacts with a protein kinase CPK1, forming a novel kinase/phosphatase complex that modulates immune responses in plants. Understanding how stress signaling networks co-operate to adjust metabolic processes is expected to provide candidates for bio-refining programs in the future.

A genomics approach to understanding interactions between forest trees and phytopathogenic fungi

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Molecular pathobiology of forest trees for a long time lagged behind parallel studies on agricultural crop pathology. Recent technological advances have significantly contributed to the observed progress in this field. The first powerful impulse was provided by the completion of the black cottonwood genome sequence in 2006. Genomes of several other important tree species will be completed within a short time. Simultaneously, application of transcriptomics and next-generation sequencing (NGS) has resulted in the rapid accumulation of the vast amount of data on molecular interactions between trees and their microbial parasites. In this study, we investigated specificity in the recognition and response mechanisms of conifer tree roots (Scots pine) following challenge with either a pathogenic (*Heterobasidion annosum*), symbiotic (*Laccaria bicolor*) or saprotrophic fungi (*Trichoderma aureoviride*). We assayed global changes in the expression of conifer tree genes during interactions with the respective fungi. The results indicated that there were specific transcriptional responses of conifer roots challenged with either mutualistic, saprotrophic or pathogenic fungi. This suggests that Scots pine trees are able to recognize all three fungi and specifically distinguish whether they are pathogenic, neutral or beneficial microbial agents. The transcriptome analysis led to the isolation of a highly-transcribed gene shown to encode a novel antimicrobial peptide (Sp-AMP). The Sp-AMP was cloned and expressed, the purified protein had antifungal activity against *H. annosum*. It was directly shown to specifically bind

soluble and insoluble β - (1,3)-glucans with high affinity. We concluded that the Sp-AMP represent a new family of anti-microbial proteins (PR-19) that are likely to act by binding fungal cell wall glucans.

Evolution and functional diversification of CYC/TB1-like transcription factor genes in regulation of Asteraceae inflorescence architecture

Sari Tähtiharju, Anneke Rijpkema, Inka Juntheikki-Palovaara, Suvi Broholm, Tianying Lan, Raili Ruonala, Victor A. Albert, Teemu H. Teeri, Paula Elomaa
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Plants of the sunflower family (Asteraceae) have dense inflorescences, capitula, that consist of morphologically and functionally different types of flowers. The marginal ray flowers are female or sterile and have a showy asymmetric corolla, while the central disc flowers are hermaphrodite and have much shorter radially symmetrical petals. Our studies using *Gerbera hybrida* as a model gave the first molecular evidence that plant-specific TCP domain (CYCLOIDEA/TEOSINTE BRANCHED1-like) transcription factors are involved in regulating flower type identity (Broholm et al., 2008). In contrast to three CYC/TB1 genes in *Arabidopsis*, Asteraceae with ten genes identified both in gerbera and sunflower shows extensive expansion of the gene family. The duplicated genes correspond to three gene lineages specific to core eudicots: CYC1, CYC2 and CYC3. Our data indicates that modification of the ancestral network of TCP factors has, through gene duplications led to establishment of novel expression domains and to functional diversification. We show that especially the CYC2 clade, with greatest number of secondary gene duplications is associated with evolution of the increased complexity of the Asteraceae inflorescence architecture. We will show data indicating both functional redundancy and diversification of the gene family members involved in regulating flower type identity, flower organ development and possibly also flowering time.

Session IV Physiology, chair: Anja Hohtola (UO)

Developmental and environmental regulation of anthocyanin biosynthesis in bilberry (*Vaccinium myrtillus* L.)

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Bilberry (*Vaccinium myrtillus* L.) is one of the best sources of anthocyanins, the main pigments in flowers and fruits. Anthocyanins, among other flavonoids are strong antioxidant compounds, which are of major interest for their health beneficial properties. Flavonoids are synthesised via the phenylpropanoid pathway and the key enzymes leading to different intermediates or different flavonoid groups are well known. In molecular level the biosynthesis of flavonoids is regulated via co-ordinated transcriptional control of the enzymes in the biosynthetic pathway by the interaction with DNA binding R2R3 MYB transcription factors, MYC-like basic helix loop helix (bHLH) and WD40-repeat proteins. Recently, a considerable amount of new information has been gathered on the developmental and environmental regulation of anthocyanin biosynthesis in fruits. New discoveries have begun to reveal links between the developmental regulatory network and the specific regulators of anthocyanin biosynthesis during fruit ripening. We have shown that a SQUAMOSA MADS-box transcription factor (*VmTDR4*) is involved in regulation of the anthocyanin biosynthesis in bilberry. In non-climateric fruits, like bilberry, the plant hormone abscisic acid (ABA) also appears to have regulatory role in ripening related accumulation of anthocyanins. Furthermore, recent results have shown that the content of anthocyanins and certain other flavonoids in both berries and leaves of bilberry increase linearly from southern to northern latitudes. The common garden and the controlled growth experiments have shown that both genetic and environmental factors regulate this phenomenon. The challenge is to understand better the complicated networks and confluences behind the developmental and environmental regulation of anthocyanin biosynthesis. Unraveling these mechanisms will increase our understanding on the roles of phenolic compounds in adaptation of plants to different latitudes.

Getting information from the outside - signal perception from the extracellular space in plants

Michael Wrzaczek

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Extracellular signals serve dual purposes. They convey information from the environment (e.g. stress perception, nutrients, etc.) but they also participate in the communication between cells and tissues. Thus the perception of extracellular peptides by plasma membrane-localized receptor proteins provides a flexible way for organisms to control developmental processes and to sense environmental cues. However, especially in plants very little is known about how extracellular peptides are regulated and activated in order to allow recognition by receptors. Other receptors might not perceive peptide ligands but rather might be modified directly by extracellular reactive oxygen species (ROS). While it has become clear that elaborate systems must exist that allow the perception of ROS in order to achieve the observed specificity of ROS responses, no receptor for ROS in the extracellular space has so far been identified.

A 20 amino acid peptide derived from the secreted plant protein GRIM REAPER was sufficient to induce ROS-dependent cell death in *Arabidopsis* leaves. The peptide bound *in vivo* to the extracellular domain of KHARON, a plasma membrane-localized, enzymatically inactive receptor-like protein kinase. GRIM REAPER is cleaved directly by a type II metacaspase METACASPASE-9 and the cleavage was essential for the induction of cell death. The secreted extracellular plant protein is processed by an extracellular protease and

is subsequently sensed by a catalytically inactive receptor-like kinase, which acts as a true receptor.

Tracking potyvirus infection in four cellular compartments

Katri Eskelin, Anders Hafren, Konstantin Ivanov, Andres Löhmus, Kristiina Mäkinen
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Potato virus A (PVA; genus *Potyvirus*) is a single-stranded positive-sense RNA (ssRNA(+)) virus. PVA infection involves four cellular compartments being nucleus, replication complex (RC), RNA granule and polysomes, in the host cells. PVA encodes at least two proteins which are translocated into the nucleus. Our working hypothesis is that PVA replication proteins NIa and NIb act together to hijack cellular machinery in the nucleus to regulate cellular functions to support the viral infection cycle. In cytoplasm ssRNA(+) viruses induce host membrane rearrangements in order to assemble viral RCs. Within these membrane-associated RCs viral proteins, together with host factors, direct viral RNA synthesis. Studies showing that only replicated viral RNAs are efficiently translated have indicated that ssRNA(+) virus translation in polysomes and replication are functionally coupled. Our novel finding is that PVA RNA can induce RNA granules that contain at least ribosomal protein P0, eIF(iso)4E, and PVA RNA. We call these structures as P0 RNA granules (P0Gs). P0Gs can be defined as PVA-induced RNA granules, which are connected to viral RNA expression and resemble cellular stress granules (SGs). We have developed methods to isolate these cellular compartments active during PVA infection and to purify viral RNP-complexes from them by an affinity chromatography method. Viral and host proteins associated with these complexes have been identified by mass spectrometry to obtain the virus-induced proteomes within these structures. This proteomics approach was initiated to obtain a comprehensive molecular understanding of potyvirus infection and to identify host factors as targets to induce resistance against PVA.

FvSOC1 regulates both flowering and vegetative growth in woodland strawberry, *Fragaria vesca* (L.)

Katriina Mouhu, Elli Koskela, Takeshi Kurokura, Marja Rantanen, Paula Elomaa, Timo Hytönen
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Detection of seasonal changes is essential for survival in plants. Day-length is a major environmental cue for the regulation of development during the year. Annual and perennial plants seem to utilize the same day-length measurement system, the CO-FT module, in regulating photoperiodic flowering response and vegetative growth responses such as tuberization in potato and bud growth cessation in poplar. In the diploid Rosaceae model plant woodland strawberry, *F. vesca*, short days induce flowering and change axillary meristem development from runner formation to side crown formation. The photoperiodic flowering response is regulated by *F. vesca* *TERMINAL FLOWER 1* (*FvTFL1*), which, however, does not affect axillary meristem fate. Our studies with other, putative flowering related genes in *F. vesca* revealed that *F. vesca* *FLOWERING LOCUS T 1* (*FvFT1*) and *F. vesca* *SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS 1* (*FvSOC1*) are activated under long days. Studies with transgenic plants suggest that *FvSOC1* is activated by *FvFT1* and that *FvSOC1* regulates flowering negatively via *FvTFL1* up-regulation. Moreover, *FvSOC1* over-expression enhanced and RNAi silencing repressed runner formation indicating a role for *FvSOC1* in regulation of axillary bud development. This regulation is

likely mediated via gibberellin as *FvSOC1* over-expression up-regulated and RNAi silencing down-regulated several genes in gibberellin biosynthetic pathway. These data indicate that *FvSOC1* mediates photoperiodic signals to control flowering through *FvTFL1* and vegetative development by gibberellin pathway activation in woodland strawberry.

Chloroplast-targeted ferredoxin-NADP⁺ oxidoreductase isoforms in Arabidopsis

Paula Mulo, Minna Lintala, Nina Lehtimäki, Jürgen Soll, Bettina Bölter
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Two leaf-type ferredoxin-NADP⁺-oxidoreductase (FNR) isoforms present in *Arabidopsis thaliana* have been shown to possess specialized properties but are nevertheless largely redundant in their function. We have defined how a drastic reduction of FNR, catalyzing the final step of photosynthetic electron transfer chain, affects phenotype of the plants by generating and characterizing FNR double mutants. Depletion of FNR in Arabidopsis double heterozygote *fnr1xfnr2* plants (F1 generation) resulted in a small and pale green phenotype and marked downregulation of photosynthetic pigment-protein complexes. The mutant plants suffered from over-excitation of electron transfer chain, which was partly compensated by induction of cyclic electron flow. The mutant plants possessed high content of xanthophylls in high de-epoxidation state, as well as high level of α -tocopherol, which apparently assist in coping with oxidative stress. We have also shown that FNR is bound to the thylakoid membrane *via* the Tic62 protein. At the thylakoids, Tic62 and both FNR isoforms form high molecular weight complexes that are not involved in photosynthetic electron transfer but are dynamically regulated by light signals and the stromal pH. Furthermore, in the absence of Tic62, membrane binding and stability of FNR are drastically reduced. We conclude that Tic62 represents a major FNR interaction partner not only at the inner envelope membrane and in the stroma, but also at the thylakoids of *Arabidopsis thaliana*. We furthermore propose that correct allocation of FNR is used to efficiently regulate Fd-dependent electron partitioning in the chloroplast, as the activity of various chloroplast enzymes has been significantly changed in the mutant plants containing only soluble FNR.

Industry Talks, chair: Kristiina Himanen (UH)

High-Throughput Plant Phenotyping – a Boost for Genomics in the 21st Century

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Due to the development of highly automated genetic analysis, plant genomics has immensely enlarged our understanding of the genetic structure of plants over the last two decades.

The fast evolving need to identify interactions between genes and environmental factors (biotic and abiotic) that brings about a certain plant phenome made it necessary to develop quantitative, reproducible and highly automated plant phenotyping systems for large plant numbers in controlled environments or green houses or under field conditions.

Phenotyping systems such as these have to integrate reproducible plant management (randomization, watering) and comprehensive imaging of root and shoot far beyond human vision (visible light, fluorescence, near infrared, infrared, X-rays, NMR, THz) as well additional chemical analysis methods. Immediate and automated image analysis of the stored images and further data transformation using plant shape and plant growth models are the important intermediate steps before undertaking statistical data analysis of the phenotyping results to characterize plant phenotypes quantitatively. Such quantitative data contributes in a decisive way to the further analysis of gene functions (tilling, QTL etc.), especially under fluctuating or stress-induced environmental conditions with a special focus on complex traits like yield or drought tolerance.

This presentation provides a survey on new phenotyping technologies in controlled environments, green houses and in the field and the close interaction between phenotyping technologies, modeling approaches and the new opportunities of fast and automated high-throughput genomics.

New Trends of Vast Specimens Genetic Variation Analyzing base on NGS

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Nowadays, researchers have diversified technologies at their disposal for collecting genetic information. As sequencing costs continue to drop, new approaches of NGS-based genetic variation excavation and genotyping are being increasingly used.

Here we introduce a new system GenomeBiomarker® which is used for breeding pedigree and natural population's genetic marker development and vast specimens' genotyping. It encompasses bin map, target region capture, reduced representation and hybridization-based approaches to discover and genotype SNPs and InDels. This system provides insight into different NGS methods, their benefits, and the role these technologies will play in the future.

Session V Ecology & Ecophysiology, chair: Riitta Julkunen-Tiitto (UEF)

Effects of large scale moth outbreaks on vegetation and fungal communities in mountain birch forest ecosystem

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Massive moth outbreaks cause large-scale damage in subarctic mountain birch forests. In addition to the mountain birches (*Betula pubescens* ssp. *czerepanovii*), also field vegetation and fungal communities change dramatically due to moth outbreak. In Finnish Lapland outbreaks of autumnal moth (*Epirrita autumnata*) are natural phenomena, but during the outbreak of 2004-2008 also winter moth (*Operophtera brumata*) participated in the moth outbreak in Kaldoaivi, Utsjoki causing the forest damage on the area of 400 km². During this moth outbreak the dwarf shrubs declined dramatically. Especially, crowberry (*Empetrum nigrum* ssp. *hermaphroditum*), which is the dominating understory plant in many mountain birch forests, suffered badly during the outbreak partly due to the fungal pathogens. If the outbreak lasted only one year, approximately half of the mountain birches died and *Empetrum* dominated field vegetation recovered mostly during the first five years after the outbreak. If the outbreak continued for two or more consecutive summers, the death of birches were often total and the field vegetation turned into grass dominated and recovery of the dwarf shrubs was very slow. Due to possible following intensive mammalian grazing, these destroyed forests may permanently turn to open tundra heath land. The as a result of dramatic decline in photosynthesis in the damaged forests, the fungal community changed as the amounts of mycorrhizal fungi decreased and decaying fungi increased remarkably. In decomposer community of the soil the proportion of bacteria increased in relation to fungi after the moth outbreak.

Variation in endophyte-mediated effects on constitutive and induced volatile emissions among fescue species

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Systemic fungal endophytes that live asymptotically within their host plants can affect plant-herbivore interactions, mainly due to endophyte-derived toxins increasing plant resistance to herbivores. Besides, induced plant defence by endophytes is another mechanism against herbivores. Volatile organic compounds (VOCs) produced by plants play a major role in plant-herbivore interactions by attracting/repelling herbivores and recruiting herbivore's natural enemies. However, little is known about the effect of endophytes on constitutive and induced VOC emissions, particularly in endophyte-grass symbiotic systems. We investigated whether and how fungal endophytes altered constitutive and induced VOC emissions in two economically important agricultural species, tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pretense*), by using the generalist aphid species, *Rhopalosiphum padi*. To compare with results from agronomic grass species, we included two genotypes of a native grass species, red fescue (*F. rubra*). We found that endophyte infection differentially affected constitutive and induced VOC emissions and that such effects varied both quantitatively and qualitatively among fescue species. In addition, aphid performance was significantly reduced on endophyte-infected plants. Ecological implication of the results is discussed in the context of the grass-endophyte symbiosis.

An improved heat-dissipation method for measuring sap flow in woody plants

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Accurate measurements of sap flow are needed e.g. for studies of plant and soil water balance, plant water use efficiency and water stress of plants. The classical method of heat dissipation presented by Granier (in 1985) is one straightforward method of measuring the sap flow flux rate in woody plants. In this method, two temperature sensors (typically within a thin metal tube) are inserted into the plant trunk. The lower of the two sensors is used to measure ambient temperature. The “down-stream” sensor (i.e. upper one) has also a heating element installed. The heat is transferred from the sensor both by thermal conductance and convection along with the xylem sap flow. Increased sap flow increases convection, and hence from the temperature difference of the two sensors, the sap flow rate can be calculated. The Granier method assumes that the temperature distribution (and hence the sap flow) is in a steady state. But actually, reaching the steady state temperature distribution may take up to several hours. Therefore the Granier method is not able to react to rapid changes in the sap flow rate; also asymmetry of responses to flow rate changes leads to biases in the measurements.

