

IX Finnish Symposium on Plant Science, May 17–19, 2010, Joensuu, Finland Abstracts



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ELINA OKSANEN & MARKKU A. HUTTUNEN (EDS)

IX Finnish Symposium on Plant Science, May 17–19, 2010, Joensuu, Finland

Abstracts

Publications of the University of Eastern Finland Reports and Studies in Forestry and Natural Sciences No 1

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Cover photo (© Elina Oksanen):

Leaves of silver birch (Betula pendula) damaged by ozone

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ABSTRACT

This Abstract book contains presentations from the IX Finnish Symposium on Plant Science (IX Kasvitieteen päivät), to be held in May 17–19, 2010, at the University of Eastern Finland, Joensuu campus.

This symposium continues the series of Finnish symposia on plant science (Kasvitieteen päivät) of which the latest one was held in Oulu in 2002. The aim of the symposium is to bring together people from different areas of plant biology research and related activities, increase the cooperation and networking among researchers, and give an insight of current status of plant biology research in Finland.

The presentations in The IX Finnish Symposium on Plant Science were positioned under eight scientific themes: Changing environment, Ecophysiology, whole-plant physiology and plant development, Stress and signaling, Omics, Genetics and evolution, Potential of novel plant biology applications, Ecosystems and biodiversity, and Plant interactions.

The symposium program includes 37 oral presentations (five keynote presentations) and 64 poster presentations and a scientific visit to Botania, the botanic garden.

Keywords: plants; changing environment; ecophysiology; whole-plant physiology; plant development; stress; signaling; omics; genetics; evolution; ecosystems; biodiversity; plant interactions

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Program

IX Finnish Symposium on Plant Science – IX Kasvitieteen päivät May 17-19, 2010, University of Eastern Finland, Joensuu Campus, <u>Carelia</u> building (Yliopistokatu 4), room <u>C2</u> and <u>Aurora</u> building (Yliopistokatu 2), room <u>AU100</u>

●●● Monday 17 May ●●●		
19:00-20:00	Registration, Poster set-up (<u>Carelia</u>)	
19:00-22:00	Get-together party, Poster set-up (<u>Carelia</u>)	
●●● Tuesday 18 May ●●●		
08:00-09:00	Registration (<u>Carelia</u>)	
09:00-10:20	Session 1: Opening session, <u>C2</u> (Chair Elina Oksanen)	
09:15-09:30	Elina Oksanen: Organizational remarks and Welcome speech Academic Rector Kalervo Väänänen: Opening of the symposium Keynote: Seppo Kellomäki: Climate change in boreal conditions – from impact studies to adaptation	
10:20-10:50	Coffee break (Carelia)	
10:50-12:20	Session 2, <u>C2</u> (Chair Elina Oksanen)	
	Keynote: Lars Gädda: Opportunities for renewal in the forest industry Keynote: Eva-Mari Aro: Solar fuels – making use of photosynthesis in biofuel production	
12:30-13:30	Lunch (<u>Carelia</u> and <u>Aurora</u>)	
13:30-14:30	Session 3: Ecosystems and biodiversity, <u>AU100</u> (Chair Heikki Simola)	Session 4: Stress and signaling, C2 (Chair Markku Keinänen)
13:30-13:50	Hanna Tuomisto: What is beta diversity and what have we learned about it in natural ecosystems?	Mikael Brosché: Use of Arabidopsis thaliana natural variation to study plant stress responses
13:50-14:10	Outi Lähteenoja: Amazonian peatlands: a scientifically neglected habitat with potentially global significance for atmospheric carbon dynamics	Saijaliisa Kangasjärvi: Protein phosphatase 2A in the cross-talk of chloroplast signaling
14:10-14:30	Mirkka Jones: Microhabitat partitioning promotes mid-elevation diversity in tropical montane forest ferns	<i>Pinja Jaspers</i> : Transcription factor interactions of the RCD1 protein mediated by the novel RST domain
14:30-15:00	Coffee break (<u>Carelia</u>)	

	Session 5: Plant interactions, <u>AU100</u> (Chair Toini Holopainen)	Session 6: Stress and signaling, <u>C2</u> (Chair Markku Keinänen)	
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15:20-15:40	Piippa Wäli: Effects of a fungal symbiont (Epichloë endophyte) on performance of a subarctic grass (Festuca rubra)	Taina Tyystjärvi: The roles of multiple group 2 sigma factors in cyanobacteria	
15:40-16:00	Minna-Maarit Kytöviita: Ecosystem effects of large herbivores		
16:00-16:20	Jaana Laamanen: New virus detections in Humulus L., Prunus L. and Allium L. species in Finland	anen: New virus detec- mulus L., Prunus L. and Elina Häikiö: Venturia tremulae infection is affected by the foliar	
16:30-18:30	Poster session I (Themes of sessions 3–6, <u>Carelia</u>)		
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19:00-22:00	Banquet (Art Museum of Joansuu htt		
●●● Wednesday 19 May ●●●			
	-	p://taidemuseo.jns.fi/in_english.htm) May •••	
08:30-10:00	-	, ,	
	••• Wednesday 19 Session 7: Changing environment,	Session 8: Novel tools/ methods in plant biology, C2 (Chair Ilkka Porali) LI-COR	
08:30-08:50	••• Wednesday 19 Session 7: Changing environment, <u>AU100</u> (Chair Heli Peltola) Elina Oksanen: Tropospheric ozone causes oxidative stress to northern decompositions.	Session 8: Novel tools/ methods in plant biology, C2 (Chair Ilkka Porali) LI-COR ci- es) r- Finnzymes	
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10:50-11:10	Heikki Simola: Changes in carbon storage of Finnish forestry-drained peatlands based on reinvestigation of old peat inventory study sites	Marjo Tuomainen (Arja Tervahauta): Proteomics of Thlaspi caerulescens accessions and inter-accession cross segregating for zinc accumulation	
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13:20-13:40	on timing of height growth cessation		
	on timing of height growth cessation and field performance of silver birch <i>Hannu Ahokas</i> : Storage protein types as indicators of ecophysio- logical adaptation of the plant	wood formation in <i>Populus Marjatta Uosukainen</i> : Cloudberry (<i>Rubus chamaemorus</i>) cultivar 'Nyby' – an example of domestication of	
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Keynote presentations (sessions 1, 2, and 13)

ABSTRACTS OF

5 ORAL PRESENTATIONS

Solar fuels – making use of photosynthesis in biofuel production

Eva-Mari Aro

evaaro•utu.fi, Dept of Biochemistry and Food Chemistry, University of Turku

A Solar fuel is a non-fossil fuel made by direct solar energy conversion. European solar fuel team aims for renewable fuels from solar energy using water as raw material. Introduction of new CO₂-lean fuel concepts based on renewable resources is spurred by worries for global warming and decreased availability of oil and gas. The two key areas in solar fuel production are supramolecular and nanostructured artificial systems, and photosynthetic microorganisms (algae or cyanobacteria) that excrete the fuel (e.g. H₂ or hydrocarbons). Synergy based on powerful biochemical and biophysical insights on natural water splitting and hydrogenases is critical for solar fuel. Microorganisms will be optimized with metabolic engineering and organism design based on molecular biology and genomic research. The development of efficient bio-inspired photocatalysis in supramolecular systems or nanostructured smart matrices involves synthetic and physical chemistry, materials research and photo-electrochemistry.