Here we present a novel improvement to the Granier method: a microprocessor-based thermostat reads the temperature difference between the two sensors, and actively adjusts the heating power of the heated element to keep the temperature difference, and therefore the heat distribution in the trunk much closer to constant, steady-state conditions. With an active control of the heating power, the time lags of the measurements are roughly half of those of the original Granier method. The new method also makes it easier to detect and measure the zero-flow conditions, and reduces the hysteresis in the sap flow measurements.

Phenological change of *Picea abies* (L.) H. Karst. piperidine alkaloids and condensed tannins

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Secondary compounds are playing important part in plant defense against herbivores and parasites. Here, we investigated how concentrations of piperidine alkaloids and condensed tannins of adult *P. abies* are changing during bud burst and shoot growth. We harvested buds while winter dormant, when primordial needles started to elongate and when bud scales were starting to open. Another three time points (needles and twigs) were collected after bud burst. Total concentration of 11 individual piperidine compounds (from which 3 were identified new to *P. abies* and 3 remain unidentified) and condensed tannins were analyzed. Concentrations of both total piperidine alkaloids and condensed tannins were highest immediately after bud burst. The individual piperidine alkaloids detected in reasonable amounts could be divided in two groups: major components and developmental phase dependent components. Interestingly the concentrations of major piperidine alkaloid compounds were found to be constant during bud development as well as needle and twig development. On the other hand one part of the developmental phase dependent compounds occurs only in early branches and are likely to be intermediates in piperidine biosynthesis, while others appear in late buds and stay somewhat constant after that, referring that these compounds are end products of biosynthesis (in the side of major components). The high concentration of total piperidine alkaloids and condensed tannins in young branches is likely to be part of plant defensive system. Constant concentrations of nitrogen-containing major components of piperidine alkaloids through development are underlining their importance for *P. abies*, although ecological meaning is yet to be solved.

Session VI Environment, chair: Pedro Aphalo (UH)

The effects of a changing climate on plant metabolisms

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Forecasted climate changes will undoubtedly affect plant life; environmental conditions are regulating factors of plant metabolisms, thus having direct or indirect consequences on plant vitality and growth (modulation of primary metabolisms) but as well on plant responses to abiotic and biotic stresses (modulation of secondary metabolisms). Our interest is more specifically focused on plants growing in the northern boreal to sub-Arctic zones, where climate changes are expected to be the highest, and where many plant species grow at the limit of their distribution zone and the balance between growth and survival is sometimes subtle.

The effects of changes in environmental conditions during the growing season have been widely studied in many types of plant species and some general conclusions have emerged. Although less frequently studied but most likely as important as changing conditions during the growing season, changing winter conditions are expected to affect the productivity and biodiversity of perennial plant species. More frequent warm spells, rain and freeze-thaw cycles may cause modification of the snowpack and formation of ground ice encasement. Such changes will lead to lower insulating properties of the snowpack and possible development of soil hypoxia under the ice layers. Plant, and especially root, maintenance metabolism during dormancy are poorly characterized, but new overwintering conditions could have important consequences on plant winter survival and spring regrowth (ex: possible storage pool depletion due to more frequent warm spells).

Results from several field experiments (modulation of UV-radiation, temperature, nutrient availability or induced ground ice encasement) will be presented and discussed.

Oligogalacturonide signalling in plant innate immunity

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Plants are continuously exposed to a wide range of both biotic and abiotic stresses, hence adaptation to ambient environment is a prerequisite for plant growth and survival. The activation of plant (and animal) innate immunity systems involves specific detection of pathogen-associated molecular patterns (PAMPs) by corresponding host encoded pattern-recognition receptors (PRRs).

Perception of PAMPs will subsequently trigger signalling cascades leading to activation of plant defences and enhancement of basal immunity towards potential pathogens. In addition to recognition of non-self such as PAMPs, plants have the ability to recognize modified-self including damage-associated molecular patterns (DAMPs). A model DAMP is Oligogalacturonides (OGs), plant cell-wall fragments released by the action of degradative enzymes secreted by the pathogen during the infection process. Release and recognition of such fragments has also been shown to trigger innate immunity responses and result in enhanced disease resistance.

We have conducted a large mutant screen for OG-insensitive mutants in *Arabidopsis* aiming to identify hypothetical OG receptor(s) as well as downstream signalling components. Interestingly, two mutant lines currently under investigation have led us to believe that miRNAs play a significant role in mediating DAMP-triggered defence signalling.

Carbohydrate concentrations and freezing stress resistance of silver birch buds grown under elevated temperature and ozone

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The effects of elevated temperature (+0.8°C), ozone concentration (1.3 × ambient ozone concentration) and their combination on over-wintering buds of *Betula pendula* were studied after two growing seasons of exposure in the field. Freezing stress resistance (FSR), carbohydrate concentrations, bud dry weight to fresh weight ratio, and transcript levels of cytochrome oxidase (COX), alternative oxidase (AOX) and dehydrin (LTI36) genes were studied in two clones (clones 12 and 25) in December.

Elevated temperature increased the bud dry weight to fresh weight ratio and the ratio of raffinose family oligosaccharides (RFOs) to sucrose, and the transcript levels of the dehydrin (LTI36) gene (in clone 12 only), but did not alter the FSR of the buds. Genotype-specific alterations in carbohydrate metabolism were found in the buds grown under elevated ozone.

In conclusion, we found evidence that increasing autumnal temperatures may have favourable impacts on metabolic processes that are generally considered to be associated with FSR, although these changes did not appear as lower electron leakage from the cells in silver birch buds. Whether this is a true response or an indication of unsuitability of the electron leakage test for dormant bud tissues remains to be evaluated. Increasing ozone concentrations do not seem to increase the risk for freeze induced damage in silver birch buds.

Adaptability of silver birch (*Betula pendula* Roth) to elevated temperature and changing water regimes

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Predicted climate change in terms of changing temperatures and precipitation regimes holds challenges for long-lived forest trees. To cope with these challenges within-population genetic variation and phenotypic plasticity are key factors. In our project we have firstly evaluated the within population variation in many morphological and physiological parameters related to photosynthesis, water use, growth and stress tolerance. Secondly, the variation in relation to adaptability to elevated temperature and different watering regimes was evaluated. For the first part of the project, a 10-year old common garden experiment with 15 genotypes was used. For the second part, ten of the 15 genotypes were cloned to study in detail their responses to ambient and elevated temperature (+1° C) in combination with three watering regimes (drought, optimal and excess) in a pot experiment. Measurements were conducted during different phases of the treatments (induction, tolerance, recovery) over two growing seasons. Preliminary results from the stress tolerance phase and from biomass harvests show good adaptive capacity for silver birch. Biomass accumulation and allocation were not strongly affected by the treatments. Watering had the strongest effect which was seen as a slightly lower biomass accumulation under low water treatment. Physiological measurements from 2011 show that plants under low water treatment had a more efficient control of water use. Furthermore, low water treatment led to smaller leaves with lower SLA. Interestingly, these same leaf morphological traits were also observed in genotypes with high biomass production in the first experiment in the field. Our data suggests that genotypes showing high biomass production might also have good adaptive capacity against stress.

Session VII Ecology & Ecophysiology, chair: Kurt Fagerstedt (UH)

‘Plant volatile-mediated interactions in a polluted atmosphere: Communication breakdown’

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Plants produce and emit a wide range of volatile organic compounds, which provide detailed information about their physiological condition. This information allows plants to communicate with other community members including neighbouring plants and the myriad of arthropods sharing their habitat. The blend of volatile compounds emitted by plants is altered by biotic and abiotic stress, while the interaction of different stresses may have complex effects on plants and their volatiles. It is well documented that herbivore-feeding induces plants to emit a volatile bouquet that is attractive to natural enemies of the herbivores and which also constitutes a warning of danger to neighbouring plants. The mechanisms underlying the signalling between plants are not well elucidated, but there is gathering evidence that both active and passive volatile-mediated interactions are ecologically significant. However, it is clear that these interactions are among the most sensitive of ecological processes and vulnerable to environmental perturbation. Oxidising pollutants may have complex effects on volatile-mediated interactions, both through exerting oxidative stress associated changes in volatile emissions and through oxidation of volatile organic compounds in the atmosphere. These routes may result in changes in the fidelity of the volatile signal and a reduction in the signalling efficiency, respectively. In this talk I will present new data on volatile-mediated interactions and detail how polluted environments, especially ozone polluted environments, may impact on these processes. I will particularly focus on the active and passive forms of plant-plant signalling and how these interactions feed into a plant’s interactions with other organisms.

Ecophysiological aspects of xylem and phloem transport

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Efficiency and safety of xylem water transport from soil to leaves is crucial for plant productivity, growth and overall performance as photosynthetic production in leaves is ultimately limited by the rate at which water can be supplied through the xylem. The water transport rate of the xylem is ultimately limited by its hydraulic conductivity and its ability to maintain water in the liquid form, i.e. without excessive cavitation.

Simultaneously to the upward water transport in xylem, the carbon assimilated by photosynthesis in the leaves is transported to the carbohydrate sinks in the respiring and growing tissue and also the soil through the phloem. Phloem transport and carbohydrate utilization in the sinks have to maintain the rate of carbon assimilation in photosynthesis, or carbohydrate accumulation will force stomatal closure and down-regulation of photosynthesis. The functioning, construction and maintenance of both of the long distance transport systems may become increasingly limiting for plant performance, while consuming a growing proportion of the assimilated resources, with increasing plant size.

Molecular responses of silver birch (*Betula pendula* Roth) and hybrid aspen (*Populus tremula* x *P. tremuloides* Michx.) to increased air humidity

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By current climate change models air humidity is predicted to rise in the northern latitudes due to increasing precipitation and global warming. Water vapor acts as a greenhouse gas affecting the energy economy of plants; still long term air humidity effects on northern trees and ecosystems are poorly known. Responses of silver birch (*Betula pendula* Roth) and hybrid aspen (*Populus tremula* x *P. tremuloides* Michx.) to elevated humidity (~7% over ambient) have been studied in Free Air Humidity Manipulation (FAHM) experiment in Estonia. Increasing humidity inhibits tree growth by suppressing transpiration, thus limiting nutrient uptake. Besides physiological effects, metabolic and genetic responses behind adaptation to altered air humidity are being studied. Birch and aspen leaves varied in GC-MS analyzed primary metabolite responses to humidification treatment during fourth growing season in 2011. Birch leaves showed a clear metabolic shift despite the genetic variation within seedlings; when cloned aspen had no clear response. Results indicate birch being more sensitive to humidity changes. Further research was conducted in growth chambers with cloned silver birch in low (60%) and high (95%) relative air humidity with low or high nitrogen fertilization. High RH induced sapling growth.

Genetic variation in leaf traits and growth within a *Betula pendula* population

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Genetic diversity within populations is important for ecosystem functioning, coping with environmental disturbances as well as practical forestry and has been studied extensively in provenance trials. These trials ignore the variation and plasticity within local populations that is important for resilience of local ecosystems. Currently, there is no information available linking genetic variation in tree processes to differences in growth for local populations, despite the need for this information in e.g. climate modelling. We have addressed this question using a field experiment with silver birch (*Betula pendula* Roth), using biomass as an indicator of performance.

The field experiment, located in Punkaharju (Finland), consists of 6 replicate blocks with 22 silver birch genotypes, micro-propagated from individuals selected randomly from a single, naturally regenerated, local forest stand. Fifteen genotypes, covering the variation in biomass in the field experiment, were selected for further studies to assess the role of physiological (e.g. gas exchange), morphological (e.g. specific leaf area) and phenological (e.g. bud burst, growing period) traits on within population variation in biomass.

From the evaluated traits, leaf morphology, specifically the ratio of leaf area and leaf dry mass (specific leaf area) was most important in explaining the variation in biomass observed in the field. However, for almost all evaluated traits there were significant differences between the genotypes and the rank of the genotypes varied over years, indicating extensive genetic variation and phenotypic plasticity. Morphological traits were found to be more important for superior growth compared to physiological and phenological traits. It is concluded that within a single population multiple combinations of traits can result in good growth, rather than a single, fixed combination of traits.

Environmental, floristic and species diversity gradients across Amazonia

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Although Amazonia has historically been considered a relatively uniform forest area, several recent studies have documented considerable floristic turnover across sites at the landscape to regional scales. Much of this variation has been related to differences in soil properties, such as nutrient content and texture. These ultimately reflect the geological history of the site and the mineralogical origin of the sediments, and these in turn are known to differ between western and central Amazonia. This can be postulated to have broad-scale consequences for species distributions, but to date there has been little information available on how the floristic–edaphic relationships that have been observed within one region scale up to the entire Amazon basin. I will address this issue using ferns as an example group. The data reveal both similarities and differences in how the flora relates to soil gradients in western and central Amazonia, and additional patterns related to climatic heterogeneity within the rain forest realm become also apparent.

Session VIII Evolution & plant systematics, chair: Johannes Enroth

Macromorphology in mosses - architectural convergence and phylogeny in simple land plants

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Perhaps the most fundamental problem in phylogeny reconstruction is distinguishing similarity due to common ancestry from similarity due to evolutionary convergence. Mosses are small and structurally simple plants with limited tissue differentiation and a highly modular organisation, which can make convergence due to similar selective pressures in unrelated groups difficult to detect. This is especially so when convergence occurs in relatively recently diverged clades and involves suites of closely correlated characters that have been subject to repeated cycles of gain and loss. Nonetheless, general architectures that enable relatively rapid macro-morphological transformations may underlie the success of some groups through facilitating speciation in response to changed selective pressures in new habitats.

Two case studies will be presented illustrating how architectural convergence in mosses can be highly misleading for taxonomy as well as highly interesting evolutionarily. The genus *Oligotrichum* s.l. (Polytrichaceae) was historically defined largely by characters of gametophytic reduction, although recent studies show that it conflates distantly related clades that have evolved to occupy similar niches in geographically isolated temperate regions of the northern and southern hemispheres. The Hypnodendrales, by contrast, are an order of mostly very robust and architecturally complex mosses, nearly all of which have one of three distinct growth forms. Although these different architectures were previously thought to provide evidence of close relationships, they have now been shown to have arisen repeatedly in response to shifts between epiphytic and non-epiphytic lifestyles in humid forests.

From strategy to action – the first steps in the ESCAPE project

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The Finnish ex-situ conservation strategy includes five targets: to (i) secure the extant collections; (ii) increase the number of conserved taxa; (iii) develop ex-situ conservation for northern species; (iv) incorporate ex-situ conservation into reintroduction programmes, and (v) increase the use of collections in education and public outreach. To achieve these targets a five-year EU Life+ project was launched in autumn 2012. Here, we present the first steps of the project: establishing a national seed bank, ensuring the storage of micropropagated tissues in a cryogenic unit, setting up assisted migration trials based on ex-situ material, and initializing ex-situ conservation of bryophytes.

Phylogenetic structure of rain forest fern communities

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In this study we investigated the phylogenetic structure of rain forest fern communities in central Amazonia and Panama. In total, 90 local fern communities were sampled and the phylogenetic history of the community members was reconstructed. We then investigated whether the phylogenetic structure of these communities varied along soil fertility and rainfall gradients. We found a negative relationship between Mean Phylogenetic Distance (MPD) and soil fertility, and a positive relationship between Net Relatedness Index (NRI) and soil fertility, but no such relationship was observed along the rainfall gradient. We also found that fern genera *Trichomanes*, *Lindsaea* and *Triplophyllum* are overrepresented in poor-soil communities, whereas *Adiantum*, *Thelypteris* and *Tectaria* are overrepresented in rich-soil communities. However, there was no clear signal along the rainfall gradient. These results suggest that edaphic preferences of rain forest ferns are phylogenetically conservative and that communities growing on rich soils are composed of more closely related species than poor-soil communities. It also appears that within the rain forest biome the edaphic gradient is more important than the rainfall gradient in determining the community structure.

Field guide and online key - improving identification tools to the ferns in Amazonia

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There is a general lack of taxonomical knowledge in Amazonia coupled with the scarcity of identification tools. To help filling this gap, we developed a field guide and an online key to the ferns and lycophytes found in the Biological Reserve of Uatumã, Brazilian Amazonia. Both comprise 120 species, which probably represents 1/5 to 1/6 of the total number of fern species in Amazonia. Each tool targets different publics. The Guide to the Ferns and Lycophytes of REBIO Uatumã – Central Amazonia is available in both printed and electronic versions and it is fully illustrated with pictures of live specimens. It focuses on diagnostic characteristics that can be observed in the field and with naked eyes and therefore, the guide is simple and easy to be used even by non-botanist. On the other hand, the online key is a multi-entry key that was developed for more advanced users, that are familiar with the terminology and morphological structure of ferns. Both strategies are complementary and aim to fill the gap in the botanical knowledge in Amazonia.