Cyanobacteria have a great potential in production of solar fuels particularly when the agricultural (ethical) consequences associated with current land-based-biofuel feed stocks are taken into consideration. Cyanobacteria (also algae and diatoms) are inherently more efficient solar energy collectors than land plants. This is due to several reasons, including the differences in light-harvesting and photoprotective mechanisms. The generation of hydrogen by photolysis of water is the most environmentally friendly way to generate this clean fuel. Cyanobacteria can split water in their photosynthesis using sunlight and thereafter, with the help of hydrogenase and nitrogenase, they are capable of hydrogen production. Such hydrogen production from substrates widely available in nature (water and sunlight) is a unique characteristic of cyanobacteria and some green alga. The nitrogenase enzyme produces hydrogen as a side product whereas hydrogenases (bidirectional hydrogenase and uptake hydrogenase) serve as electron valves or hydrogen recyclers. Genetic engineering of cyanobacterial hydrogenases, electron transfer pathways and light-harvesting antenna systems has been shown to increase biohydrogen production. Therefore a thorough understanding of the biology of cyanobacterial hydrogen production, which is intimately linked with photosynthetic processes and nitrogen metabolism, is a prerequisite for metabolic engineering of cyanobacteria for commercially profitable biohydrogen production.

Opportunities for renewal in the forest industry

Lars Gädda

lars.gadda•forestcluster.fi, Forestcluster Ltd, Snellmaninkatu 13, 00171 Helsinki

My presentation will address four main topics relevant for the Finnish Forest Industry:

- Forest Industry outlook shortly
- Tree, a renewing raw material with lot of opportunities
- Innovation, towards the future biosociety; an opportunity
- Future product and business opportunities

The first topic shows the relevance of Forest Industry from a European business point of view.

The second topic shows the interesting and beautiful structure of tree and how we learn more about tree raw material and its applications when we move from macro-, to micro- and nano-scale and finally deal with molecular and polymer structures.

The third topic deals with Innovation and how different prerequisites must be fulfilled before an Innovation can occur.

The fourth topic deals with practical new application opportunities we already have and can foresee in different value chains.

GM trees: influence on target and non-target organisms and ecosystem processes

<u>Joakim Hjältén</u>, E. Petter Axelsson, Gilles Pilate, Thomas Whitham, Anders Wennström, Carri LeRoy, Riitta Julkunen-Tiitto joakim.hjalten•vfm.slu.se, Swedish University of Agricultural Sciences, Dept of wildlife, fish, and environmental studies, Sweden

The intent with genetic modification of trees is to increase yield or quality without negative effects on non-target organisms or important ecosystem processes. We tested this in lab and short-term field studies with two types of transgenic poplars. One hybrid poplar (*Populus tremula* × *tremuloides*) type expressing a synthetic cry3Aa gene derived from *Bacillus thuringiensis*, which provides resistance against Coleopteran species and one hybrid type (*P. alba x tremula*) with reduced modified synthesis of lignin (CAD or COMT) which improves pulping performance. As control, we used the respective isogenic wildtype clones.

We found that Bt poplars clearly have the expected target effects in the lab. The survival of leaf beetles and the degree of leaf damage inflicted by them were clearly lower on Bt poplars than on the wildtype. In addition, we found strong indications that Bt poplars grow better than the wildtype in the presence of leaf beetles (but slightly less in the absence of them!)

We also detected some non-target effects. In decomposition experiment with leaf litters in streams we found that macrofauna assemblages differed between Bt and wildtype litter, but we detected no differences in decomposition rates. By contrast, we found reduced decomposition rates in one of the lignin lines but no effects on the macrofauna assemblages. These differences in decomposition could be due to the detected differences in secondary chemistry in leaves from GM and wildtype trees.

Furthermore, non-target slugs consumed more leaf area from Bt aspen then the wildtype but their survival was unaffected. The higher consumption from Bt leaves might be explained by the observed changes in the concentration of phenolics in the Bt leaves and this have implication for the discussion on accumulation of secondary pest species on Bt plants. Vole consumption of twigs did not differ between lignin and wildtype poplars.

In summary, we found strong indications that the Bt aspens had the intended target effects. However, our studies also demonstrate that genetic modification of trees can indeed affect non-target herbivores and ecosystem processes under natural conditions. One likely explanation for these effects are induced differences in plant chemistry, which could be due to random insertions altering the expression of existing genes in the receiving plant, or regulatory elements in the plant DNA influencing the expression of the introduced genes. Furthermore, our studies indicates that metabolic changes caused by genetic modifications are not restricted to living leaves, but can be maintained beyond the lifetime of a leaf and influence leaf litter quality.

Climate change in boreal conditions – from impact studies to adaptation

Seppo Kellomäki seppo.kellomaki•uef.fi, University of Eastern Finland, Finland

In Finland, the studies on climate change were launched in late 1980s and early 1990. From the very beginning, impacts of the climate change on trees and forests were in the focus of these studies. In 1990s and early 2000s, large-scale experiments were established to define, how the changes in temperature and precipitation and the elevation in atmospheric carbon may effects on the physiological processes and growth of trees and on the dynamics of forest and forest ecosystems. These studies are of importance to understand how forests are growing and developing under climate change and how forests are adapting to the changing conditions. Knowledge based on impact studies is a key to understand how to tune the forest management to meet the future requirements.

Concept of adaptive management refers to the management, which aims at moderating or offsetting the potential damages or taking advantage of opportunities created by the prevailing environmental conditions. Adaptation occurs through adjusting and modifying the management to meet the requirements "to moderate or offset the potential damage or to take advantage of opportunities created by a given environment".

Adaptive management is a way to make the structure and the consequent functioning of the forest ecosystem to resist harmful impacts of prevailing environmental conditions, and to utilise the opportunities created by the prevailing environmental conditions. Adaptive management of forests is affected by the vulnerability of trees and forest ecosystem. Vulnerability refers to the extent to which trees and forest is susceptible to sustaining damage from climate change. It is a function of the sensitivity, adaptive capacity and exposure of the system to climate change. In this context, sensitivity refers to the degree to which systems will respond to a given change in climate, including beneficial and harmful effects. Adaptive capacity refers to the degree to which adjustments in practices, processes, or structures can moderate or offset the potential for damage or take advantage of opportunities created by a given change in climate.

Responses, tolerance and acclimation to multiple interacting abiotic and biotic stresses

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Plants under natural environments encounter a plethora of various abiotic and biotic stresses throughout their life cycle. Often, several stresses can simultaneously affect plant performance. Understanding and predicting plant responses to multiple simultaneous stresses is complicating as various stresses can influence plants interactively, and thus, simple additive approaches fail to predict plant reactions to stress combinations. Furthermore, exposure to a mild stress typically elicits acclimation responses that render the plant more tolerant to a more severe level of the given stress. Such stress priming may also enhance plant tolerance to a subsequent different type of stress, and enhance plant tolerance to multiple co-occurring stresses. Apart from plant capacity to acclimate to any given stress and stress combinations (phenotypic plasticity), there is a huge variability in plant tolerance to given stresses and stress combinations (genotypic plasticity). Variations in stress tolerance are associated with whole plant traits such as overall size of carbohydrate pool, biomass partitioning as well as leaf-level traits such as foliage longevity, mechanical resistance, the level of secondary defense compounds and antioxidants.