Session IX Physiology, chair: Jaakko Kangasjärvi (UH)

Regulation of energy flows in the photosynthetic machinery

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In the higher plant thylakoid membrane, several co-operating regulatory mechanisms maintain the functionality of the photosynthetic light reactions under continuously changing light environment. In the center of this regulatory network are the phosphorylation of several core proteins of photosystem II (PSII) and the major chlorophyll a/b light harvesting antenna LHCII, the non-photochemical energy dissipation (NPQ) and the proton gradient-dependent control of electron transfer. Failure in any of these regulatory mechanisms results in damage of either the photosystem II or photosystem I. Traditionally, LHCII phosphorylation has been connected to the “state transitions”, an acclimatory mechanism, to changing light quality. The PSII core protein phosphorylation, in turn, has been connected to the turnover of the D1 protein of PSII under high light stress. A striking feature of PSII-LHCII phosphorylation, however, is that phosphorylation is present under all naturally relevant light conditions yet relative phosphorylation level between different PSII-LHCII phosphoproteins varies continuously according to the prevailing light intensity. Such behavior of thylakoid protein phosphorylation challenges the traditional “state transitions” and D1 turnover models as well as makes it difficult to integrate the role of thylakoid protein phosphorylation to a bigger picture on regulation of the photosynthetic machinery. During the past 10 years, knock out mutants of the key regulatory mechanisms of light acclimation have become available, making it possible to place the thylakoid protein phosphorylation in to the network of reactions responsible for dynamic light acclimation of the photosynthetic apparatus. Our earlier model on interaction of LHCII phosphorylation with NPQ, ensuring the redox balance under fluctuation intensity of light remaining severely incomplete with respect of dynamic light acclimation and protection of both PSII and PSI. Accumulation of experimental evidence made it possible to include in the model the proton gradient-dependent regulation of electron transfer, taking place concomitantly with NPQ, and the regulation of PSII core protein phosphorylation strict interaction with LHCII phosphorylation. Moreover, the intricate feature of the thylakoid membrane at all light conditions, the PSII turnover, has been integrated into the model to build up a solid and holistic picture on regulation of the photosynthetic machinery according to internal and external cues.

Immune reactions are intertwined with photosynthetic electron transport in

Arabidopsis

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The plant cell not only uses its chloroplasts for the house-keeping needs such as energy production and metabolism, but also employs them in many signalling pathways as signal receptors, mediators or amplifiers [1]. For example, early on during the immune responses provoked by pathogen infections, the changes occur in the operation of photosynthetic electron transfer chain (ETC) located in chloroplasts. Presumably, the controlled destabilization of ETC by a plant serves the purpose of generating specific reactive oxygen species [2, 3]. In turn, the accumulation of these species in the chloroplast leads to the reprogramming of gene expression in the cell’s nucleus with large consequences for plant adaptation and development. The mechanisms of propagation of such signal, particularly of

its transfer across the membrane barriers between the organelles, are largely unknown. In our studies we address the role of chloroplasts in plant immunity by inducing defense reactions in *Arabidopsis* and by closely monitoring the state of the chloroplast ETC *in vivo* in the course of the plant's response. Such monitoring is made possible by measuring chlorophyll fluorescence – a real-time non-invasive means to get insight into molecular dynamics of the ETC. Our studies of a number of *Arabidopsis* mutants with altered immune responses shed light on the new components of the above-described signalling.

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Silver birch (*Betula pendula*): a novel model tree for molecular genetics

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The aim of our research is to understand molecular mechanisms controlling tree development. We will explore natural variation in forest trees to identify novel genetic regulators of tree architecture, cambial activity and wood quality. Our model organism is an important forestry tree, silver birch (*Betula pendula*), whose small genome is currently being sequenced. This tree is monoecious, and already very young trees can be induced to flower under greenhouse conditions. Birch will bring the power of inbreeding and short generation times into tree genetics, enabling exploitation of advanced crossing schemes for gene mapping.

The main focus of our study is a collection of naturally occurring tree mutants; a diverse set of trees with atypical wood quality, cambial activity, branching pattern, or secondary metabolite content. We are currently most advanced in characterisation of one of these mutants, “swirly birch”, with a striking growth pattern. Its stem and branches grow initially straight upwards, but when they get longer they are not able to stay upright, and start to bend downwards. Excitingly, this tree has an intriguing stem phenotype, where the tension wood formation is mutated into a rotating, “swirly” pattern. We aim, through whole genome sequencing approach, map the causative recessive mutation behind the “swirly” phenotype, and study function of the identified gene in transgenic trees.

Besides its fascinating basic science aspect (what makes a tree a tree?), understanding molecular mechanisms regulating tree development has also significant applied value. Historically, due to their large size and long generation times, trees have not been easily accessible for traditional breeding. Detailed knowledge about regulatory mechanisms controlling tree traits will provide us tools for the domestication of forest trees. Boosting efficient production of wood in commercial forests is essential for sustainable management of natural resources. With birch as our model, our project represents a novel approach of tree genetics with potential for ground-breaking insights into tree development and breeding.

Growth Dynamics of Vascular Cambium

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Large proportion of biomass on Earth is stored as wood. Wood originates from the proliferate activity of vascular cambium. Several phytohormones have been shown to regulate cambial cell proliferation, however the molecular and cellular mechanism of cambial activity has remained largely unknown. On one hand this is due to a long lifespan of tree species making it challenging to approach the scientific questions with molecular genetics, and on the other hand due to technical difficulties to study vascular cambium with modern microscopy techniques.

In order to overcome these challenges we have established *Arabidopsis thaliana* root cambium as model system to study cellular and molecular processes during the secondary growth. In addition to the routinely used molecular genetics tools, we have been able to develop confocal microscopy imaging techniques to follow cambial cell proliferation in live tissues in the transparent root of *A. thaliana*. We have developed and utilized modern lineage tracing techniques to understand cellular dynamics in root cambium. This will help us to locate the cambial stem cells. Phytohormones cytokinin and auxin have been implicated as central regulators of cambial cell proliferation. Our data indicate that the interaction of these two hormonal signaling pathways is important to set the position of the cambial cells. Additionally, our group is using forward and reverse genetics approach, as well as transcript profiling of cambial cells to identify novel cambial regulators. The latest advances of these research lines will be presented at the meeting.

POSTER ABSTRACTS

A) Evolution & plant systematics

A1: Archaeobotany – macrofossil plant remains from historical gardens

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Archaeobotany is a multidisciplinary field of plant science working between botany and archaeology and interpreting the results with cultural aspects. Macrosubfossils, commonly macrofossils or macroscopic plant remains, tells to archaeobotanist, how people has been using plants in the past or how people's behavior has influenced on the local vegetation of the area studied. Plant macrofossils are usually seeds, seed coats, fruits or parts of these or parts of leaves or stems. The material for macrofossil analysis comes often from archaeological excavations and plant remains are linked to archaeological context and dated with archaeological methods. Conventionally analysis of macrofossil and microfossil, like pollen, has been used for investigating the cultivation history. In cases of archeophytes archaeological data can give information when plant species not native to country in question has been introduced to new areas. Macro- and microfossils can also be studied from biological or geological premises when studying environmental changes in the past or making climate reconstructions.

Garden history can also be studied with archaeobotanical methods. In this PhD thesis plant remains are studied from relatively young soils of historical gardens. Soil samples are taken from gardens from five different levels on average using an end-filling open-ended sampler. Macrofossils found from samples are dated using radiocarbon dating. With these methods this work is aiming to enable macrofossil studies also without excavations. Results will be compared with macrofossil analysis done from samples taken from excavation sites near the study area. Also written documents with botanical, archaeological, and historical references are studied and the taxa found from soil samples will be compared to historical data of the taxa grown in the study sites earlier. When studying seeds in soils archaeobotany does not search for live seed banks but archives kept in soils, though some old seeds in those are still able to germinate. Still macrofossil analysis and seed bank studies can be done simultaneously side by side like macrofossil and pollen analysis.

A2: Newly discovered diversity in the tropical fern family Metaxyaceae

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For a long time, *Metaxya rostrata* HBK. was considered to be the only species in the monotypic fern family Metaxyaceae. A second species, *M. lanosa* A.R.Sm. & Tuomisto was described in 2001 based on morphological features and the rbcL gen. Its authors suggested that there may still be more than one species masquerading under the name *M. rostrata*. We have now carried out a more thorough analysis using specimens from Central America (Panama and Costa Rica) and Amazonia (Peru and Brazil). Phylogenetic analyses using the rbcL and the trnG genes divided the specimens into five clades, one of which corresponded to *M. lanosa*. All Central American specimens of *M. rostrata* were placed in one clade, and those from the Contamana area of Peru in another. The remaining specimens were split between two clades, both of which have a broad geographical distribution in western and central Amazonia. *Metaxya lanosa* also occurs in that same area, but is restricted to a different kind of habitat, namely white sand forests rather than typical rain forest. Further studies are needed to clarify if there are ecological differences also between the sympatric *M. rostrata* clades.

A3: Oil body morphological character evolution in liverworts, a phylogenetic perspective

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Extant liverworts include approximately 8000 species and are found on all continents, occupying arid to aquatic niches. Fossil records together with evidence from phylogenetic studies suggest that the first land plants, which evolved approximately 470 million years ago, had a liverwort affinity. These findings indicate that metabolic pathways essential for survival under stress conditions on land must have evolved at a very early stage. It also implies that liverworts might have played an important role in creating a more suitable environment for the subsequent evolution of other land plant groups and that they may help us to understand the vegetative and reproductive changes that favoured the successful radiation of land plants. One of the putative evolutionary innovations of liverworts is the presence of oil bodies in the cells of both gametophytes and sporophytes. They are intracellular organelles bounded by a single unit membrane containing lipophilic globules suspended in a proteinaceous matrix. These highly distinctive organelles are found in the cells of more than 90 % of liverwort species. Although morphological and chemical variations of the oil bodies have been widely used in liverwort taxonomy and chemosystematics and many of their secondary metabolites are known to be bioactive and are considered as potential sources of medicines, the origin, development and function of the liverwort oil bodies still remain poorly understood. In this study, we reconstruct the most complete phylogenetic hypothesis of oil body evolution using multigene sequence data. In the phylogenetic context we examine oil body morphological character evolution by reconstructing ancestral shifts in character states. Our results provide first evidence on the evolutionary transformations of the oil body morphological characters and their significance in taxonomy and systematics in liverworts.

A4: What is the reality of a gene?

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Biological systems are extremely complex and have emergent properties that cannot be explained, or even predicted, by studying their individual parts. The facts that the boundaries of transcriptional units are far from clear; in fact, whole chromosomes if not the whole genome seem to be continuums of genetic transcription, many examples of gene fusion are known, and the structure of the gene, its functional status can also be inheritable, and, epigenetic extra-genomic modes of inheritance, called genetic restoration, seem to be a rather common phenomenon, meaning that organisms can sometimes rewrite their DNA on the basis of RNA messages inherited from generations past. The gene concepts require different prerequisites or complementation by different 'worlds'. The gene that is manipulated is dependent on living organisms that are competent to integrate it into their own genome. Genes are not causes, but conditions for life processes. But their functions cannot be deduced from the latter; hence, there must be a 'world' of processes where proteins are at its disposal. The functions of gene products need to be integrated into the entirety of an organism, by the organism itself. Proteins allow for the expression of phenotypic characters, they are not their causes. Instead of investigating the living in accordance with its nature, one forces upon it a lawfulness alien to it. Our thinking should not also be adequate to ask where an organ of a living being originates instead of what purpose it serves.

A5: Extant and fossil microfungi on plant exudates and honeydew

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The fungi comprise one of the most diverse groups of organisms on Earth, but several fungal groups remain little studied. Especially data on evolution, distribution and ecology of many microfungi is scarce. Even though several fungal species grow on plant surfaces, resin and other plant exudates are challenging substrates for fungi because of their toxic components and the lack of available water. However, certain fungal groups have specialized in utilizing resinous microhabitats. In my PhD project I will study dark filamentous microfungi growing on plant exudates and honeydew excreted by sap-feeding insects. Several well-preserved fossils of microfungi belonging to hyphomycetes and sooty moulds have survived as inclusions in amber. Information from the amber fossils can be combined with molecular data collected from the extant species, and together they can shed light on the evolution and biogeography of extant fungal groups growing on plant surfaces.

A6: From strategy to action – the first steps in the ESCAPE project

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The Finnish ex-situ conservation strategy includes five targets: to (i) secure the extant collections; (ii) increase the number of conserved taxa; (iii) develop ex-situ conservation for northern species; (iv) incorporate ex-situ conservation into reintroduction programmes, and (v) increase the use of collections in education and public outreach. To achieve these targets a five-year EU Life+ project was launched in autumn 2012. Here, we present the first steps of the project: establishing a national seed bank, ensuring the storage of micropropagated tissues in a cryogenic unit, setting up assisted migration trials based on ex-situ material, and initializing ex-situ conservation of bryophytes.

A7: Phylogenetic structure of rain forest fern communities

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In this study we investigated the phylogenetic structure of rain forest fern communities in central Amazonia and Panama. In total, 90 local fern communities were sampled and the phylogenetic history of the community members was reconstructed. We then investigated whether the phylogenetic structure of these communities varied along soil fertility and rainfall gradients. We found a negative relationship between Mean Phylogenetic Distance (MPD) and soil fertility, and a positive relationship between Net Relatedness Index (NRI) and soil fertility, but no such relationship was observed along the rainfall gradient. We also found that fern genera *Trichomanes*, *Lindsaea* and *Triplophyllum* are overrepresented in poor-soil communities, whereas *Adiantum*, *Thelypteris* and *Tectaria* are overrepresented in rich-soil communities. However, there was no clear signal along the rainfall gradient. These results suggest that edaphic preferences of rain forest ferns are phylogenetically conservative and that communities growing on rich soils are composed of more closely related species than poor-soil communities. It also appears that within the rain forest biome the edaphic gradient is more important than the rainfall gradient in determining the community structure.

A8: An automated mass-digitization for natural history collections

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Aim of digitization of natural history collections is to create databases with all the information the specimens contain. Openly and freely available databases enable the efficient use of collections in taxonomic and environmental research, education and decision making. Digitization may also decrease handling and transportation of often vulnerable physical specimens.

Digitarium is a joint initiative of the Finnish Museum of Natural History and the University of Eastern Finland. It aims to mass-digitization - it has been estimated there are about 20 million specimens in public collections in Finland.

At Digitarium, all specimens are imaged, which allows the subsequent steps to take place without concern of space or time. The entire imaging process is being automated with a modular, motored conveyor belt system that brings the specimens under digital cameras. Software using image pattern recognition and sensors controls placement of specimens under the cameras, and triggers them automatically. Specimens are tagged with QR-code and unique URI before they are placed on the conveyor belt, enabling the basic metadata to be available for subsequent steps of data entry. An operator is required for tagging and loading / unloading the trays on belt. Presently, our process may handle up to 1,000 samples per day, which translates to 200,000 samples per year.

Data entry especially from old, hand-written specimen labels needs human knowledge and experience. We are presently experimenting with crowd-sourcing in order to scale the data entry up.

A9: Micromorphology of leaves in the genus *Larix*

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We have studied the anatomical characters of leaves in long and short shoots of various taxa of the genus *Larix*. Previously the first author studied taxonomy of *Larix* species cultivated in Finland and most trees proved to be hybrids. This finding urged on a further and more profound study on characters possibly useful in distinguishing species and different varieties. We chose to study morphological characters of the needles which are far less used in studies of *Larix* than cones and long shoots. One aim of this study is to find out whether these characters are consistent enough to be used in a phylogenetic analysis of the genus *Larix*.

The material consists of specimens collected from trees cultivated in Finland and herbarium material of native taxa originating from Siberia, Far East and Canada. The anatomy of needles was studied based on cross sections prepared by embedding specimens in paraffin. Needle surfaces were viewed using SEM. The anatomical characters studied were the general shape of the needle, wax, epidermis, stomata, hypodermis, resin ducts and vascular bundle. The characters were measured and/or coded.

Some of the useful characters are width and thickness of leaf, diameter and location of resin ducts, size and shape of epidermal cells, size, shape and abundance of hypodermal cells, abundance and location of stomata, conspicuousness of keel, shape of leaf margin, and abundance of wax. Generally, leaves in short shoots are more differentiated than those in long shoots. Interestingly, in some *Larix* taxa leaves of long and short shoots are micromorphologically similar and in some other taxa not.

A10: Field guide and online key - improving identification tools to the ferns in Amazonia

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There is a general lack of taxonomical knowledge in Amazonia coupled with the scarcity of identification tools. To help filling this gap, we developed a field guide and an online key to the ferns and lycophytes found in the Biological Reserve of Uatumã, Brazilian Amazonia. Both comprise 120 species, which probably represents 1/5 to 1/6 of the total number of fern species in Amazonia. Each tool targets different publics. The Guide to the Ferns and Lycophytes of REBIO Uatumã – Central Amazonia is available in both printed and electronic versions and it is fully illustrated with pictures of live specimens. It focuses on diagnostic characteristics that can be observed in the field and with naked eyes and therefore, the guide is simple and easy to be used even by non-botanist. On the other hand, the online key is a multi-entry key that was developed for more advanced users, that are familiar with the terminology and morphological structure of ferns. Both strategies are complementary and aim to fill the gap in the botanical knowledge in Amazonia.

B) Applied Plant Sciences

B1: Virus-induced gene silencing for *Gerbera hybrida*

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Virus-induced gene silencing (VIGS) is a reverse genetic approach, which uses the plant's natural viral immune mechanisms and allows identifying gene functions conveniently and quickly. As a model in Asteraceae flower development and secondary metabolism, *Gerbera hybrida* has many advantages, bearing highly organized head-like inflorescences, and being rich in unique metabolites such as gerberin and parasorboside. For reverse genetics, *Agrobacterium*-mediated stable transformation, however, is labour-intensive and time-consuming. In order to develop a tobacco rattle virus (TRV) based VIGS for *Gerbera hybrida*, we attempted to improve TRV VIGS vectors, to select TRV sensitive cultivars, and to optimize agroinfiltration conditions. Intensive silencing symptoms were achieved both in green tissues and floral tissues, demonstrated by knocking down genes involved in chlorophyll biosynthesis (*GPDS*, *GCHL-H*, and *GCHL-I*), flower pigmentation (*GCHS1*) and organ development (*GGLO1*). Unexpected off-target silencing of *G2PS1* was observed by the silencing of *GPDS*. This off-target silencing was shown to be caused by photo-bleaching, as photo-bleaching induced by norflurazon herbicide treatment also resulted in *G2PS1* silencing without an effect in *GPDS* expression. We have shown the feasibility of VIGS for functional studies in gerbera, but our results also pointed that the selection of marker genes should be critically evaluated.

B2: Does light spectral quality affect survival and regeneration of potato (*Solanum tuberosum* L.) shoot tips after cryopreservation?

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Cryopreservation is one of the most powerful methods for long-term storage of plant germplasm in minimal space and maintenance requirements. Cryopreserved germplasm is stored at ultra-low temperature, in or above liquid nitrogen to maintain the viability of the material and to enable regeneration after thawing.

In vitro plants are generally not autonomous for photosynthesis, they depend on a source of organic carbon such as sucrose. For this reason, the effect of light spectral quality is often underestimated when growing plants *in vitro*. Plant development and physiology are strongly influenced by the light spectrum. Light spectral composition is a source of information affecting morphogenesis: germination, de-etiolation, elongation growth, flowering, secondary metabolism and overall plant form. Light spectral quality has been used to control the growth and morphogenesis of potato tissues and organs *in vitro* (Wilson et al 1993, Aksenova et al 1994, Seabrook 2005).

For cryopreservation of potato shoot tips of *in vitro* plants are used. In the present study the effect of light spectral quality on survival and regeneration of potato after cryopreservation is studied.

B3: Phytoremediation and heavy metal detoxification mechanisms in *lupinus* and *cannabis* plants grown on soils polluted with Cr, Cu and As

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Chromated copper arsenate (CCA) is a wood preservative, which has contaminated wood processing areas with three toxic elements; chromium (Cr), copper (Cu) and, arsenic (As). Six greenhouse and three Petri dish experiments were organized between 2007-2013 as an object to study phytoremediation potential and detoxification mechanism of high-biomass crops grown under different Cu, Cr and As levels.

Most heavy metals are phytotoxic and strong oxidants. A variety of enzymes are involved in tolerance/defence mechanisms such as peroxidases, catalase, superoxide dismutase (SOD) and glutathione (GSH). GSH is an essential metal chelator, being an important antioxidant as well as a precursor for phytochelatins (PC). PCs are known to be responsible for metal tolerance in many plants. In addition, architectural changes in plants are common in abiotic stresses. Our results include for example germination, biomass production, photosynthesis, water potential and heavy metal accumulation into plant parts as well as alterations to root architecture, root anatomy, H₂O₂ production and heavy metal localization. In addition, the production of antioxidant enzymes, glutathione and the permeability of cell membranes has been studied spectrophotometrically.

B4: Split luciferase complementation assay to study protein-protein interaction *in planta* for Gerbera MADS domain proteins using binary Gateway vectors.

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We developed a split luciferase assay to examine protein-protein interactions *in planta* using agro-infiltration for tobacco (*Nicotiana benthamiana*) leaves (Fig.1). In this method, the N- and C- terminal fragments of *Renilla reniformis* luciferase are translated as parts of bait and prey proteins, respectively. When the proteins interact, split luciferase becomes activated and emits light which can be measured by a luminometer. Split luciferase activity was measured in leaf discs 2-7 days after agro-infiltration. DNA vector expressing both bait and prey genes was constructed in binary vectors. We detected hetero- and homo-dimer protein-protein interaction levels between MADS domain proteins, which are involved in gerbera flower development.



Figure 1: *Renilla reniformis* luciferase expressed in tobacco (*Nicotiana benthamiana*) leaves using agro-infiltration.

B5: Assaying plant ribosomes with Asymmetric flow field-flow fractionation (AsFIFFF)

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Ribosomes are responsible for translation of cellular mRNAs into proteins. Upon translation initiation, the 40S and the 60S subunits bind to mRNA and form a translation competent 80S ribosome. The distribution of mRNAs among polyribosomes (several ribosomes bound to mRNA) and monosomes (one ribosome bound to mRNA) are indicative of their translational activity: translationally active mRNAs are associated with polyribosomes. Internal and external stimuli affect the translatability of the mRNAs. Therefore, changes in the ratio of free subunits, monosomes and polyribosomes are informative about the physiological state of the cell.

Our aim was to study the suitability of asymmetric flow field-flow fractionation (AsFIFFF) for ribosome purification and profiling. In AsFIFFF, the separation takes place in an open channel. Thus, elution is gentle and keeps the analyzed molecules in their native conformation. Crude preparations of ribosomes for the analysis were prepared from *Nicotiana benthamiana* plants using ultracentrifugation. The AsFIFFF analysis contains the injection/focusing and the elution steps. We optimized the steps to enable separation of contaminating components of the preparations from ribosomes. After the optimization of AsFIFFF conditions the peak containing free RNA and free protein that co-sedimented with the ribosomes during ultracentrifugation could be separated from the main peak containing ribosomes. The main peak was broad and had several sub-peaks. Treatment of the sample with RNaseA and EDTA verified that this peak contained the ribosomal subunits and intact ribosomes. Finally, we collected fractions from AsFIFFF and analyzed their RNA and protein content. Analysis of ribosomal RNA (rRNA) verified the position of the subunits and intact ribosomes in the fractograms. Furthermore, positive results from the RT-PCR analysis of several host genes from the main peak showed that ribosomes remained bound to mRNAs during the AsFIFFF analysis. Analysis of the protein content in various fractions correlated with the RNA data: the protein profiles of the main peak differed from the peak representing the free protein and RNA. In conclusion, our results show that AsFIFFF is well suited to study ribosome composition as well as changes in the ratios of subunits, monosomes and polyribosomes during different growth conditions of plants.

B6: Interaction of the potyvirus protein HCpro with the cellular microtubule-associated protein HIP2 is required for efficient virus infection in plants

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Microtubules (MTs) maintain the overall shape of non-dividing plant cells by regulating cell wall synthesis and organizing cell organelles and cellular membranes. A new role of cortical MTs in conveyance and amplification of signalling in development and in biotic and abiotic stresses is emerging.

Here we provide data on involvement of a MT-associated protein in infection with a potyvirus, *Potato virus A* (PVA), representing the largest family of plant-infecting RNA viruses. Our results showed that HIP2 of potato (*Solanum tuberosum*) is a MT-associated protein homologous to Arabidopsis SPIRAL2 (SPR2). Silencing, complementation and localization experiments demonstrated that SPR2 and HIP2 share similar functions at cortical MTs. The helper component proteinase (HCpro) of PVA, *Potato virus Y* and *Tobacco etch virus* interacted with HIP2 of potato and tobacco (*Nicotiana tabacum*) *in planta*. Interactions of HCpro with HIP2 were localized on MT and MT-intersections in cell cortex in PVA-infected leaves. Accumulation of PVA was significantly reduced in the *HIP2*-silenced leaves of *N. benthamiana*, which indicates that HIP2-HCpro interaction supports virus infection. The region of HCpro controlling interaction with HIP2 was determined and mutated, which reduced fitness of PVA and caused novel necrotic symptoms in tobacco plants. Analyses of gene expression showed that ethylene- and jasmonic acid-inducible pathways were induced, whereas transcription of genes associated with MTs, growth and metabolism was down-regulated. These results implicate for the first time a role for MTs in potyvirus infection and suggest that HCpro interacts with MTs to impede antiviral defence.

B7: A chemotaxonomic investigation of the carnivorous plant family

Sarraceniaceae L.

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Sarraceniaceae is a New World carnivorous plant family comprising three genera. These are *Sarracenia* (ca. 11 species from the east coast of North America), *Heliamphora* (ca. 23 species from South America) and *Darlingtonia* (monotypic from California). *Darlingtonia* and *Sarracenia* occur in nutrient poor swamps and *Heliamphora* on tabletop mountains and thus they developed carnivory that is the attraction, capture, and digestion of insects to supplement their nutrient uptake.

There are several reports on natural compounds of Sarraceniaceae e.g. occurrence of sarracenin in *Sarracenia*. Our main interest was to investigate the distribution of coniine alkaloids in Sarraceniaceae. It had been reported that *S. flava* contains coniine, a naturally occurring alkaloid possessing insect stunning properties and which is found in *Conium maculatum* and several *Aloe* species, too. In this study we have examined 59 different accessions within 11 *Sarracenia* and other species within Sarraceniaceae. Compounds were extracted from freshly ground plant material using basic water-dichloromethane extractions. The extracts were analyzed using GC-MS and data was analyzed using NIST08 and in-house generated spectral libraries. The data from accessions were combined and compared with each other.

B8: Cytokinins and ethylene in tree biotechnology

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The plant hormones cytokinin and ethylene are essential regulators of plant growth and development. Woody raw material for industrial use will increasingly rely on high efficiency forest production systems using trees with enhanced growth rate and other quality traits. Thus, the main objective is to find and establish ways to improve wood growth and quality and to provide a generic biotechnology tool applicable also for other tree species by cytokinin and ethylene biotechnology in trees. We have identified several genes that regulate various hormone-responsive wood properties, and have generated and screened for transgenic poplar lines and phenotypes overexpressing various cytokinin and ERF (Ethylene Response Factors) signalling enhancer genes. Under greenhouse conditions, the lines overexpressing both cytokinin and *ERF* genes show up to 50% enhanced growth rate. In addition, overexpression of several *ERFs* results in modified wood chemistry. Based on our preliminary observation cytokinins and ethylene are possibly strongly connected to each other, and our aim is to further study interaction between these two hormones. Conventional tree breeding is time consuming in the aim to gain more productive genotypes. Therefore we have established generic tools to speed up this process significantly by tree biotechnology. Additionally, these biotechnologies could facilitate maximal wood productivity at lesser land area, which potentially reduces further need to exploit natural forests areas.

B9: Breeding of the common bean (*Phaseolus vulgaris* L.) using local germplasm and marker information

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Nicaragua is located in the Mesoamerican center of genetic diversity for *Phaseolus* species. However, common bean genetic resources have been undervalued and then not utilized in active breeding programs. In the present project, we evaluated the genetic diversity of forty representative bean landraces using microsatellite markers. The results revealed that there is a higher level of genetic diversity than reported before and this is partially associated to important agronomic traits. Of this collection, two promising landraces were chosen and crossed. Parental polymorphic markers showed that we succeeded in reshuffling most of the genetic diversity. The most segregating F_1 individuals were selected to produce the F_2 generation. Computer simulations demonstrated that this selection could improve the genetic gains of yield under the pedigree method in breeding programs. Then, F_2 individuals were evaluated and selected under field conditions using marker information and mixed model analyses. Our preliminary results suggest that selected F_2 individuals have a higher grain yield than the check cultivar and both parents. Also, they have a similar level of resistance to common diseases as other released cultivars in the region. In addition, most individuals hold acceptable seed colorations for national and international markets. This approach contributes to the conservation of bean genetic resources by means of their active utilization and also increases the number and diversity of genetic materials exploited as formal cultivars; something justified within the frame of the current climate change and potential shifts in market preferences.

B10: Regulation of canopy structure in timothy (*Phleum pratense* L.) by vernalization, photoperiod and GA

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Timothy is widely grown perennial forage grass in Finland and other temperate areas. The canopy consists of three tiller types (VEG, ELON, GEN) and is harvested two to three times per growing season for silage. Timothy is a long day plant that flowers when the critical day length has been exceeded. Our earlier results have shown that also vernalization has a positive effect on the flowering in timothy. Flowering is regulated by several endogenous and environmental factors, and numerous genes have important role in the complex regulation system. The genomic information available for timothy is very limited compared to other monocots and grass species. To study the regulation of canopy structure in timothy at physiological level, observations and tiller counting were done after vernalization, GA and photoperiod treatments. In addition five cDNA libraries were sequenced, annotated and sequence information was used further for qPCR analysis. Vernalization released stem elongation and resulted in dramatic change in the proportion of ELONG and GEN tillers. SD (12 h) was a strong inhibitor of stem elongation and flowering. In SD GA was only able to induce modest stem elongation, which did not result in the development of ELONG or GEN tillers. LD (16 h) was sufficient enough to induce production of ELONG and GEN tillers, and GA application combined with vernalization resulted in higher stem elongation. Several putative homologs of timothy flowering genes were identified, such as *VRN1*, *VRN3*, *LpMADS10* and *PpD1*. qPCR results supported physiological observations showing that the expression of flowering inducer *VRN1* peaked at the time of the veg/gen transition, but also *VRN3* was needed for the development of ELONG and GEN tillers. These results demonstrate the essential role of vernalization and photoperiod in the regulation of canopy structure in timothy.

B11: Modelling tree structure – tapering trunks and complex crowns

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The technical quality and therefore economic value of a tree trunk depends on its branchiness and tapering. Most of Finland is covered in commercial forests with the main aim to make profit from timber production. Global change is influencing tree growth. Optimizing future forest management can be done based on models that includes trunk quality determining economic value and physiological processes described mechanistically, allowing extrapolations into future climates. PipeQual (Mäkelä et al. 2003. Forest Ecology and Management) is suitable for this purpose. The aim of this research was to develop models for *Betula pendula* on crown structure and for *Picea abies* on trunk tapering with the goal to later include these into future PipeQual versions. Our approach to model *Betula* crowns was empirical, and we chose and potentially modified statistical models on branch angle, size and height distribution published earlier (Mäkinen et al. 2003. Forest Science; Lintunen et al. 2011. Canadian Journal of Forest Research) to better fit to the PipeQual context. For *Picea* trunk tapering we instead chose a more mechanistic approach based on two key assumptions: 1) Bending moment in a given trunk section depends on the product of wind drag and lever length (distance from the given trunk section to the drag) 2) Maximal resistance of trunk section against bending moments scales with the third power of trunk diameter. Thanks to the mechanistic modelling approach the results can be applied easily to the existing *Pinus sylvestris* PipeQual and to the currently developed *Betula* PipeQual.

Incorporating the developed models on crowns and tapering will make more realistic predictions on future forests possible.

B12: Transcriptomics of pinosylvin biosynthesis in Scots pine

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Scots pine (*Pinus sylvestris*) is an economically important forest tree species in Finland. Its heartwood is naturally decay resistant and the most durable wood produced by local tree species. The natural decay resistance of Scots pine heartwood is an important wood quality trait and it has reasonably high heritability. The decay resistance correlates with extractable heartwood compounds (stilbenes and resin acids). Trees with the highest heartwood extractive concentrations are the most decay resistant. Scots pine stilbenes (pinosylvin and its monomethyl ether) are synthesized during heartwood formation and as a response to biotic or abiotic stress in other pine tissues. They are phenolic compounds formed by a branch of the phenylpropanoid pathway. The pathway converts phenylalanine to Scots pine stilbenes with involvement of several key enzymes: phenylalanine ammonia lyase (PAL), coumaroyl-CoA ligase (4CL), stilbene synthase (STS) and pinosylvin methyl transferase (PMT). In this study, we utilized large scale RNA sequencing to investigate the induction of candidate genes in stilbene biosynthesis pathway during both developmental and stress induction. The candidate *STS* gene was rapidly induced on both conditions. However, *PAL* and *4CL* were either constitutively expressed or slightly induced in stress condition, but not during heartwood formation. Interestingly, the expression pattern of the *PMT* candidate gene was not suggestive of involvement in stilbene biosynthesis at all, although the gene has been described as encoding a pinosylvin methyl transferase activity. Mapping of our reads to the pine EST collection (*DFCI Pine Gene Index*, 77.326 contigs) and searching of *O*-methyl transferases encoding sequences coexpressed with *STS*, uncovers a novel candidate for the true *PMT*.