This presentation describes recent quantitative studies carried out in our lab on multiple stress responses in plants with varying constitutive stress tolerance. Quantitative stress doze vs. plant response relationships have been achieved by monitoring stress-elicited release of volatile organic compounds from plants. In particular, any stress triggers rapid emissions of volatile products of lipoxygenase pathway (LOX products, various C6 aldehydes, also called green leaf volatiles) associated with oxidative burst, as well as elicitation of a variety of defense pathways leading to longer-term emissions of methylsalicylate, and a variety of mono- and sesquiterpenes. Our studies demonstrate that these emissions scale positively with the strength of different stresses, such as drought, ozone, heat, herbivory stress and with these stress combinations. Both priming to mild stress as well as overall increase of constitutive stress tolerance reduces plant stress responsiveness. The main view put forward in this presentation is that developing quantitative stress doze vs. plant response relationships in a variety of model species is needed for construction of stress models capable of predicting plant performance in multistress environment.

Ecosystems and biodiversity (session 3)

ABSTRACTS OF

3 ORAL PRESENTATIONS

AND

5 POSTERS (1 – 5)

Microhabitat partitioning promotes mid-elevation diversity in tropical montane forest ferns (Pteridophyta)

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Plant species richness often peaks at mid-elevations in tropical montane forests. This pattern has been attributed to multiple causes whose relative importance remains unclear, in part because detailed ecological studies are lacking.

We examined the role of local environmental variation in promoting montane forest fern diversity at mid-elevations, using local ecological data and an extensive elevational range dataset from Bolivia. We recorded fern community composition and environmental descriptors in transects at a fixed elevation (c. 1650 m, close to the elevation at which fern diversity peaks in the eastern Andes) at Tunquini Biological Station in the Bolivian Yungas. We partitioned variation in fern composition into fractions best explained by different environmental datasets (soils, forest structure, topography, microclimate). We then linked species' local environmental distributions, as evidenced in these analyses, to their elevational ranges in Bolivia.

Local microclimatic and topographic differences, in particular, had strong impacts on community variation. Soil and forest structural variation also made a significant contribution. Species' local microclimatic distributions were significantly linked to their broad-scale elevational range distributions: low-elevation species reached higher on more humid slopes, and high-elevation species reached lower on drier slopes.

In sum, microhabitat partitioning may be important in enabling the local coexistence of a diverse fern flora. Moreover, fine-scale variation in microclimate may be key to the mixing of lowland and highland species pools in mid-montane forests, thereby contributing to commonly observed mid-elevation diversity peaks.

Amazonian peatlands: a scientifically neglected habitat with potentially global significance for atmospheric carbon dynamics

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Amazonian peatlands have been regarded merely as an anecdote, quite to the contrary of Southeast Asian peatlands that are known to cover extensive areas, harbour large quantities of carbon and support a specialised fauna and flora. We have now documented for the first time the existence of raised peat bogs in Amazonia. Our field observations furthermore suggest that Amazonian peatlands may be an equally important carbon sink and potential source as Southeast Asian peatlands. Also, Amazonian peatlands seem to have both animal and plant species that are known to have restricted ranges and therefore the existence of peatland habitats may change the way how Amazonian biogeography should be understood.

What is beta diversity and what have we learned about it in natural ecosystems?

Hanna Tuomisto

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A wide variety of different phenomena have been called beta diversity over the years. I recently made an extensive literature review on the topic, and found that beta diversity is being defined in dozens of different ways in the current ecological literature.

Many of the definitions actually lead to quantifying phenomena that are not correlated with each other. Therefore, when studies reach conflicting conclusions about "beta diversity", the underlying reason may fall into one of three categories:

- 1) there are real biological differences among organism groups or geographical regions targeted in the studies;
- 2) there are real differences in the sampling setup of the studies that cause beta diversity to differ, or
- 3) one or both of the studies actually did not measure beta diversity but some other phenomenon.

As a consequence, it is rather difficult to obtain an overview about what has been learned about patterns in beta diversity, and about the processes that affect beta diversity in nature. In my presentation, I will briefly justify the use of one particular definition as true beta diversity. I will also provide an overview of the knowledge accumulated on true beta diversity in tropical rain forests, especially in Amazonia.

(1) Growth and resistance to pathogens and herbivores in different genotypes and sexes of Salix myrsinifolia

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Salix myrsinifolia is a dioecious willow species. In dioecious species female and male reproductive organs exist in different individuals. As a pioneer species, *S. myrsinifolia* has many different asexual ways to reproduce in addition to sexual reproduction. It reproduces from seeds, root and stump seedlings, cuttings and layers. Often one genotype broadens into its surroundings. Previous studies done with dioecious species have reported differences in growth, pathogen and herbivore resistance, as well as in resource allocation between sexes. Differences in resource allocation have been thought to occur because reproduction demands more resources from females, leaving males more growth and maintenance oriented. Interaction of sexes with pathogens and herbivores are also found to vary.

In this experiment we examined if different sexes of *S. myrsinifolia* differ in their growth and resistance against herbivores and pathogens. For the experiment, cuttings from ten clones of each sex, 40 cuttings of each clone, were sampled from genotypes found in different areas in Joensuu and Kaavi in eastern Finland. Cuttings were planted randomly in an old field in Kaavi. Length and basal diameter of the longest shoot, number of shoots and leaf area were used as parameters for growth. For the pathogen and herbivore damage, we calculated and measured rusts and herbivore inflicted damage from the leaves.

Our preliminary results show that there seems to be more differences between genotypes than between sexes in growth parameters and rust damage. That is, we found significant differences between genotypes, inside one sex and between genotypes from different sexes. Herbivore damage did not significantly vary between sexes or genotypes. Results are from the first two growing seasons.

(2) Effects of reindeer grazing on bryophyte diversity in subarctic tundra at different spatial scales

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The unproductive ecosystems of the arctic and subarctic regions have been the subject of growing interest because of their vulnerability to the intensifying human impact. The effect of herbivores on plant communities is known to be substantial in unproductive tundra, but most of the research has concentrated on the direct effects on forage plants. However, the less studied indirect effects of grazing may be more profound for the functioning of the ecosystems. The role of bryophytes as mediators of grazer effects on other plants and soil has been discovered only recently. Often neglected in studies of plant communities, bryophytes can constitute a considerable part of the arctic plant communities in terms of biomass, diversity, and function. Disturbance by large herbivores may affect bryophyte communities and diversity indirectly, but few studies have focused on this question.

We studied the effects of the semi-domesticated reindeer (*Rangifer tarandus*) on bryophyte species richness and diversity in two regions in northern Finland and Norway differing in productivity. Bryophyte species and species covers were determined at five tundra sites, each with a reindeer exclosure fence. A hierarchical set of sampling units – sites (1 ha), plots (0,25 m²), and subplots (25 cm²) – was used to study the spatial patterns of diversity.

Species accumulation curves for all levels of sampling units were constructed for the two grazing treatments at the two levels of productivity. The data were also modelled by generalised linear models and nested analyses of variance. Although few significant effects of grazing on species richness or Simpson's diversity index were found, there was an obvious trend for an interaction of grazing and productivity. Grazing appeared to increase diversity at sites of the productive region and decrease it at unproductive sites. The differences were most clearly demonstrated at the plot level.

The results conform to the hypothesis that grazing enhances diversity in productive ecosystems and reduces it in unproductive ecosystems. The increase or decrease in diversity seems to manifest itself also in changes of the spatial heterogeneity of bryophyte species assemblages at a relatively small scale. The observed effect may be due to physical disturbance, changes in microenvironmental conditions, or changes in competitive relationships in the plant community, which are affected by changes in nutrient cycling. Further research on the effects of grazing on the species composition of bryophytes, as well as the mechanisms of the effects, is needed to better understand the role of bryophytes in the intricate processes of the apparently simple tundra ecosystems.

(3) Goose grazing impacts on salt marsh vegetation

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The occurrence of low-growth early successional plant species in dynamic coastal habitats is strongly suppressed by competitive exclusion by taller species. Grazing by increased populations of wild geese may potentially to hinder vegetation succession at early successional stages and, therefore, aid the existence of subordinate plant species.

The impact of summertime grazing by the greylag goose (*Anser anser*) on salt marsh vegetation was studied in a five-year exclosure experiment on the coast of the Bothnian Bay, which is undergoing isostatic land uplift. A special reference was to a small halophytic graminoid, *Puccinellia phryganodes*, which is critically endangered in the Baltic Sea area. Grazing was assumed to benefit low-growth early successional plant species by depressing the invasion and growth of taller graminoids.

Goose grazing reduced the biomass and height of vegetation as well as suppressed the performance of taller graminoids, thus benefiting subordinate species. The impact of grazing was most pronounced in the earlier successional stage. The cover of *P. phryganodes* and small forbs was slightly higher in the grazed vs. non-grazed experimental quadrats. However, grazing was not able to markedly slow down the overall decline of *P. phryganodes* or hinder the increase in the cover of the taller graminoids. The results indicate that primary succession of vegetation driven by isostatic land uplift may progress very rapidly in low-lying Bothnian Bay coastal meadows in spite of relatively intensive grazing by geese.

Sufficiently intensive management that includes the upper part of the shore, for example by livestock grazing, is likely to be increasingly important in order to maintain plant biodiversity and populations of threatened early successional species in coastal meadows.

(4) Plant species of mires, eskers and herb-rich forests continue declining

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The fourth national red-listing in Finland is currently in process and will be published in the end of year 2010. The status of each native vascular plant species has been evaluated by using the guidelines and quantitative criteria developed by IUCN (International Union for Conservation of Nature). If the population reduction of a species exceeds the threshold of 30 % in the time scale of three generations, the species should be classified as at least vulnerable (VU). The estimated reduction may be based on changes in actual population size (measured in numbers of reproducing individuals), number of occurrences or other usable index appropriate for the species. The criteria allow certain amount of uncertainty, and methods involving estimation, inference and projection are acceptable in preparing the assessments.

To assess the trends of certain common forest and mire plants we analyzed the extensive long term data sets (National Forest Inventories and BioSoil) collected by Finnish Forest Research Institute. Frequencies were used as an index of change. According to the results the populations of many common plant species have declined clearly more than the 30 % threshold for the lowest threat category, vulnerable (VU). This is the case with several plant species in both forests and mires. Human influence, like changes in forestry practices and atmospheric deposition, is likely to be the main cause of the decline.

(5) Vegetation change indicates the active area of spruce-swamp buffer zone of peatland forestry

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Forestry operations increase leaching of solids and nutrients from the catchment. Peatland buffers with sedimentation ponds are established to prevent leaching of these effluents to water courses from forest management area. Solids are sedimented to the pond and nutrients bound by soil micro-organisms and vegetation while part of the nitrogen (N) is released in gaseous form to the atmosphere.

We followed changes in plant species composition and cover (%) in a spruce swamp buffer (0.35 ha, representing 0.5 % of the catchment area). In addition, we measured N content of ground vegetation biomass excluding trees and surface peat at different distances from the water inflow. We also estimated the potential release of N and phosphorus (P) from the tree stand that was gradually killed by flooding after buffer construction in 1997.

The incoming water flow from the forestry areas increased the cover of indicator species of flooding such as Calamagrostis purpurea, Juncus filiformis, Viola palustris and Sphagnum riparium. In contrast, the cover of forest dwarf shrubs and mosses (e.g. Vaccinium myrtillus and Pleurozium schreberi) decreased. The total N content of the shoot parts of ground vegetation and that of surface peat appeared to be similar in the buffer and the reference area. Thus, changes in species composition, biomass or vegetation N content did not indicate increased nutrient availability or nutrient binding capacity in the buffer. Probably the buffer did not receive significantly increased nutrient flow from forestry; e.g. the N concentration in the water inflow in the spruce swamp buffer was typical for natural humic waters. The concentrations of P were typical for surface or vadose waters in drained peatlands. In addition, nutrient concentrations were periodically lower in the inflow water than in the outflow, which may indicate nutrient release from the buffer: Dying and decomposing tree stand could potentially release over 50 kg N and more than 4 kg P during years of their complete decomposition within the buffer.

Buffers should cover 1 % of the catchment, because relatively large buffers bind nutrients and solids better than small ones. However, buffers are usually realized smaller than recommended. In addition, water flows may cover the planned buffer area only partly and the active buffer area is smaller than its geographic area. Spread of flood tolerant species and decrease of forest dwarf shrubs and mosses could be used as a field tool, when the success of the water protection operation is evaluated.

Stress and signaling (sessions 4 and 6)

ABSTRACTS OF

7 ORAL PRESENTATIONS

AND

10 NUMBERED POSTERS (6 - 15)

Use of *Arabidopsis thaliana* natural variation to study plant stress responses

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Short and high pulses of the atmospheric pollutant ozone cause the formation of reactive oxygen species (ROS) in the apoplastic space of leaves. Numerous similarities between the plant responses to ozone and pathogens suggest that ozone triggers hypersensitive response-like programmed cell death (PCD). The model plant *Arabidopsis thaliana* has been instrumental for studying mechanisms regulating plant ozone sensitivity. For example mutants, like *vtc1* and *rcd1*, screened on the basis of their ozone sensitivity, have helped to understand the role of ascorbic acid and to identify proteins involved in plant hormonal signaling during oxidative stress.

The genetic variation that exists among naturally occurring populations of *Arabidopsis* is a so far largely untapped source of genetic information in ozone research. Many *Arabidopsis* ecotypes have been collected from wild populations growing throughout the world and phenotypic variation among ecotypes should reflect also in the genetic variation that is important for adaptation to specific conditions. We have screened a large collection of *Arabidopsis* ecotypes for ozone sensitivity and they display a large range of ozone induced damage, ranging from extremely tolerant to hypersensitive ecotypes. The mechanism behind ozone sensitivity of different ecotypes seems to be largely determined by stomatal regulation of ozone uptake.