B13: Effects of hydrothermal treatment on cell wall components and enzymatic digestibility of wheat straw

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Second generation biorefineries utilize non-food parts of plants, such as stems and leaves of cereals, for fuel and chemical production. Enzymatic hydrolysis of plant cell wall polysaccharides is an important step in this process. As such, plant cell wall structure is often resistant to the enzyme treatment and various pretreatment techniques have been developed in order to enhance the digestibility. In this work, effects of the hydrothermal pretreatment on cell wall components and enzymatic digestibility of wheat straw were studied using chemical analysis, microscopy and commercial enzyme preparations. The hydrothermal pretreatment caused solubilisation of arabinoxylan and phenolic compounds in a temperature and hold-time dependent manner. Effects on the cellulose and lignin contents were minor. Microscopy revealed intensification of staining of cellulose and relocalisation of xylan in the cell walls of pretreated samples. Phloroglucinol staining and autofluorescence imaging suggested changes in distribution and structure of the cell wall phenolic compounds. In the enzymatic hydrolysis, the highest yields were obtained from the samples with a low xylan and diferulate content.

B14: Selenium modulates the activity of chloroplastic fructose 1,6-bisphosphatase with synergistic effects on carbohydrates and nodulation in alfalfa (*Medicago sativa* L.)

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Selenium (Se), an essential micronutrient in humans and animals, is required by several selenoproteins such as glutathione peroxidase for important antioxidation roles. Selenium is circulated in the food chain via dietary plants. Studies have reported on positive effects of Se on growth, stress tolerance and energy metabolism in plants. However, its classification as a plant micronutrient remains in doubt. This study investigated effects of Se on fructose 1,6-bisphosphatase (F1,6-BPase), along with carbohydrate metabolism and nodulation in alfalfa. Separate experiments for nodulation studies (pot) and carbohydrates studies (nutrient solution) with different Se (0, 1, 5, 10 and 15 μM Na_2SeO_4) and N (2 and 10 mM) concentrations were conducted. Plants in perlite (pot) were inoculated with *Sinorhizobium meliloti* and data on growth and other physiological parameters were analyzed at different time points. Selenium addition (15 μM) increased enzyme activity about 50 % but the effect diminished with time. Similar increases in soluble sugars (45 %) and starch (30-55 %) accumulation in shoots and roots were recorded. In addition, low Se (1 and 5 μM) increased nodule number (NN) about 40 % and 62 % respectively. However, NN decreased with plant growth, and the effect of Se on nodule fresh weight (NFW), nitrogenase activity and nitrogen (N) concentration in plants was not significant ($p \geq 0.05$). Selenium had a negative effect on dry matter (DM) yield. These results suggest that the improved energy balance in plants may have beneficial effects on nodulation. Further studies on the role of Se in N_2 -fixation are needed.

C) Physiology

C1: Paraquat and the power of the dark side

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In plants it is widely accepted that the toxicity of paraquat (methyl viologen; MV) requires light and occurs via chloroplast ROS generation. However, MV is also toxic in non-photosynthetic organisms, via mitochondrial ROS production. To address alternative mechanisms in plants, MV responses were assayed in the dark. MV significantly restricted seedling growth in the dark in Col-0 and the MV tolerant *rcd1* mutant. Increased sugar concentrations, which increase mitochondrial activity, enhanced MV toxicity in the dark. RNAi knockouts of the mitochondrial specific MnSOD were more sensitive to MV under dark condition suggesting that MV induces ROS production in plant mitochondria.

To further define MV response mechanisms in the dark and light, several genetic screens were performed. First, 93 *Arabidopsis* ecotypes were tested, demonstrating a high level of natural variation in *Arabidopsis* MV response. Trends in MV sensitivity were the same in the light and dark although a few ecotypes behaved differently. Recombinant inbred lines of Kondara x Ler were used for QTL mapping; this indicated that loci regulating ROS response in the dark and the light were distinct. Additionally, a reverse genetic screen of 53 single mutants and 36 *rcd1* double mutants were tested demonstrating roles for stress hormones and ROS related genes. In addition, exogenous SA, MeJA, ABA and ACC increased plant MV resistance. Reverse genetic screens also demonstrated a partially distinct profile of loci controlling MV responses between light and dark conditions.

The role of ascorbate peroxidases (APXs) was tested. Only the cytosolic *apx1* mutant exhibited enhanced sensitivity to MV. APX protein levels were unchanged in plants with different MV tolerance such as Col-0 and *rcd1* or between several sensitive and tolerant ecotypes. ET-induced MV tolerance was dependent on stromal and thylakoidal APX; other hormone-induced MV tolerance (SA, MeJA, ABA) was APX-independent.

C2: Phylogenetic analysis of SLAC1 protein family structure and predicted function in land plants

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The S-type anion channel SLAC1 has been shown to be responsible for rapid stomatal closure in response to various environmental factors in *Arabidopsis thaliana*. The molecular mechanisms governing SLAC1 regulation have been extensively studied but more research is needed to understand its role in plant ecophysiology. Our aim is to understand the significance of SLAC1-dependent stomatal regulation under natural conditions. We are elucidating the presence and structure of SLAC1 protein family and related stomatal functions in several plant species for which genome sequence information is available. Available sequence information and stomatal conductivity measurements suggest that *Populus* species may have lost the functionally important features of SLAC1 and thus have altered ability to regulate their stomatal aperture. Here we have conducted a phylogenetic analysis of SLAC1 protein family and measured stomatal sensitivities against different stimuli

in numerous plant species. The aim is to link the variation in protein structure and conservation to the differences in stomatal regulation between various plant species.

C3: Transformation of Norway spruce (*Picea abies*) embryogenic tissue culture line with three xylem-expressed heme peroxidases to affect lignification

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Class-III secretory plant peroxidases are considered to participate, among other multiple functions, in the formation of lignin and xylem cell walls during plant cell development. Earlier, we have cloned and sequenced three xylem-expressed heme peroxidases from Norway spruce (*Picea abies*) (Marjamaa et al. 2006, Plant Mol. Biol. 61: 719-732). All the three translated proteins, PA-PX1, PA-PX2 and PA-PX3, contain the conserved amino acids required for heme binding and peroxidase catalysis. The peroxidases begin with putative secretion signal leader peptides but diverge substantially at protein phylogenetic level, grouping to two subclusters when aligned with other class-III plant peroxidases. We postulate that at least one of these new xylem-expressed peroxidases, the high-cationic PA-PX1 xylem peroxidase is secreted and localized to the spruce tracheid cell walls where this enzyme is possibly involved in lignin biosynthesis. In the present work, these peroxidase genes were used, in sense and antisense direction together with ampicillin resistance gene, to biolistically transform an embryogenic Norway spruce tissue culture line with the aim to affect lignification in the xylem cell walls. After transformation, transformed tissues were selected on Basta plates, grown further to torpedo stage, changed to a maturation medium, and after a drying stage and cold treatment at +6°C for five weeks, plantlets could be recovered and transplanted to soil and grown further in the greenhouse. Next, once the plantlets have grown somewhat, we can analyse the xylem for possible changes in lignin amount and composition.

C4: Cysteine-rich receptor-like kinases (CRK) in ROS signaling in *Arabidopsis thaliana*

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The receptor-like kinase (RLK)/Pelle gene family constitutes a major gene family in land plants with respectively over 610 members encoded in the *Arabidopsis* genome. RLKs are implicated in all aspects of the plant life cycle from embryogenesis to responses to the environment. CRK (Cysteine-rich RLK)/DUF26 (Domain of unknown function 26) sub-family with 44 members belong to one of the largest RLK sub-families in *Arabidopsis*. CRKs possess two copies of the DUF26 domain in their extracellular domain which contains the C-X8-C-X2-C motif, which might have a function in redox regulation and/or protein-protein interaction. CRKs have been shown to be transcriptionally induced by oxidative stress, pathogen attack and salicylic acid. Many CRKs, including CRK6 and CRK7, have been shown to be transcriptionally induced after ozone (O₃) exposure. Interestingly, most of the 44 CRK knock-out plants lead to sensitivity to extracellular reactive oxygen species (ROS), resulting at least in subtle lesion formation, upon O₃ treatment, indicating their involvement in ROS signalling. In addition, the sensitivity of the CRK knock-out plants to xanthine/xanthine oxidase (X/XO), which induces extracellular ROS production, has been tested. Typical RLKs are transmembrane proteins that sense signals with their extracellular domain and relay them to the interior of the cell through their intracellular kinase domain *via* phosphorylation events. To verify the functionality of corresponding kinase domains, kinase domains of CRK6

and CRK7 has been expressed in *E. coli* and kinase activity has been assessed. Moreover the identification of the interacting proteins and downstream targets of the CRKs is also crucial for the understanding of the ROS signaling. We expect the split-ubiquitin membrane yeast two-hybrid technology to be valuable for the identification of potential interacting partners of the CRKs. The results will offer new and exciting perspectives in ROS signaling and the study of the role of CRKs in plants.

C5: Characterization of Flower related Ubiquitin Proteasome System components and their targets

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Molecular regulation of flowering time integrates environmental and endogenous signals to promote flower development and thus contributes to the reproductive success of the plant. Regulated protein degradation by the Ubiquitin Proteasome System (UPS) plays an important role in plant signaling. Our working hypothesis is that as much as transcription factors are expressed in tissue specific manner, they will also be repressed at protein level in developmental and spatial context. In most cases the mechanism of this instability has not been characterized but regulated protein degradation by Ubiquitin (Ub)/26S Proteasome will likely be involved. Only a fraction of all the proteins involved in plant UPS have been characterized to date. In this project our main objective is to identify Flower related Ubiquitin Proteasome System components that we call the FUPS. We have set up different screens to identify these flower specific protein degradation components and aim to characterize them at molecular level. This study will provide the flower development research with functional data about upstream regulatory mechanisms and downstream molecular networks. Ultimately these data will lead to the establishment a knowledge database about “Flower related Ubiquitin Proteasome System”.

C6: The Role of Cysteine-rich Receptor-like Protein Kinases in ROS Signaling in *Arabidopsis thaliana*

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Plants monitor their surroundings by sensing temperature, light, nutrients, humidity, air pollutants, pathogens etc., and adapt to the changing conditions by altering their metabolism and defense accordingly, which demands complex signaling processes. Receptor-like protein kinases (RLKs) are sensor proteins which are involved in many important signaling pathways in plants, such as in growth and development, self-incompatibility and bacterial resistance as well as in oxidative stress signaling.

The production of reactive oxygen species (ROS) is a common response to many stresses in plants. The production and the action of ROS are critical to the stress recognition and the coordination of the plant's response to those stresses. ROS is also an important regulator of plant development and controls processes such as root development, pollen tube growth but also cell expansion. In contrast to ROS as signaling molecules, plants can suffer from oxidative stress when ROS production exceeds antioxidant capacity and can ultimately lead to cell death by ROS toxicity.

ROS are sensed via yet unidentified mechanisms. We have found several Cysteine-rich RLKs (CRKs), which respond to ROS. Up-regulated gene expression and ROS sensitive phenotype of the corresponding knock-out plants suggests that these proteins have a role in

ROS-mediated signal transduction pathway. We propose that the members of the CRK subfamily could act as ROS “receptors” by sensing ROS through redox modifications of the extracellular domain which could activate receptor complex and lead to signal transduction and response. Phenotypic analysis of the knock-out plants and the overexpression plants under various conditions including ozone, pathogen, paraquat, X/XO, etc., have provided more information about the biological relevance of these proteins and supported our hypothesis.

C7: Changes in Photosystem II heterogeneity under changing climatic conditions

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Recent climatic change are mostly related to global warming which is accompanied by increased frequency of high temperature regimes, decreased availability of water (water stress/drought) and increased salt concentration (salinity) in the soil. Photosynthesis is one of the most important metabolic processes in plants and its study provides information about the general “health” of plants. Photosystem II complex in the photosynthetic machinery is not homogenous in nature and differs in its structure and function both. This diverse nature of PSII is known as Photosystem heterogeneity. Two major types of PSII heterogeneity are: PSII antenna size heterogeneity (characterized by α , β , and γ centres) and the PSII reducing size heterogeneity. On the basis of differences in antenna size, α , β and γ centers have been defined and on the basis of acceptor side function, Q_B -reducing and Q_B -non-reducing centers have been defined. Extent and nature of PSII heterogeneity may vary under different physiological conditions i.e. salinity stress, temperature stress etc. The results revealed that at high temperature as well as high salinity, there is a change in the relative amounts of PSII α , β and γ centers. As judged from the complementary area growth curve, it seemed that the PSII $_{\beta}$ and PSII $_{\gamma}$ centers increased at the expense of PSII $_{\alpha}$ centers. The reducing side heterogeneity was also affected as shown by an increase in the number of Q_B -non-reducing centers. The work is a significant contribution to understand the basic mechanism involved in the adaptation of crop plants to stress conditions.

C8: Biosynthesis of 4-Hydroxy-5-Methylcoumarin in *Gerbera hybrida*

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Plants produce a great variety of secondary metabolites which have both biological and pharmaceutical properties. Plant polyketide derived compounds are one of the largest and most diverse groups of plant secondary metabolites. Polyketides have important roles in plants acting, e.g. defense and antimicrobial agents (Koskela et al. 2011). They also have essential roles in medicine due their pharmaceutical properties. Polyketide synthases are closely related enzymes, which catalyze from one up to eight condensing reactions between malonyl-CoA and variety of CoA-thioester starters (Austin & Noel 2004). Previously we have characterized a 2-pyrone synthase (2PS) as a type III polyketide synthase from the ornamental plant *Gerbera hybrida*. 2PS is involved in the biosynthesis of antifungal compounds gerberin and parasorboside (Eckermann et al. 1998, Koskela et al. 2011). *Gerbera* is also known to contain 4-hydroxy-5-methylcoumarin. The biosynthesis of the coumarin is still unclear. Previous research indicates that it is derived from the acetate-malonate pathway (Inoue et al. 1989). The model suggests that the enzyme uses acetyl-CoA and four malonyl-CoA molecules to produce a pentaketide which is further reduced by another enzyme to finally produce the chemical of interest. The enzyme catalyzing this reaction remains still unknown but it is thought that it comes from the closely related type III polyketide synthases in *Gerbera*.

C9: Post-translational modifications of ferredoxin-NADP⁺ oxidoreductase isoforms in *Arabidopsis thaliana* chloroplasts

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Post-translational modifications of proteins mediate fast responses to external and internal stimuli in plants by changing the activity, half-life and localization of proteins as well as their interaction with other molecules. Phosphorylation is a well-established modification affecting the activity of a multitude of chloroplast proteins, whereas acetylation, methylation, nitrosylation and glycosylation of chloroplast proteins have gained attention only during the recent decade. Ferredoxin-NADP⁺-oxidoreductase (FNR) catalyzes the last electron transfer reaction of the light reactions of photosynthesis producing reduced NADPH. *Arabidopsis thaliana* has two genes encoding different FNR isoforms (FNR1 and FNR2) in its genome. Both of the isoforms are present as soluble proteins in chloroplast stroma as well as attached to thylakoids. Additionally, both FNR1 and FNR2 appear as two distinct spots (the acidic and basic forms) in 2D-SDS-PAGE, which indicates that both of them are post-translationally modified. Our results show that both of the chloroplast FNR-isoforms FNR1 and FNR2 are uniformly N-glycosylated. We have also shown with MS analysis that both FNRs are differentially methylated and acetylated, which could give rise to change in the pI of the proteins. Additionally, tyrosine nitration seems to occur in both isoforms, whereas the most common form of modification, phosphorylation, is completely absent. The physiological significance of these modifications will be discussed.

C10: Involvement of ROS, a class III peroxidase and the mitochondrial protein TSPO1 in the response of *Physcomitrella patens* to the fungal elicitor chitosan

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Physcomitrella patens wild type (WT) and two gene knockout mutants ($\Delta PpTSPO1$ and $\Delta PpPrx34$) were studied for their biotic stress responses (Lehtonen *et al.* 2012, MPMI 25: 363–371). Chitosan treatment caused an instant oxidative burst of extracellular superoxide in *P. patens* WT. In contrast, the previously characterized mutant $\Delta Prx34$ lacking the gene for the chitosan responsive secreted class III peroxidase (Prx34) resulting in an increased susceptibility to fungal infection was incapacitated in superoxide production. The genes encoding NADPH oxidase (NOX), alternative oxidase (AOX), plastidial lipoxygenase (LOX7), and a translocator protein TSPO1 involved in mitochondrial tetrapyrrole transport and abiotic stress were induced in chitosan treated *P. patens* WT. However, in the $\Delta PpTSPO1$ mutant expression levels of *PpAOX*, *PpLOX7* and *PpNOX* were constitutively elevated and superoxide production upon chitosan treatment was doubled compared to *P. patens* WT. Unexpectedly, the $\Delta PpTSPO1$ mutants were more sensitive to fungal infection than WT suggesting uncontrolled damage by reactive oxygen species. Our results show that moss responds to a common pathogen elicitor with an oxidative burst, which involves a class III peroxidase, similar to vascular plants. Our results also indicate a role of mitochondrial PpTSPO1 in maintenance of redox homeostasis during biotic stress besides its known involvement in abiotic stress adaptation.