Of the ecotypes tested Cvi-0 has by far the strongest phenotype. Our future goal is to fine map QTLs and characterize corresponding genes from the ozone sensitive Cvi-0. In addition QTL mapping will be performed in several recombinant inbred populations to identify ozone QTLs. We will also map QTLs for other stress related traits.

Venturia tremulae infection is affected by the foliar chemical composition of European aspen (Populus tremula) and hybrid aspen (P. tremula x tremuloides) leaves

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Potted two-year old seedlings of eight hybrid aspen (*Populus tremula x tremuloides*) and two native European aspen (*P. tremula*) clones were studied over one growing season (2003) using the open field ozone exposure system in Kuopio, Finland. The impacts of moderately elevated ozone (1·5 x ambient) and different soil nitrogen levels (60 kg N ha⁻¹ yr⁻¹ and 140 kg N ha⁻¹ yr⁻¹) on aspen shoot blight (*Venturia tremulae* Aderh.) infection were studied. The differences among clones in the severity of infection symptoms, foliar phenolic composition (salicylates, flavonoids and condensed tannins) and leaf anatomy were assessed.

Significant differences in both ozone sensitivity and susceptibility to *Venturia tremulae* infection among clones were found. Hybrid aspen had higher amounts of total foliar phenolics and was less susceptible to *Venturia* infection than European aspen. High concentrations of condensed tannins were associated with both ozone and *Venturia* tolerance. The ozone-sensitive hybrid aspen clones and the more *Venturia*-prone European aspen clone contained high amounts of salicylates, indicating no role of salicylates in mitigating oxidative stress, whether caused by ozone or pathogen attack.

Nitrogen amendment did not affect the ozone sensitivity of the clones. No effects of nitrogen were found in the concentrations of foliar phenolics or *Venturia* susceptibility of the clones.

Transcription factor interactions of the RCD1 protein mediated by the novel RST domain

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[This abstract is published as a printed version only.]

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Protein phosphatase 2A in the cross-talk of chloroplast signaling

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Light modulates developmental programs, metabolic pathways and defence reactions throughout the life cycle of plants. In chloroplasts, light-induced photochemistry, metabolic imbalances and formation of reactive oxygen species initiate signals, which converge with cytoplasmic signaling networks to modulate transcriptional activity in the nucleus. We have taken a reverse genetic approach to identify novel components in the cross-talk of chloroplast signaling.

Mutants deficient in serine/threonine protein phosphatase 2A (PP2A) subunits showed peculiar light-intensity-dependent phenotypes, and were chosen for further analysis. A PP2A regulatory subunit B was identified as a cytoplasmic component in the cross-talk between light acclimation and disease resistance in *Arabidopsis thaliana*. Knock-down mutants for *PP2A-B* show constitutive defence responses, alterations in antioxidant metabolism, enhanced photo-oxidative stress and age-dependent cell death when grown under moderate light regimes. Another highly similar PP2A subunit seems to have a distinct function in the regulation of cellular energy metabolism. Intriguingly, however, genetic analysis indicates that these two PP2A regulatory B subunits are functionally interconnected with each other. The current aim of our research is to identify the specific PP2A-B-dependent target processes of PP2A. A combination of interaction studies and phenotypic analysis of Arabidopsis mutants is expected to specify the mechanisms of PP2A function in plants.

[Compared to the printed version, this abstract is slightly expurgated.]

Membrane attachment of ferredoxin-NADP⁺ oxidoreductase regulates photosynthetic activity

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Precise management of the regulatory circuits between photosynthetic electron transfer and various downstream processes is required for the optimal fitness of the plant. Since ferredoxin-NADP+ oxidoreductase (FNR) enzyme mediates the transfer of electrons from ferredoxin to NADP+, and it can be found as a membrane-bound and as a soluble protein in the stroma, it is an important mediator of reducing power providing the transition from exclusively membrane-bound light reactions to the stromal metabolic pathways.

In the present study, we provide evidence that FNR is bound to the thylakoid membrane via the Tic62 protein, which has been earlier shown to be a 62 kDa subunit of the translocon at the inner envelope of chloroplasts. At the thylakoids, Tic62 and FNR form high molecular weight complexes that are not involved in photosynthetic electron transfer but are dynamically regulated by light signals and the stromal pH. Structural analyses revealed that Tic62 binds to FNR in a novel binding mode for flavoproteins, with a major contribution from hydrophobic interactions. Moreover, in 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 envelope and in the stroma, but also at the thylakoids of *Arabidopsis thaliana* and perhaps all flowering plants. We furthermore propose that correct allocation of FNR is used to efficiently regulate Fd-dependent electron partitioning in the chloroplast.

[Photo and caption removed.]

The roles of multiple group 2 sigma factors in cyanobacteria

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Cyanobacteria are eubacteria that perform oxygenic photosynthesis like plants. The initiation of transcription, mediated by RNA polymerase holoenzyme, is the main determinant of gene regulation in eubacteria. The core of RNA polymerase exhibits the RNA polymerase activity, while the σ factor is responsible for the recognition of promoter sequences.

Cyanobacteria typically encode many group 2 σ factors that resemble the essential group 1 \circ factor. Three out of four group 2 σ factors in *Synechocystis* sp. PCC 6803 can be inactivated simultaneously in any combination. All inactivation strains grow well under standard conditions, but *Synechocystis* cells do not acclimate to different abiotic stress conditions as efficiently as the wild type strain. According to our results SigB factor is important for acclimation to high temperature and high salt stresses as it is required for normal activation of heat shock genes, especially the *hspA* gene. The SigC factor is involved in acclimation to low inorganic carbon at high temperature or in low CO₂ atmosphere. The SigD and SigB factors are important for light regulation. Our results show that group 2 sigma factors are partly functionally redundant, especially the structurally most similar SigB and SigD factors. Furthermore, the results reveal complicated regulatory networks between the different sigma factors.

Involvement of 1,3- β -D-glucanases in dormancy cycling in *Populus*

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Trees growing in northern latitudes evolved the capacity to become dormant and freezing tolerant, allowing survival through extreme winter conditions. These regulated changes involve complex interactions between environmental and cellular factors, thus securing the proper timing of dormancy induction and release. A key feature of the dormant state is the physical obstruction of symplasmic paths by deposits of callose (1,3- β -glucan) on sieve plate pores and plasmodesmata (PD). During chilling these callose deposits are enzymatically removed by 1,3- β -D-glucanase (glucan hydrolase family 17, *GH17*). Our aim is to elucidate the mechanisms that regulate the activity of genes encoding PD-localised 1,3- β -D-glucanases, as these might regulate dormancy cycling.