C11: Natural variation in ozone induced cell death in *Arabidopsis*

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Plants constantly face both biotic and abiotic stresses in nature. To increase their fitness plants have developed ways to survive through stressful periods. One of the ultimate ways is programmed cell death (PCD), which for example prevents the spread of some pathogens by restricting access to nutrients. One signal intermediate in plant defence, including PCD, are reactive oxygen species. The atmospheric pollutant ozone (O₃) can be used to study signalling activated by reactive oxygen species and treatment of plants with short, high concentrations of O₃ activates PCD. The molecular responses to acute O₃ exposure are overlapping with responses to other stresses. We have used acute O₃ exposure to trigger PCD in more than 350 natural accessions of *Arabidopsis thaliana* and in 400 multi-parent advanced generation inter-cross (MAGIC) lines. Earlier studies have shown phenotypic variation in response to O₃ exposure between a set of natural accessions and that stomata play an important role in plant O₃ responses by varying the speed of stomatal closure between accessions. We have mapped genetic regions involved in natural variation of O₃ induced cell death and stomatal signalling by association mapping and QTL mapping of MAGIC lines. The underlying genetic variation has proven to be complex.

C12: Effects of drought stress on polyamine metabolism in Scots pine embryogenic cell culture

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Polyamines (PA) are small cations existing in all living organisms and having a fundamental role in maintenance of viability and growth of the cells. PAs are involved also in stress related processes in plants. We have proved Scots pine (*Pinus sylvestris* L.) embryogenic cell culture to be a favourable experimental platform to study PA metabolism (Vuosku et al 2012). In the present study we developed the method to study the effect of drought stress on PA metabolism in Scots pine embryogenic cells. Drought stress was caused by 0%, 6% and 14% polyethylene glycol (PEG) 6000 treatments. The concentrations of free and soluble conjugated PAs as well as the expression of PA metabolism (*ADC*, *SPDS*, *ACL5*, *DAO* and *PAO*) and stress related genes (*LEA* and *CAT*) were measured. The changes in PA metabolism were combined with the data on growth rate and cell viability to elucidate the effects of drought stress on Scots pine embryo genic cells both on physiological and molecular level. According to preliminary results PEG treatment decreased viability of the cells but did not affect growth rate. The expression of the PA biosynthesis genes was repressed under drought stress, whereas the expression of the PA catabolism and stress related genes was not affected.

Vuosku J. et al (2012). *Tree Physiology* 32(10): 1274-1287

C13: The N-Terminus of PaRBOH1 from *Picea abies* is a Phosphorylation Target

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Lignin biosynthesis during secondary plant cell wall formation is a process of oxidative polymerization of monolignol radicals activated by H₂O₂-requiring peroxidases and/or O₂-requiring laccases. One possible source of apoplastic reactive oxygen species (ROS) are NADPH oxidases, known as respiratory burst oxidase homologs (RBOHs) in plants. RBOHs are a regulatory merging node in the ROS generation network by integrating phosphorylation and Ca²⁺-signaling, a function determined by multiple serine, threonine and tyrosine amino acid residues as well as by Ca²⁺-binding EF-hand motifs in their N-terminal domain. RBOHs are also a known target for Ca²⁺-dependent protein kinases. The full-length sequence of a NADPH oxidase PaRBOH1 has been isolated previously from an extracellular lignin-producing tissue culture line of *Picea abies* (L.) Karst.

We performed *in silico* analysis of PaRBOH1 to predict possible post-translational modifications. In order to determine whether the predicted phosphorylation events really happen, the initial 402 amino acids of the N-terminal part have been heterologously expressed in *E. coli* with an additional 6xHis-tag at the C-terminus. The purified peptide has then been used as a substrate in radioactive *in vitro* kinase assays with several types of spruce protein extracts as kinase sources, and Ca²⁺ supplementation or depletion. We have been able to purify ³³P-labelled PaRBOH1 N-terminal peptide from the kinase reaction mix, which indicates that the peptide was indeed subjected to phosphorylation(s). Currently we are conducting non-radioactive kinase reactions followed by mass spectrometry analysis in order to resolve the phosphorylation number and sites in the peptide.

C14: Virulence mechanisms of the soft rot pathogen *Pectobacterium*

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The soft rot bacteria of the genus *Pectobacterium* are an economically important group of plant pathogens characterized by their massive production of plant cell wall-degrading enzymes, which are aggressively used to decay plant tissue for nutrient acquisition. We have sequenced the genome of a well characterized soft rot model strain SCC3193. Extensive phylogenetic analysis demonstrates that SCC3193, previously identified as *Pectobacterium carotovorum*, instead belongs to the poorly characterized species *Pectobacterium wasabiae*, which has recently been reported from potato in increasing numbers worldwide. We have identified features unique to *Pectobacterium wasabiae* as well as potential novel virulence determinants in the genome of SCC3193. We have experimentally verified that two partially redundant type VI secretion systems and the putative regulator SirB contribute to virulence of SCC3193. Genomic analysis of SCC3193 has also revealed other interesting traits that may be related to life in planta including a predicted benzoic acid/salicylic acid carboxyl methyltransferase of putative eukaryotic origin. We have shown that this enzyme has the ability to methylate salicylic acid, a crucial component in plant defense responses. Thus, *Pectobacterium* could use this enzyme during colonization of the host plant to modify salicylic acid-dependent defenses.

C15: ERD15 - an attenuator of abscisic acid responses in *Arabidopsis thaliana*

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Adaptation to changing environments is a prerequisite for plant growth and survival. Tolerance to stresses can be enhanced by metabolic and physiological adjustments mediated by plant hormones. One of the central phytohormones controlling plant stress responses is abscisic acid (ABA) which has a wide range of essential functions in plant growth and development. *ERD15* (*Early Responsive to Drought 15*) was characterized as a regulator in ABA signalling in stress responses in *Arabidopsis thaliana*.

In silico analysis revealed that ERD15 belongs to a small protein family, ubiquitous in but restricted to plant kingdom. In each species from green algae to higher plants the family contains typically two to three members. The origin of ERD15 can be estimated to coincide with the early diversification of land plants from green algae.

ERD15 contains a previously characterized PAM2 motif that enables the interaction with the C- terminus of poly(A)-binding proteins (PABP). Interestingly, analysis of ERD15 family proteins revealed two additional, highly conserved motifs. The first of these was designated PAE1 (PAM2 Associated Element 1) and the second QPR. No function has so far been assigned to PAE1 and QPR motifs, but their evolutionary conservation and occurrence throughout the ERD15 protein family indicate a functionally important role.

C16: The Role of F-box-proteins in Stress Signaling in *Arabidopsis thaliana*

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Induced defenses and stress responses play a major role in plant disease resistance and are regulated by a network of interconnected signal transduction pathways with the plant hormones ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) as crucial mediators. These specific hormone-mediated signaling cascades trigger distinct sets of stress-related genes leading to tolerance or resistance. F-box proteins (roughly 700 in *Arabidopsis*) are important components in these stress responses. They act as members of SCF protein complexes, which target substrate proteins for modification and degradation and thereby allow plants to respond rapidly to environmental stress factors. They are involved in various processes including plant metabolism, hormonal responses as well as responses to environmental stresses. The objective of this study is to identify and characterize specific F-box proteins involved in stress signaling and components interacting with these F-box proteins. To elucidate the function of F-box proteins belonging to subfamily C3 in the regulation of stress responses, we employ a reverse genetic screening approach and characterize T-DNA mutant lines of this subfamily of F-box proteins for their performance under abiotic (cold, ozone, drought) and biotic (*Erwinia carotovora*, *Botrytis cinerea*, *Alternaria brassicicola*) stresses. Mutants with altered stress response phenotypes will be subjected to microarray analysis to elucidate the response pathways a given F-box protein is involved in. So far, more than 50 F-box mutant lines were screened with the pathogens *Erwinia carotovora* and *Botrytis cinerea* and cold, ozone, salt and different hormones. We have found many candidates which are showing clear phenotype to pathogen (*Erwinia*), ozone, and hormone (ABA) treatments. Our research is focusing on these confirmed candidates.

C17: Temperature controls flowering in *Fragaria vesca* through *FvTFL1*

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Photoperiod and/or temperature control seasonal flowering in perennials. However, temperature regulation of flowering is not well studied at the molecular level. *Fragaria vesca* is a perennial, facultative short day (SD) plant in which the homolog of floral repressor TERMINAL FLOWER1 (*FvTFL1*) causes seasonal flowering¹. In nature flower induction occurs in the autumn under SD at intermediate temperatures. However, at cool temperatures flower induction occurs independently of photoperiod, and high temperature inhibits flowering in both SD and long day (LD). We show that the critical temperature to induce flowering under LD is close to 13°C. At 10°C, plants flowered at the same time in both SD and LD, whereas at 13°C, a few plants remained vegetative under LD. SD was obligatory to flowering at 16°C. At 23°C, plants did not flower at all. The expression of *F.vesca* homolog of florigen, *FLOWERING LOCUS T* (*FvFT1*), was not clearly affected by temperature indicating that temperature regulation of flowering does not require changes in the expression of *FvFT1*. In contrast, *FvTFL1* expression showed clear correlation with flowering results in all treatments. The expression was low in both photoperiods at flower inductive temperatures of 10 and 13°C, although weak photoperiodic effect was remaining at 13°C. However, at 16°C *FvTFL1* was highly up-regulated specifically under LD, and at 23°C, high mRNA levels were found in both photoperiods. Furthermore, the over-expression of *FvTFL1* inhibited flower induction at

cool temperature. Our results suggest that in addition to photoperiodic flowering¹ *FvTFL1* is a key floral repressor also in temperature mediated flower induction.

References: 1) Koskela et al. 2012. *Plant Physiol* 159: 1043-1054.

C18: Perception and downstream signaling of apoplastic reactive oxygen species

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Reactive oxygen species (ROS) have important roles as signaling molecules in plant stress responses and development. Ozone (O₃), which degrades to ROS in the extracellular space of plant cells, can be used as a tool to unravel in planta ROS signaling processes. O₃ enters into the apoplast of plant cells through stomata and initiates ROS signaling pathways leading to stomatal closure. Forward genetic screens for O₃ sensitivity have led to significant molecular insight into regulation of stomatal function. The aim of this project is to identify novel components in early stomatal signaling downstream of apoplastic ROS. This study focuses on characterization of two novel O₃ sensitive mutants with altered stomatal function, *rcd7* and *suu*. RCD7 and SUU are kinases, which regulate O₃- and CO₂-induced stomatal closure. A mutant screen is also being conducted to discover components involved in stomatal Ca²⁺ signaling, and to elucidate how they are involved in ROS-dependent regulation of stomatal movements. Although the Ca²⁺ dependence of stomatal closure mechanisms has been demonstrated, mutants with impaired guard cell Ca²⁺ sensors and Ca²⁺ channels have largely remained unidentified. The screen utilizes transgenic Ca²⁺ indicator plants to allow identification and visualization of Ca²⁺-related phenotypes. Understanding the regulation of stress responses and stomatal movements by ROS has important implications for agriculture and applied research towards improving crop quality.

C19: Genetic analysis of a T-DNA tagged 59_1 mutant altered in OG-responses and disease resistance

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Pathogens are one of the most important biotic factors limiting growth, productivity and distribution of plants. Being immobile plants have evolved a sophisticated array of adaptive strategies which allow them to recognize the presence of potentially harmful microbes and trigger the responses required for optimal growth and survival. Many necrotrophic plant pathogens produce plant cell wall degrading enzymes which are crucial virulence factors releasing oligogalacturonide (OG) fragments from plant cell walls that plants can recognize as DAMPs and activate innate immune responses. In recent years there has been a wealth of studies to elucidate defence responses triggered by OGs, however, very little is still available about perception of OGs and the following downstream events. Therefore, to be able to dissect the initial stages of OG signal transduction and identify the particular signalling intermediates that lead to the activation of the distinct pathways involved in plant innate immunity, we employed a high-throughput genetic screen in the model plant *Arabidopsis thaliana*. The screen took advantage of *Arabidopsis* mutant pools generated by T-DNA activation tagging. This approach results in two types of outcomes; either the gene is inactivated by the insertion, or a nearby gene is activated from the strong 4x35S enhancer at the right border of the T-DNA (Weigel et al 2000). OG-triggered growth retardation of

Arabidopsis seedlings was used as a primary screen for mutants insensitive to OGs followed by screening of associated pathogen sensitivity/resistance phenotypes. Several tens of mutants were isolated from T-DNA activation tagging pool. Mutated genes were identified by genomic DNA walking techniques and 59_1 mutant has been selected for extensive genetic analysis.

C20: Genomics and characterization of plant associated yeasts of the genus *Taphrina*.

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Taphrina are early diverging Ascomycete yeasts related to the eukaryotic model organism *Schz. pombe* (Fission yeast) and mammalian lung pathogens (*Pneumocystis spp.*). The majority of circa 100 known members of the genus *Taphrina* are pathogens of woody plants. They are widely distributed and have a characteristic dimorphic life cycle switching between a saprobic unicellular haploid yeast stage and a parasitic multicellular dikaryotic filamentous stage on their host plants. The molecular interaction of plants with yeasts is understudied making *Taphrina* species attractive model organisms. *Taphrina* cause significant damage to economically important trees such as peach (*Prunus spp.*) and birch (*Betula spp.*). They characteristically induce tumor formation, notably *T. deformans* causing leaf curl on peach and *T. betulina* causing Witch's broom on birch (Tuulenpesä in Finnish). The production of plant hormones by some *Taphrina* is well documented and widely assumed to be involved in pathogenesis and tumor formation. But generally molecular mechanisms of *Taphrina* pathogenesis remain unknown. We are currently working to develop model pathosystems with *T. betulina*, *T. deformans* and their respective host plants, which have been or are currently the subjects of genome sequencing projects. We have produced a draft genome sequence *T. betulina*; analysis is underway in order to deepen our knowledge on *Taphrina* cell biology, discover pathogenicity genes, and promote the development of molecular methods. Candidate genes for plant hormone biosynthesis have been identified and are currently under study. Our characterization of *Taphrina* species and analysis of the *T. betulina* draft genome will be presented.

C21: RCD1 and recognition of poly(ADP-ribosyl)ation in *Arabidopsis*.

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Plants are continuously exposed to abiotic and biotic stresses that endanger their survival. To cope with all these challenges, plants have developed a repertoire of tightly controlled signaling pathways. Protein posttranslational modifications (PTMs) are the most rapid response to stress or metabolic changes in all eukaryotic cells. Poly(ADP-ribosyl)ation is known to be involved in plant stress response (1, 2); however, no target proteins or proteins involved in recognition of poly(ADP-ribose) chains have been described.

Radical-induced Cell Death 1 (RCD1) protein and its closest homolog Similar to RCD One 1 (SRO1) are the only *Arabidopsis* proteins containing WWE domain of unknown function (3). Here we show that WWE domain specifically recognizes and binds poly(ADP-ribose) chains. Moreover, the RCD1 protein itself is tightly controlled by PTMs *in planta* and is regulated at the protein level in response to stress. These data provide novel insights into the biochemical function of RCD1, a central integrator of plant stress and developmental response.

1. De Block M et al, 2005, *Plant J*, 41, 95-106.
2. Vanderauwera S et al, 2007, *Proc Natl Acad Sci*, 104, 15150–15155.
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C22: Norway spruce xylem plasma membranes in monolignol transport studies

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Plant secondary cell wall lignification is a process that includes several steps. Monolignols, the lignin monomers, are first synthesized in the cytoplasm of lignifying cells via the phenylpropanoid pathway, and then transported to the cell wall, where they are polymerized into lignin. The transport through the plasma membrane has for long been a riddle. In 2010 Miao and Liu (PNAS, 107:22728) found that the transport of a major monolignol, coniferyl alcohol, is ABC-transporter-mediated in *Arabidopsis thaliana* leaf tissue, and in 2012 Alejandro et al. (Current Biology, 22:1) found an ABC-transporter that is responsible for the transport of another monolignol, p-coumaryl alcohol, in *Arabidopsis*. However, the question still remains if these same transporters are functioning in the developing xylem tissue and/or in other plant species. We are testing this by using developing xylem of an economically important gymnosperm species, Norway spruce (*Picea abies*), where coniferyl alcohol is the native monolignol. We have optimized a generally used plasma membrane vesicle enrichment protocol, two-phase partitioning (Widell & Larsson 1981, *Physiologia Plantarum*, 51:368), for spruce xylem material. The enrichment of plasma membranes can be shown by enzyme marker tests (e.g. glucan synthase marker for plasma membranes). We are following the transport of radioactively labeled coniferyl alcohol and its glucoside, coniferin, into the vesicles of enriched plasma membrane fraction. In contrast to the results by Miao and Liu (2010), our preliminary results do not show any ATP-dependent transport activity for coniferyl alcohol. However, coniferin transport was detected and this transport was ATP-dependent. The results must still be confirmed with appropriate repetitions, and the transport of coniferyl alcohol tested with some other energizers in addition to ATP.