We have studied a group of glucanase family genes in Populus that show sequence homology to birch lipid-body associated glucanases as well as Arabidopsis cell wall and PD associated glucanases. Our results show that number of Populus glucanase genes is differentially expressed under LD conditions and in response to SD and chilling. Antibody made against P. trichocarpa PtGH17 101 peptide recognized approximately a 37 kD protein in active shoot apical meristem (SAM) in hybrid aspen P. tremula x tremuloides. The level of this protein decreased during SD induced dormancy development and increased again after chilling treatment that released buds from dormancy, suggesting a role in dormancy cycling. However, 2D western blotting revealed that proteins in LD grown active meristem and released, chilling treated meristem have different pIs, suggesting a posttranslational modification or that they are different proteins. Identification of these proteins will be done by sequencing. In addition to GH17 domain, glucanase proteins are characterized by N-terminal signal peptide or C-terminal GPI anchoring, suggesting diverse localization within a cell or in the apoplast. This prompted us to study whether different glucanases take part in dormancy cycling by acting at various sites within the cell. The preliminary results with PtGH17-GFP fusion proteins show that although transcription pattern of PtGH17_65 and PtGH17_101 is similar and both are induced by chilling, PtGH17 65 is mainly associated with the plasma membrane whereas PtGH17_101 is also localized in ER. These results suggest that dormancy associated callose turnover might require action of several glucanases that act in concert at different sites within the cell.

(6) Magnetic field protects plants by suppressing generation of singlet oxygen by charge recombination reactions

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Light is the energy source of photosynthesis but light also damages the photosynthetic machinery in a reaction termed photoinhibition. The target of photoinhibition is Photosystem II (PSII). To maintain photosynthetic activity, plants need to repair photoinhibitory damage by continuously synthesizing new D1 protein of PSII.

Photoinhibition enhances the production of reactive oxygen species, especially singlet oxygen (${}^{1}O_{2}$) in the light. The conversion of normal triplet oxygen to the excited singlet state occurs when oxygen reacts with an excited triplet state of a photosensitizer molecule like chlorophyll. In theory, triplet chlorophyll may be formed either from singlet excited chlorophyll via intersystem crossing or by charge recombination, but the importance of the two routes in ${}^{1}O_{2}$ production is not known.

An external magnetic field lowers the triplet yield by allowing only the middle sublevel of the radical pair to become populated. Thus, magnetic fields would be expected to protect plants by decreasing the production of the harmful ${}^{1}\text{O}_{2}$, if the recombination route is a significant producer of ${}^{1}\text{O}_{2}$. The effect of the magnetic field is specific to the charge recombination mechanism and does not affect the yield of intersystem crossing.

We measured photoinhibition under strong light in intact leaves of pumpkin (*Cucurbita maxima*), and found that oxygen evolution activity consistently decreases more slowly in a magnetic field of 0.1–0.2 T than in the absence of a field. The result indicates that charge recombination reactions produce chlorophyll triplets, these triplets react with oxygen to produce ${}^{1}O_{2}$, and ${}^{1}O_{2}$ is harmful to photosynthesis.

The protective effect of the magnetic field was observed when the synthesis of the D1 protein was allowed to occur during the photoinhibitory illumination, but the effect disappeared if lincomycin was used to block synthesis of the D1 protein. This result indicates that ${}^{1}\text{O}_{2}$ produced by recombination reactions does not have a direct role in the damaging reaction of photoinhibition of PSII but instead ${}^{1}\text{O}_{2}$ interferes with chloroplast protein synthesis. Similar results concerning the target of the ${}^{1}\text{O}_{2}$ -induced damage were obtained by measuring photoinhibition in the *Arabidopsis thaliana* mutant vte1 which lacks α -tocopherol, an important scavenger of ${}^{1}\text{O}_{2}$. The vte1 mutant was found to be more sensitive to photoinhibition than the wild type in the absence but not in the presence of lincomycin.

(7) Characterization of cysteine-rich RLKs in *Arabidopsis* thaliana

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Membrane-bound receptors play fundamental roles in all organisms by recognizing signals from the environment and other cells and activating downstream signaling cascades.

In plants, receptor-like protein kinases (RLKs) have important functions in development, hormone signaling, stress adaptation and pathogen responses. The *Arabidopsis* genome encodes a huge number of RLKs, most of which are not functionally characterized. One of the largest RLK subfamilies is the CRK (cysteine-rich RLKs)/DUF26 (domain of unknown function 26) sub-family with 44 members. In their extracellular domain, the CRKs possess two copies of the DUF26 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.

Recently, we have found that several CRKs are transcriptionally regulated in response to ozone (O₃). O₃ induces apoplastic reactive oxygen species (ROS) production similarly to other stresses and is used as a tool to study ROS perception and effects of apoplastic ROS. The production and action of ROS are critical to stress recognition and the coordination of the stress responses. Loss-of-function mutations in two CRKs lead to sensitivity to extracellular ROS. The CRK mutants show enhanced cell death and increased H₂O₂ accumulation compared to Col-0. In addition to their O₃ sensitivity, CRK knock-out plants are sensitive to xanthine / xanthine oxidase (X/XO), which induces extracellular ROS production similar to ozone and pathogen attack. The downstream components and targets for the CRKs will be identified with appropriate methods including yeast two-hybrid analysis.

(8) Complex signaling interactions of two highly similar regulatory B subunits of protein phosphatase 2A

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Serine/threonine protein phosphatase 2A (PP2A) family members carry out crucial functions in the regulation of signaling through phosphorelay cascades in animals and plants. The predominant form of PP2A is heterotrimer, consisting of a catalytic subunit C, a scaffold subunit A, and a highly variable regulatory subunit B, which is thought to determine the target specificity of subunit C in the PP2A holoenzyme. We have taken a reverse genetic approach to identify PP2A subunits that specifically modulate the ability of plants to tolerate environmental stresses.

A specific B subunit of PP2A was identified as a cytoplasmic component in the cross-talk between light acclimation, disease resistance and ageing in the model plant *Arabidopsis thaliana*. Preliminarily, another highly similar PP2A-B' subunit seems to have a distinct function in the regulation of cellular energy metabolism. Whereas knock-down mutant for PP2A-B shows age-dependent formation of yellowing lesions, knock-out *pp2a-b'* mutant exhibits no distinct visual phenotype when grown under normal growth conditions. Under stress conditions, however, enhanced accumulation of anthocyanins becomes apparent in *pp2a-b'* leaves. Intriguingly, the cell death phenotype of *pp2a-b* becomes partially suppressed in a *pp2a-b pp2a-b'* double mutant. Currently, we are working on the subunit composition and signaling interactions of the PP2A holoenzymes to reveal the functional specificities of the regulatory B subunits in *Arabidopsis thaliana*.

[Compared to the printed version, this abstract is slightly expurgated.]

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(9) Characterization of *Arabidopsis thaliana* mutants with differing amino acid profiles

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The *Arabidopsis* mutant *rcd1* (*radical-induced cell death1*) was originally isolated on the basis of its sensitivity to ozone. An *rcd1* suppressor mutant line 311 was isolated on the basis on its tolerance to ozone. Both *rcd1* mutant and the suppressor mutant 311 have higher proline content than the wild type. The proline contents in *rcd1* mutant and the suppressor mutant 311 were about 80 % higher and about 40-fold higher than in the Col-0 wild type, respectively.