C23: Different diurnal patterns of stomatal conductance under blue, green and red irradiation: roles of cryptochromes and phototropin

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Light signals have different entry points to the control of stomatal conductance, including direct effects via phototropins in guard cells and more indirect effects via cryptochromes and mesophyll-based processes such as the control of carbon dioxide levels in the sub-stomatal chamber. Little is known about the dynamic relative contribution of these processes during the course of the photoperiod. To better understand how stomatal conductance is regulated through the different photoreceptors at different times of the day, we designed the following experiments. *Arabidopsis* (*Arabidopsis thaliana*) wild type Col-0, mutants, *cry1 cry2* lacking cryptochromes 1 and 2, and *phot1 phot2* lacking phototropins 1 and 2 were grown in growth room at 23°C, 67% RH and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR from fluorescent tubes (07:00-19:00) for 3 weeks. Seedlings were subsequently irradiated with 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of red, blue or green LEDs for one 12 hour photoperiod (07:00-19:00). Stomatal conductance and photosynthesis of each plant was continuously recorded using an infra-red gas analyser. The time course of stomatal conductance of wild type and photoreceptor mutants was compared during the first hour of each coloured light treatment (07:00-08:00), to investigate how cryptochromes and phototropins participate in stomatal opening in the morning. Differences in the time of maximum stomatal opening under red, blue and green light were observed, and their dependence on cryptochromes or/and phototropins was assessed.

C24: The role of *dnd1* in reactive oxygen species signaling

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The increase of cytosolic Ca^{2+} is vital event in the plant defense signaling cascades. The 'Defense, no death' (*dnd1*) mutant plants have a null mutation in the gene encoding the plasma membrane localized Ca^{2+} -conducting CNGC2 channel. It displays high SA concentration, spontaneous cell death, dwarf phenotype, ozone tolerance, and display no hypersensitive response (HR) to infection by some pathogens, etc. Although previous studies show that Ca^{2+} is essential signaling molecules, however under ozone treatment the pleiotropic phenotypes of the *dnd1* mutant allow alternative interpretations. In order to interpret *dnd1* and the role of Ca^{2+} in senescence and ozone signaling, 22 double and several triple *dnd1* mutants were obtained. Important key genes involved in SA signaling, JA signaling, ethylene signaling, ABA signaling were selected in this study. Several important defense signaling genes are found to be expressing significantly different from these mutants. Furthermore, all these mutants were exposed by ozone, and damage and gene expression were quantified, to study the role of Ca^{2+} and cross talking with other hormone in plant defense signaling.

D) Ecology & Ecophysiology

D1: Effect of increased and decreased air humidity on light sensitivity of shoot hydraulic conductance of deciduous trees

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Acclimation of the light sensitivity of the hydraulic conductance of shoots of silver birch (*Betula pendula*) and hybrid aspen (*Populus x wettsteinii*) to the air humidity of the growth environment was studied. The light sensitivity of shoots grown in three different air humidities was found to vary. Amongst the shoots grown in current natural air humidity, only the hydraulic conductance of the whole shoot and that of the leaf blades of birch upper foliage were significantly light sensitive. Amongst the shoots grown in decreased air humidity, hydraulic conductance of the whole shoot, the leaf blades, and the stem and petioles of birch upper foliage, the conductance of the whole shoot and the leaf blades of birch lower foliage, and the conductance of the whole shoot of aspen upper foliage were light sensitive. In increased air humidity all the shoots had forfeited significant light sensitivity. We predict that light sensitivity will become more widespread among species in the districts where air humidity decreases as a result of global climate change, and vice versa. Low white light caused the same increase in hydraulic conductance as high white light, and blue and white light caused a two times greater increase in conductance than red light. Thus, the air humidity of the growth environment does not remarkably modify the mechanism of light sensitivity.

D2: Solar ultraviolet-B radiation ameliorates some effects of drought on birch (*Betula pendula* Roth.) seedlings

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We aimed to assess whether solar-ultraviolet radiation (UV) when combined with water deficit will create either an additive, synergistic, or ameliorative effect on the severity of stress imposed on silver birch (*Betula pendula* Roth.) seedlings. To test this, plants were grown in a replicated fully-factorial experiment under nine treatment combinations filtering ultraviolet-A and ultraviolet-B from solar radiation together with differential watering to create water deficit conditions over two months. The effects of solar UV radiation on growth and seedling morphology were evident after the first month of treatments (June), and mostly persisted, without increasing, over the second month. The removal of solar UVB and UVA&B reduced seedling growth and leaf size under water deficit, compared with seedlings receiving the full spectrum of solar radiation; whereas well-watered seedling growth was largely unaffected by UV-filter treatment. There was no interactive effect of filter-by-water treatment for leaf morphology, nor pigmentation, although epidermal flavonoid content was reduced by UVB- and UVA&B- treatments and by water deficit. The treatment combination differentially affected water relations, with seedlings under near-ambient UV exhibiting the largest predawn-to-midday leaf water potential difference. Treatment differences in gas exchange were small, but instantaneous water use efficiency was highest in seedlings under water deficit and near-ambient UV; a response that was consistent with other water-use traits. Overall, these results reveal that the strong negative effects of water deficit are partially ameliorated by solar UV radiation, whereas well-watered birch seedlings are only very slightly disadvantaged by receiving solar UV radiation.

D3: Intra- and inter-plant communication in Birch (*Betula* spp. Betulaceae)

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Under herbivore attack, plants release a wide diversity of volatile organic compounds (VOCs) which can act as direct or indirect chemical defenses. Additionally, VOCs play an important role as airborne signals that are perceived by neighboring undamaged plants or undamaged organs of the same individual. Inter- and intra-plant signaling via airborne cues are important processes that prime or induce plant defenses against herbivores, being faster and independent of vasculature connections. Silver birch (*Betula pendula*, Betulaceae) trees are important species in the subarctic ecosystems of Northern Scandinavia and host numerous herbivore species, which can lead to total destruction of leaf foliage. Hence, rapid activation and display of defenses are crucial for the threatened plant and its neighbors. In this conference the results of experiments conducted in greenhouse conditions to assess the priming or induction of defenses via airborne signals will be presented. Birch trees and larvae of *Epirrita autumnata*, the autumnal moth, comprised the main study system. The herbivores were added to three branches and allowed to feed for five days, undamaged branches of the same individual and undamaged trees were placed adjacent to the infested branches, avoiding physical contact. After that, the undamaged branches and trees were challenged with generalist herbivores to assess the VOC emission over different periods of time. The results show that the VOCs released from damaged tissue can prime the emission of specific volatile compounds in undamaged tissue of the same individual, and that the response is stronger during the first 18 h after the challenge. A major number of primed compounds were observed in neighboring trees, mainly after 36 h after the challenge. It is unknown what the molecular mechanisms of plant signaling are, but the benefits are clear. Further discussion about the significance of these processes will be presented.

D4: Lower Water Use Efficiency in PIP2;2 Aquaporin Knockout Mutants

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Aquaporins are small membrane integral proteins facilitating the passage of water and certain small molecules through the lipid bilayer. They are classified into the following four main groups depending on the membrane location: plasma membrane intrinsic proteins, tonoplast intrinsic proteins, nodulin-26-like proteins and small intrinsic proteins. Using knockout mutants I focus on the plasma membrane intrinsic proteins PIP1;2 and PIP2;2 and whether their role in controlling hydraulic conductance has an effect on the entire plant.

As a result of knocking out aquaporins, one would expect to see altered water relations caused by a reduction in water flow. This would lead to plant responses aimed at maintaining a positive water balance including lower stomatal conductance which in turn would restrict photosynthesis.

To test this hypothesis I grew single knockout mutants of *Arabidopsis thaliana* together with the wild type plants under standard greenhouse conditions. I measured leaf level gas exchange of the 3-4 week old plants (IRGA, LICOR 6400). These mutants as well as wild type lines were also grown on petri dishes to allow analyses of the root system which may display altered growth to compensate for the effect of the mutation.

A preliminary analysis of the gas exchange data has shown a significantly lowered water use efficiency of the PIP2;2 mutants compared to the wild type.

D5: Gas exchange of silver birch (*Betula pendula*) provenances in a common garden experiment

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The ability of forest trees to acclimate to environmental conditions is of increasing interest as a determinant of ability to acclimate to climate change. We study geographical variation in acclimation capacity of silver birch (*Betula pendula* Roth.) in a common garden experiment with a total of 26 genotypes representing 6 provenances, from latitudes 60°, 61°, 62°, 65°, 66° and 67°. The common garden has been established with micropropagated plantlets in Joensuu in central Finland (62°) in late summer 2010. Gas exchange was measured from the short-shoot leaves by Licor-6400 three times during the first growing season in June 2011. Chlorophyll was measured with CCM-200+ from the same leaves during each measurement round. Additionally, light response curves for some genotypes were measured from long-shoot leaves in summer 2012. The results from 2011 showed differences between provenances. Plantlets from 62° had the lowest total photosynthesis and plantlets from 60°, 62° and 67° the lowest stomatal conductance. Chlorophyll content in the leaves was lower in plantlets from 65° than in other provenances. Photosynthesis, stomatal conductance or chlorophyll content did not correlate with biomass accumulation measured as height growth and as number of leaves.

D6: Differences in phenology and growth between Finnish silver birch provenances

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Under rapid climate change phenotypic plasticity, rather than genetic diversity, will play a crucial role in allowing plants to survive in adverse environmental conditions. Very little is known of plasticity of trees, and one of the key questions is whether the northern tree populations can adapt quickly enough to cope with the rapid environmental change. If there are latitudinal differences among tree provenances, the global warming may affect populations at the northern and southern edges of the boreal forest in a different way. We have established a multi-site common garden experiment with silver birch (*Betula pendula* Roth) in three sites: southern (60°N), central (62°N) and northern (67°N). Altogether 26 randomly selected genotypes from six provenances representing a latitudinal cline from 60°N to 67°N were planted in 2010. Growth and phenology of the clonally reproduced birches have been studied during 2011-12. The southern provenances grew the best at all sites, but the survival and growth of northern provenances is decreased when transferred to the south. The timing of phenological events did not show a clinal trend as expected, but the three most northern provenances differ from the southern provenances. Results from the first two years indicate that the northern birch provenances cannot benefit from extended growth period as much as the southern ones due to strong photoperiodic control of growth.

D7: Analysis of mycorrhizal colonisation of Scots pine roots by electrical impedance spectroscopy

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The goal of this work was to investigate whether colonization of the roots of Scots pine (*Pinus sylvestris* L.) seedlings with symbiotic mycorrhizal fungi would affect the electric impedance spectra (IS) of the root systems. The pine seedlings were raised in long-day and high temperature (LDHT) for nine weeks after germination. Two lots of the seedlings were inoculated with *Hebeloma* sp. (H) and with *Suillus luteus* (SL) during the growing phase whereas one lot was left as a non-mycorrhizal control (O). The electrical impedance spectra of the root systems were measured with a three-electrode measuring setup at the frequency range from 5 Hz to 100 kHz. Two stainless steel electrodes (voltage and current) were set in the stem and one stainless steel plate electrode in soil at the bottom of the pots. IS were analysed by a classification method using CLAFIC algorithm that employs principal component analysis (PCA). According to our results the seedlings by fungal treatments (O, H and SL) could be classified in different categories according to their IS.

D8: Using fluorescence measurements to detect spring recovery of spruce and pine

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Fluorescence measurements provide a simple and practical means of measuring the photosynthetic activity of plants; therefore the measurements can be used to assess the spring phenological development. We measured a number of Norway spruces (old trees and crafts) as well as Scots pines (grafts) during the spring 2013 in Haapastensyrjä nursery, Loppi, Southern Finland. We then fitted models based on ambient air temperature to predict the spring development. Here we present the results of our measurements and modeling work, indicating differences in the spring development of these two common conifer species. Preliminary results have indicated that spruces have much faster reaction to changing temperature conditions, making it more vulnerable to frost damage.

D9: Northern plants and climatic warming – modelling the ecophysiology of overwintering

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The environmental conditions of plants are projected to change globally during the coming decades due to climate change. The changes are predicted to be especially pronounced in the north during the winter. The change in climate will bring about novel combinations of environmental factors not experienced by contemporary plants. Experimental determination of the effect of various climate change scenarios on plants are often limited by the resources and effort needed to carry out multiple treatments. The use of ecophysiological process-based dynamic models can help overcome this shortcoming. The successful use of dynamic models in climate change research requires that the models are properly tested and parameterised by experimental data. By modifying phenological dynamic models of the annual cycle of forest trees, we developed dynamic models for the overwintering of perennial field layer plants in the boreal zone with the purpose of identifying functional overwintering types that can be used especially when modelling the effects of changes in wintertime conditions. The models were parameterised by using data from a series of growth chamber experiments with plants representing different functional groups from dwarf shrubs to hemicryptophytes, overwintering rosette plants, and grasses. Our results show that there are differences among both plant species and functional groups in the timing of growth onset following warm periods in the winter. Three main types of responses regarding timing of growth onset can be seen. Differential responses among species may result in changes in species composition. Dynamic process-based models of the overwintering responses of plants can be incorporated in geographical models and thus used to refine predictions of the consequences of climate change on the vegetation.

D10: Impact of short- and long-term foliage herbivory on the emission of volatile organic compounds from silver birch

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Plants emit large quantities of volatile organic compounds (VOCs) to the atmosphere when exposed to insect herbivory. In this study, we analysed insect herbivory-induced VOC emissions from silver birch (*Betula pendula* (Roth.)) in laboratory and field experiments. Short-term VOC responses to foliage herbivory were monitored by Proton Transfer Reaction Mass Spectrometry (PTR-TOF-MS) while larvae of *Erannis defoliaria* were feeding 24h on silver birch foliage at laboratory condition. Online monitoring showed rapid induction in green leaf volatiles (GLVs) and methyl salicylate, whereas terpenoids showed gradual increase due to herbivory. Long-term effects of herbivory on VOCs were analysed from three genotypes (gt14, gt15 and Tuusniemi provenance) of silver birch in the field over two growing seasons. In the field experiment, two-year-old trees were infested with two moth species (*Agriopsis aurantiaria* and *E. defoliaria*) in the summer of 2011 and VOCs were measured by bag enclosure method. Trees were infested again with caterpillars of the same moth species in the summer of 2012 and VOCs were sampled from shoot and pots with roots. Herbivory induced the emission rates of sesquiterpenes from all genotypes whereas micropropagated trees had higher total VOC emission compared to Tuusniemi provenance regardless of herbivory.

D11: Multiple Roles for UV RESISTANCE LOCUS 8 in Regulating Gene Expression and Metabolite Accumulation in *Arabidopsis* under Solar UV Radiation

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Photomorphogenic responses triggered by low fluence rates of ultraviolet-B radiation (UV-B, 280-315 nm) are mediated by the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8). Beyond our understanding of the molecular mechanisms of UV-B perception by UVR8, there is still limited information on how the UVR8 pathway functions under natural conditions. Here, wild-type *Arabidopsis thaliana* and *uvr8-2* were used in an experiment outdoors where UV-A (315-400 nm) and UV-B irradiances were attenuated using plastic films. Gene expression, PYRIDOXINE BIOSYNTHESIS 1 (PDX1) accumulation and leaf metabolite signatures were analyzed. The results show that UVR8 is required for transcript accumulation of genes involved in UV protection, hormone signal transduction and defence against herbivores under solar UV. Under natural UV-A irradiance, UVR8 is most likely to interact with UV-A/blue light signaling pathways to moderate UV-B driven transcript and PDX1 accumulation. UVR8 both positively and negatively affects UV-A-regulated gene expression and metabolite accumulation, but is required for the UV-B induction of phenolics. Moreover, UVR8-dependent UV-B acclimation during the early stages of plant development may enhance normal growth and fitness under long-term exposure to solar UV.

D12: Sex-related differences in growth and phenolic defense in a dioecious species *Populus tremula* under different levels of nitrogen and phosphorus

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Nitrogen (N) and phosphorus (P) are key nutrients that limit the productivity of terrestrial ecosystems. Because of their important roles in plants' primary and secondary metabolism, alterations of their cycles due to anthropogenic activities may not only impact ecosystem productivity, but also plant-herbivore interactions, which in turn affect competitive interactions between plants. Changes in resource availability may particularly influence sexual dimorphism of the species because males are expected to invest more resources in growth, and accordingly less in defensive compounds such as phenolics, than females do. The aim of this study was to investigate the individual and interactive effects of N and P on sex-related differences in the allocation of *Populus tremula* L. to growth and secondary metabolites. Individuals of twelve clones (six of both sexes) of *P. tremula* were grown under two levels of N and P (low/optimum for P was 13ppm/446 ppm, respectively, and low/optimum for N was 12 ppm/400 ppm) and their four combinations. Biomass and concentrations of phenolic compounds were determined after six weeks. Our results showed that in both sexes, N limitation decreased seedlings' biomass whereas P-level had no effects. As expected, males accumulated significantly more biomass than females but maintained lower concentrations of phenolics than females. The effect of N and P on constitutive defence varied across plant organs and across the main groups of phenolics. These results suggest that females of *P. tremula* are better chemically defended than males, since phenolic glycosides are important defenses against generalist herbivores.

D13: Predicting soil properties from floristic composition in western Amazonian rainforests

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Soil quality is an important determinant of primary productivity and species distribution patterns. However, soil data are laborious to collect, and laboratory analyses of soil samples are expensive. In many parts of the world, indicator plant species are therefore used as proxies to infer soil quality. We investigated how this idea might work in Amazonian lowland rain forests. We used the species of Melastomataceae plant family as indicator species and applied two different numerical methods, k-nearest neighbour estimation and calibration via weighted averaging, for making predictions of surface soil Ca, K, Mg, Na, the sum of these four cations, P, Al, pH and loss on ignition (a proxy for carbon content).

The result was that the two prediction methods performed about equally well and produced for Ca, K, Mg and the sum of cations estimates that were accurate enough for practical applications.

We can conclude that it is possible to estimate important soil characteristics on the basis of plant species composition. Further development of the method should include basic taxonomic work on the group of indicator plant species, selection of a restricted and more easily identifiable set of indicator species and refinement in the laboratory analysis of plant-available soil nutrients.

D14: Solar blue light plays a key role in tuning flavonoid accumulation in leaf epidermis of outdoor-grown pea (*Pisum sativum* cv. Meteor)

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Phenolic compounds are plant secondary metabolites that are synthesized under different types of stress, e.g. in UV-B exposure. One of the largest groups of phenolics is flavonoids. They function in plants as protective pigments and antioxidants. Here we show that blue light functions independently, without UV-A, in flavonoid biosynthesis. We germinated and grew pea plants outdoors under five different spectrally modifying filters: polyester to remove UV-B, MHCM09B to remove UV-B and UV-A < 340 nm, Rosco 226 to remove UV-B and UV-A, Rosco 312 to remove UV-B, UV-A and blue light; and polythene as a control that transmits all wavelengths. Pea plants had significantly less protective flavonoids in their leaf epidermis under blue light removing filters. The total proportion of all 21 flavonoids in the leaf dry mass was fairly constant in all the treatments, although concentrations of individual compounds varied significantly. The removal of blue light mainly reduced the content of Quercetin and increased the content of Kaempferol derivatives. The total concentration of flavonoids was highest when UV-A was removed entirely due to the higher content of one flavonoid: quercetin-3-O-(*p*-coumaroyl)-sophoroside-7-O-glucoside. UV responses lacked almost completely, most likely due to acclimation and high amount of PAR. Additionally, leaf position and age modulated the concentration and location of phenolic compounds. It seems that plants regulate the contents of individual compounds in a very complex manner in different light conditions and phases of plants development.

D15: Variation in forest understorey structure on two geological formations in western Amazonia

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Non-inundated western Amazonian lowland rain forests are mostly found to grow on two geological formations – Pebas formation and Nauta formation. Pebas formation is geologically older and its sediments have finer texture and contain more plant nutrients than those of Nauta formation. In terms of plant species composition they have been found to support quite distinct forests. The existence of possible structural differences between these forests has been very little studied and the structure of forest understorey has not been investigated at all. We collected field observations from forests on both Pebas and Nauta formation about forest structural features that were assumed to be relevant for the habitat quality of forest understorey organisms. These features are tree stem density in three stem diameter classes (diameter at breast height, DBH, below 2.5 cm, between 2.5 and 10 cm, and above 10 cm) and canopy openness at the height of human eye measured with a canopy scope. In order to have a control that the field inventory sites really represent floristically different geological formations, we also recorded indicator plant species, terrestrial pteridophytes and *Iriartea deltoidea* palm, in the field inventory sites.

Our results showed very little if any structural differences between the forests, even though floristically they were clearly different.

Amazonian forest structure appears to change partly independently of changes in plant species composition.

D16: Dynamic relationship between the VOC emissions from a Scots pine stem and the tree water relations

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The stems of coniferous trees contain huge storages of oleoresin, a viscous mixture of mono- and sesquiterpenes and resin acids. Once in contact with air, volatile parts of oleoresin evaporate, and the residual compounds harden to make a solid protective seal over damaged tissues. Volatile monoterpene emissions from woody plant parts are poorly known and that's why we started to measure them. We measured simultaneously monoterpene emissions from Scots pine (*Pinus sylvestris*) stem and shoot, stem diameter changes, sap flow, foliage transpiration, and several other variables to learn about their connections. All the measurements were done at SMEAR II measuring station in spring 2012.

The results showed interesting issues. VOC emissions from stem peaked strongly on a warm day in mid-April. High temperature was not the only reason behind high emissions, but peaks coincide with pronounced xylem diameter shrinkage and low transpiration. The tight connections between transpiration, sap flow and tree diameter broke down at the same time. We interpret the phenomenon as a phase when a tree shifts from winter state to active growing period state.

We also found that stem hydraulic conductance increased almost linearly from the beginning of April until early June. Our results show that monoterpene emissions from tree stems are connected to the tree water relations, and that they are substantial during the early spring when the foliage is still rather inactive.

D17: Genetically determined partition of belowground resources affects the growth performance of Norway spruce

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In boreal forests, the functional importance of mycorrhizal short roots of trees is high since they form the interface for water and nutrient exchange. Mycorrhizas use fungal specific enzymes in the mobilization of nutrients from litter and soil organic matter and the mycorrhizal species composition has a major influence on nutrient foraging abilities of trees in nutrient poor boreal soils. We have evidence of intraspecific variation in the root associated ectomycorrhizal community structure among differentially growing Norway spruce (*Picea abies*) clones, and among spruce genotypes in general. Our recent results show that also the seedling short root formation is genetically controlled by trees, and that the short root formation seems to interconnect with later growth performance of spruce. In long-term perspective, the genetically controlled variation in fine root structures and belowground biomass allocation may result in differences in taxonomical and functional diversities of mycorrhizal fungi. In turn, increasing ECM diversity can bring along more functional abilities for the host tree and create a belowground virtuous circle of accumulating good in heterogeneous forest soil.

D18: The effect of habitat on polyphenol content in crowberry (*Empetrum hermaphroditum* Hagerup)

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The polyphenol content in plants can develop variably according to the microclimate and the soil conditions of the habitat. We examined the polyphenol concentrations in leaves, stems, roots and fruits of crowberries, that were growing in sunny but nutrient limited habitats on islands of three different lakes and compared them to samples collected from more nutritious mainland habitats.

Individual anthocyanins, flavonols and phenolic acids from each plant part were analyzed by HPLC-DAD and UHPLC-qtof MS and proanthocyanidins (condensed tannins) by acid-butanol – method.

The polyphenol composition was plant part specific and the amounts of phenolics varied both among sampling sites and among the lakes. The highest concentrations of berries were found from populations at Lake Rikkavesi, of leaves from populations at Rikkavesi and Koitere, and of stems and roots from populations at Lake Pielinen. The variation in the levels of several compounds was higher among populations at Lake Koitere and Lake Pielinen than at Lake Rikkavesi. This variation was suggested to be a result from differences in the habitats. However, the crowberry populations on nutrient limited rocky islands did not differ from those of more nutritious mainland ones, unlike supposed.

E) Environment

E1: Due to salt deicing in wintertime in Finland maritime littoral biotope is mimicked on road pavement borders

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Some littoral plant species, especially *Leymus arenarius*, *Allium schoenoprasum*, *Plantago maritima*, and *Lotus corniculatus* began to spread on narrow border stripes next to the pavement of roads since the late 1960s. Winter deicing with salt (95 – 98% NaCl) was started on the major roads of Finland in the 1960s. Fifty-eight soil samples of the root layer of two roadside stands of *Leymus arenarius* were collected at intervals of about a month during the unfrozen season in the 1990s. These samplings cover 3 entire seasons plus parts of 2 seasons from one stand in Vihti, Ab, road no. 120, and another in Tammela, Ta, road no. 2. The pooled annual data of each stand show pH values that reach the maxima about one month after the frozen season (the highest measured value, pH 9.25, in April), then sink towards neutrality until October (the lowest value, pH 7.04). The pH rise in the topsoil in early spring is explained by the observed fact that Cl⁻ is leached faster than Na⁺. Cl⁻ is known to be accumulated in ground water. Ion exchange apparently tends to retain Na⁺ as Na-silicate in topsoil sand. The conductivity data showed U-shaped distribution, being high (the highest value, 827 µS/cm) in March, sinking usually below 100 µS/cm by October, and rising again along with the following salt deicing period. From the narrow zone next to the pavement, these conditions eliminate perennial species which do not tolerate Na-alkalinity or Cl⁻. Comparative conditions are generated on sandy or gravelly sea shores, temporarily wetted by marine water with pH usually >8. Temporal floods, wind and sun shine on sea shore are mimicked by rainwater running beyond the pavement borders, wind generated by vehicles and treeless, sunny stripes of the roads, respectively. A single plant of the nonnative *Medicago sativa*, a neutrophilic species cultivated abroad, survived over two decades in a site on road no. 2 in Tammela until a construction of the road destroyed it.

E2: Identifying genes regulated by ExpA independently of RsmA in

***Pectobacterium wasabiae* SCC3193**

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Pectobacterium wasabiae SCC3193 is a model for virulence regulation research in soft rot bacteria. Regulating gene expression as a response to environmental signals is necessary for host infection, and can be achieved by utilizing two component systems (TCS). TCS typically consist of signal binding sensor kinases, and DNA binding response regulators relaying sensor kinase input to gene expression. Regulation can be further fine-tuned by for example RNA binding post transcriptional regulators.

Two expression regulators of virulence related genes in SCC3193 are; ExpA, a response regulator of the ExpA/ExpS TCS; and RsmA, an RNA binding regulator. Studies suggest that ExpA homologues primarily affect gene expression via RsmA activity. However, the extent of this regulon overlap is not clear.

To assess how much ExpA regulation of gene expression is routed via RsmA in *P. wasabiae* we performed a gene expression microarray analysis of *in-vitro* cultured SCC3193 wild type, an *expA* mutant, an *rsmA* mutant, and an *expA rsmA* double mutant. Microarray data for various genes was confirmed through quantitative RT-PCR, along with assays

linking transcriptomic changes to phenotypes regarding growth rate, PCWDE production, and virulence *in planta*. Conclusively, a majority of ExpA regulation seems routed via RsmA. However, we identified genes involved in the electron transport chain, metabolism and oligogalacturonide uptake and utilization, which ExpA seems to influence independently of RsmA.

E3: Variation of ozone sensitivity between *Arabidopsis* accessions identify a novel stomatal regulator

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Short and high pulses of the air pollutant ozone cause the formation of reactive oxygen species in the apoplastic space of leaves and in sensitive species leads to cell death. We have screened a collection of *Arabidopsis thaliana* accessions and recombinant inbred lines for ozone sensitivity and they display a large range of ozone-induced damage, ranging from tolerant to extremely sensitive. The development of recombinant inbred lines allows the identification of the genes regulating ozone sensitivity in natural populations through quantitative trait loci (QTL) mapping.

The *Arabidopsis thaliana* accession Cvi-0 (originally collected at the Cape Verde Islands) has increased ozone sensitivity, more open stomata and a reduced stomatal response to changes in CO₂ concentration. Through QTL mapping, generation of near isogenic lines in the background of Col-0 and fine mapping we have placed the QTL responsible for altered stomatal function to the bottom of chromosome 2. S2, a near isogenic line in Col-0 with the chromosome 2 QTL from Cvi-0, displays the same CO₂ phenotypes as the parental Cvi-0. We are studying the role of S2 in stomatal conductance in response to various environmental treatments (including elevated CO₂) and in response to known signaling molecules including the major regulator of stomatal function the hormone abscisic acid. Furthermore, complementation lines where candidate genes are transformed from Cvi-0 to Col-0, and vice versa, are characterized as final proof that the correct gene has been identified.

E4: Overexpression of *ERD15* enhances pathogen tolerance in *Arabidopsis*

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ERD15 (Early Responsive to Dehydration 15) is rapidly induced in response to various abiotic and biotic stress stimuli in *Arabidopsis*. Overexpression of *ERD15* has been shown to change the responsiveness of the transgenic plants to the phytohormone abscisic acid (ABA) resulting for example in impaired stomatal closure in response to ABA, ozone and darkness. Despite the impaired stomatal closure, *ERD15*oex plants demonstrate enhanced resistance to phytopathogens *Pectobacterium carotovorum* and *Pseudomonas syringae* even if the infection is done by spraying and more bacteria initially enter the plant apoplast when compared to wild type plants. Interestingly, already 2 hours after bacterial inoculation this difference has evened and after 2 or 5 days, depending on the pathogen, *ERD15*oex plants demonstrate enhanced tolerance. The observed tolerance seems to constitute of rapid and clearly increased accumulation of reactive oxygen species (ROS) as well as altered expression of antioxidant genes rather than activation of specific defense pathways.

E5: The WRKY superfamily of transcription factors plays a central role in plant response to environmental stresses

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The function of WRKY transcription factors has been mainly associated with plant defense but recent studies suggest additional roles in regulation of other physiological processes including senescence and abiotic stress responses. We explored the role of two related group III WRKYs, WRKY70 and WRKY54 in osmotic stress tolerance. These two transcription factors are positive regulators of plant defense integrating signals from different hormonal pathways as well as co-operating as negative regulators of salicylic acid (SA) biosynthesis and senescence. We employed single and double mutants as well as an overexpressor line to explore their role in osmotic stress response. The double mutant exhibited clearly enhanced tolerance to osmotic stress, suggesting that WRKY70 and WRKY54 co-operate as negative regulators of stress tolerance. In contrast, both single and in particular the double mutants showed reduced induction of the majority of genes responsive to osmotic stress, suggesting a positive role for these WRKYs in regulation of osmotically induced genes. Similarly, accumulation of the osmoprotectant proline was reduced in the double mutant. Part of the observed suppression of gene expression and proline accumulation could be explained by increased SA levels and was relieved by introduction of the allele deficient in SA biosynthesis. The enhanced tolerance to osmotic stress in the double mutant could be correlated with improved water retention and enhanced stomatal closure suggesting that WRKY70 and WRKY54 negatively regulate stomatal aperture. Moreover, stomatal closure was also promoted by the increased SA levels. These results show that WRKY70 and WRKY54 co-operate as negative regulators of stomatal closure and consequently osmotic stress tolerance in *Arabidopsis*, and suggest that WRKY70 and WRKY54 have an important role not only in plant defense but also in abiotic stress signaling.

E6: Monoterpene emissions of a temperate heath ecosystem –the effects of long term CO₂ enrichment, warming and drought periods

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We investigated emissions of monoterpenes, the most common group of biogenic volatile organic compounds, at a semi-natural coastal heath ecosystem dominated by the dwarf shrub *Calluna vulgaris* and the grass *Deschampsia flexuosa*. The study was conducted in Denmark, at the CLIMAITE experimental site where the ecosystem had been under elevated CO₂ (510 ppm), night-time warming and extended summer drought periods for six years prior to the measurements. For first time, the combined effects of realistic, long-term climatic manipulations on monoterpene emissions were studied in this ecosystem. Samples for monoterpene analysis with TD-GC-MS were collected with the conventional push-pull system with adsorbent tubes and a transparent chamber in seven campaigns over one growing season.

The results show that the temperate heath ecosystem is a considerable source of monoterpenes. The emissions correlated with the abundance of *C. vulgaris* but not with *D. flexuosa* or other grasses or herbs. The mean total monoterpene emission over the season in the untreated plots was $20 \pm 6 \mu\text{g m}^{-2} \text{h}^{-1}$. The effect of the treatments varied over the season but in the most of the campaigns, elevated CO₂ and extended drought periods decreased the emissions either alone or together. Night-time warming increased the emission in one campaign but decreased or increased the emission in concert with other treatments in others. According to these results, the ongoing climate change will alter monoterpene emissions from temperate heaths. Increasing CO₂ concentration and extended droughts will decrease the emissions while increasing minimum temperatures at night-time may overcome some of the decrease.