In our research project, we will gain insight to the molecular basis of regulation of proline metabolism in Arabidopsis, and elucidate the role of proline in abiotic stresses, especially regarding the signaling components involved. The main objectives are to perform positional cloning of the gene causing proline accumulation in the rcd1 suppressor mutant line 311, and characterize the mutant, specifically in relation to oxidative stress responses, including salt and drought tolerance. We have backcrossed the line 311 to the Col-0 wild type, and analyzed the first F2 generation for the presence of rcd1 mutation, total carbohydrate content and amino acids. The plants without the rcd1 mutation were screened, and two groups of mutants were chosen for a closer look. There were only three plants with higher proline content than in the Col-0 wild type, and those also had higher content of arginine than the wild type. Instead, there was ¼ of plants with lower total amino acid content and higher total carbohydrate content than in the Col-0 wild type. Those mutants were also characterized by a lower content of glutamine than in the Col-0 wild type. The low total amino acid mutants were separated from the rest of the F2 plants in the PCA analysis of the F2 generation based on their amino acid profile.

(10) Induction of pinosylvin synthesis in Pinus sylvestris

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Natural decay resistance of Scots pine (*Pinus sylvestris*) heartwood is an important wood quality trait with reasonably high heritability. Decay resistance correlates with extractable heartwood phenolics (stilbenes and resin acids), the trees highest in heartwood extractives being most resistant. Exploitation of the heritable variation in Scots pine heartwood in breeding is hampered by the fact that the trait is expressed only in mature trees. Our aim is to find genetic causes for the variation, making early selection of seedlings with DNA markers possible.

The stilbenes pinosylvin and its monomethyl ether are synthesised under both developmental and stress induced control in Scots pine. Our aim is to determine if these two processes have common genetic determinants, i.e. are regulated by converging signal transduction pathways. The kinetics of induction for the gene encoding pinosylvin synthase (*PST-1*) is rapid and occurs within hours of induction under UV. We are proceeding in determining if the induction is dependent on translation, which would infer involvement of specific transcription factors not present in uninduced tissue. Both the *PST-1*gene itself and the possible upstream regulators are first candidates for genes responsible for inherited variation in the response.

(11) Redox-dependent changes in tyrosine phosphorylation of *Pisum sativum* root proteins

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Plant adaptation to environmental changes and also intercellular communication are realized through the functioning of the cascades of the signaling pathway network. Protein phosphorylation, which is the most important and well studied posttranslational modification (PTM), is a key reaction of signaling systems. Protein tyrosine phosphorylation is a critical stage in the regulation of cell proliferation and differentiation (Hunter, 1995, Zhang et al., 1998). The level of tyrosine phosphorylation is reversibly controlled by the activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). In plants, protein tyrosine phosphorylation was shown to be involved in various physiological and biochemical processes.

Protein activity is regulated by more than one type of PTMs, which can be competitive.

The study of the role of protein PTMs and their interactions is a major topic of modern research on cellular activity. In literature, besides the revelation of redox-sensitive plant proteins it is absent data about redox-sensitive phosphotyrosine proteins (and changes in their tyrosine phosphorylation level (TPL)), which are critical for signal transduction and cell metabolism regulation by norm and adaptive reactions. In this study we have used 2D-electrophoresis together with immunochemical approach to demonstrate changes in plant protein tyrosine phosphorylation by in situ action of different redox-agents on excised roots of pea plants. More then 50 phosphotyrosine proteins were detected. Among revealed proteins, whose TPL was redox regulated, a part of proteins were identified using MALDI-TOF mass-spectrometry (MS) or tandem mass-spectrometry (MS/MS). These proteins were associated with a variety of functions, including energy- and material metabolism and signal transduction.

(12) Protein phosphatase 2A subunit B as a regulator of defense pathways in *Arabidopsis*

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Recognition of an invading pathogen activates phosphorelay cascades where controlled activities of protein kinases and protein phosphatases lead to a rapid induction of specific defence reactions in plants. We found that a specific regulatory B subunit of protein phosphatase 2A (PP2A-B) is required for accurate light acclimation and jasmonic acid (JA) and salicylic acid (SA) dependent disease resistance in Arabidopsis thaliana. Knock-down pp2a-b mutants show age-dependent formation of yellowing lesions when grown under moderate light intensity. Promoter::GUS analysis indicates activity of PP2A-B promoter in patches that highly resemble the yellowing lesions on pp2a-b mutant leaves. On ultra-structural level, symptoms of cell death appear particularly in the spongy mesophyll tissue of visually presymptomatic pp2a-b leaves. The cell death phenotype is accompanied by accumulation of reactive oxygen species through a pathway that requires the activity of CONSTITUTIVE EXPRESSION OF PR GENES 5 (CPR5). Moreover, similarly to cpr5, pp2a-b shows constitutive activation of SA- and JAdependent defense pathways, and resistance against biotrophic and necrotrophic pathogens. Currently, PP2A-B-dependent signalling interactions are being studied to reveal how the action of PP2A-B prevents unnecessary defense reactions in Arabidopsis thaliana.

[Compared to the printed version, this abstract is slightly expurgated.]

(13) A gene-centered approach to study regulation of transcription in *Arabidopsis thaliana*: Yeast 1-hybrid

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Understanding the transcriptional regulation of gene expression requires knowledge of the binding specificities of transcription factors. The question of the binding specificities can also be asked in a gene-centered way: Which transcription factors bind to the promoter region of the gene in question? Yeast 1-hybrid method enables screening of large pools of proteins for interaction with specific DNA sequence. We applied Clontech's Yeast 1-Hybrid System to study plant stress signaling. Eight short DNA sequences considered to be potential response elements were selected for screening against two protein collections.

Half of the DNA sequences were selected based on a bioinformatic model, which predicts potential response elements related to signaling through RCD1 (RADICAL INDUCED CELL DEATH1), a protein shown to interact with many transcription factors. The other half of the sequences was selected for being enriched in the promoter regions of genes induced by one hour ozone treatment. Drought responsive element (DRE), known to interact with transcription factor DREB2A, was used as a positive control. One protein pool was created through a cDNA library prepared from stressed and hormone-treated plants; the other pool contained 38 transcription factors shown to interact with RCD1.

The overlap between the results of the two protein pools was low. Neither did the results confirm the predictions of the bioinformatic model. The poor overlap between the pools is probably because of the way cDNA library is created; highly transcribed genes are overrepresented in the library, as genes transcribed in lower level may be very few. However, some interesting results were obtained: Disease resistance protein HSPRO2 might have a DNA binding functionality with preference towards sequence tgggcc, and a W-box present in the promoter of Cysteine-rich receptor-like kinase 5 (CRK5, At4g23130) appears to be favored by closely related WRKY transcription factors WRKY54 and WRKY70.

Neither the specificity nor sensitivity of this method is optimal, but it is applicable method for gene-centered approach in the quest for new transcription factors or response elements. The strength of the method is the ability to screen large protein collections in one experiment, but results need to be verified with complementary methods.

(14) Ozone-triggered rapid stomatal response involves production of reactive oxygen species and is controlled by SLAC1 and OST1

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The air pollutant ozone can be used as a tool to unravel *in planta* processes induced by reactive oxygen species (ROS). Here we have utilized ozone to study ROS-dependent stomatal signaling. We show that ozone-triggered rapid transient decrease in stomatal conductance (RTD) coincided with the burst of ROS in guard cells. RTD was present in 11 different *Arabidopsis* ecotypes suggesting that it is a genetically robust response. To study which signaling components or were involved in RTD, we tested 44 mutants deficient in various aspects of stomatal function. This revealed that the SLAC1protein, essential for guard cell plasma membrane S-type anion channelfunction, and the protein kinase OST1 were required for the ROS-induced fast stomatal closure. We showed physical interaction between OST1 and SLAC1 and provide evidence that SLAC1 is phosphorylated by OST1. Phosphoproteomic experiments indicated that OST1 phosphorylated multiple amino acids in the N-terminus of SLAC1.

Using TILLING we identified three new *slac1* alleles where predicted phosphosites were mutated. Lack of RTD in two of them, *slac1-7* (S120F) and *slac1-8* (S146F), suggested that these serine residues were important for the activation of SLAC1. Mass-spectrometry analysis combined with site-directed mutagenesis and phosphorylation assays, however, showed that only S120 was a specific phosphorylation site for OST1. Absence of the RTD in dominant negative mutants *abi1-1* and *abi2-1* suggested also a regulatory role for the protein phosphatases ABI1 and ABI2 in ROS induced activation of the S-type anion channel.

(15) SRO protein family: analysis of protein structure and function

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The RCD1 (RADICAL-INDUCED CELL DEATH1) protein is an important regulator of stress and developmental responses in *Arabidopsis thaliana*. A loss-of-function mutation in *RCD1* results in highly pleiotropic phenotypes including increased sensitivity to apoplastic ROS, tolerance to chloroplastic ROS, altered hormonal and abiotic stress responses (1, 2).

RCD1 is a member of the plant specific SRO protein family which is highly conserved among land plants. All SROs are multidomain proteins and can be divided into two types according to their domain structure. Type A SROs contain the C-terminal RST domain responsible for interaction with transcription factors (2), the poly-ADP ribose transferase (PARP) domain and the N-terminal WWE domain whereas type B SROs possess only the PARP and RST domains. The roles of the WWE and PARP domains of SRO proteins and their functions in stress responses are unknown.

High conservation of the PARP domain of SRO proteins with PARP-1 and PARP-2 of *Arabidopsis thaliana* suggested that SRO proteins have PARP or mono(ADP-ribosyl) transferase (mART) activity. To test the predicted activity we expressed the full-length RCD1 protein and a truncated form containing the PARP and RST domains as GST-tagged proteins in *Escherichia coli*. The recombinant proteins were tested for NAD binding and PARP activity. The results of biochemical analysis of RCD1 show that the protein does not have PARP or mART activity. The bioinformatic analysis of the PARP-like domains of all SRO proteins revealed the loss of residues critical for NAD binding and suggests that, despite the presence of the highly conserved PARP domain, SRO proteins do not possess PARP activity. Nevertheless, the function of SRO proteins is critical for plants and may be related to transcription factor regulation and complex formation.

- 1. Overmyer et al. 2000, Plant Cell, 12, 1849–1862
- 2. Jaspers et al. 2009, Plant J, 60, 268–279.

Plant interactions (session 5)

ABSTRACTS OF
4 ORAL PRESENTATIONS
AND

7 NUMBERED POSTERS (16 - 22)

Plant-plant communication in a changing troposphere: Does ozone facilitate a communication breakdown?

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Since pre-industrial times the tropospheric ozone concentration has risen, and is predicted to continue doing so for some time to come. This can have a number of direct adverse effects on plants, including phytotoxicity. However, very limited information is available on the indirect effects of ozone on plants – for example, the effects that degrading plant emitted volatiles has on plant defence. Studies in our laboratory have suggested that degradation of volatiles by ozone may negatively affect responses to plant host complexes by foraging predators and parasitoids, but that to some extent they can still utilise the volatile cues available, albeit a different blend to that usually encountered. Plasticity in behavioural responses and usage of compounds that are less reactive with ozone could be important here. Motivated to further test the effects of ozone on interactions mediated by plant volatiles we began to explore the effects on plant–plant communication – a process unlikely to display the same type of behavioural plasticity that can be observed in studies with insects.

During the last few years a number of studies have convincingly demonstrated the existence of plant-plant communication, with the majority of these studies focussing on communication via volatile organic compounds. The responses in receiver plants can be rather subtle, making observation challenging. One of the clearer observations has been with Lima bean, Phaseolus lunatus. Lima bean plants, in receipt of a volatile signal from a damaged conspecific neighbour, increase production of extra-floral nectar. To date the majority of studies on plant-plant communication have been conducted under laboratory conditions, with a small but increasing number of studies demonstrating an effect under field conditions. These field based observations have been over short distances. Volatile chemicals are naturally diluted in the air, but with tropospheric pollutants, such as ozone, reaching high levels - there is a further and previously untested factor. We used a Lima bean based system to explore the effects of ozone on plant-plant communication. We will review the previous work in this field, present our new experimental data and discuss the ecological, agricultural and evolutionary implications of this work.

Ecosystem effects of large herbivores

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Grazing by large mammalian herbivores can influence the structure and productivity of plant communities. Grazing directly reduces plant biomass and alters plant community structure by affecting plant competitive interactions. Herbivores can indirectly affect plant communities by influencing plant–soil interactions and below-ground competition.

Summarizing our results from long-term field experiments in subarctic and arctic and their adjoining laboratory tests show that herbivory changes the quality of soil organic matter and the quantity of carbon with concomitant changes in the soil decomposer organisms such as bacteria and fungi. Long-term defoliation in field creates changes in the soil with negatively feedback on subsequent plant nutrient uptake and plant growth. However, there was no evidence that plant species richness affected the growth response of the plant community to defoliation history of the soil. Furthermore, we did not find any interaction between defoliation and plant species richness, which indicates that the resistance to defoliation was even less affected by plant species richness than species composition. In contrast to numerous studies emphasizing the role of defoliation and nutrient enrichment, our results indicate that trampling may have larger and defoliation and fertilization smaller roles than anticipated in explaining reindeer grazing effects in subarctic. Below-ground competition and competition for mycorrhiza-mediated resources may be an important factor underlying seedling establishment and plant performance in grazed subarctic and arctic systems with cascading effects on plant community composition, diversity and ecosystem productivity.

New virus detections in *Humulus* L., *Prunus* L. and *Allium* L. species in Finland

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Certified plant production produces high quality, disease and pest free material from fruit, berry and landscaping plants for nursery production. National Programme of Plant Genetic Recourses, where MTT has the responsibility of coordination work and preservation of agricultural and horticultural plants, started in 2003. Nuclear plants and nuclear plant candidates for certified production as well as plant accessions selected to long term preservation are treated and preserved by *in vivo*, *in vitro* or cryopreservation in MTT Plant Production Research at Laukaa. In 2005–2009, some viruses from new host plants and even new plant viruses were detected in Finland from plant genera *Humulus*, *Prunus* and *Allium*. Studied clones were under the process for certified production, for the long term preservation or for commercial production.

Finnish hop (Humulus lupulus L.) clones collected from eastern Finland and selected to long-term preservation, were screened of Apple mosaic virus (ApMV). The tests were made by ELISA and results were confirmed by PCR. Likewise ApMV Apple chlorotic leaf spot virus (ACLSV) was screened by ELISA and PCR on plum (Prunus domestica L.) cultivars growing in Finland but originating from Estonia. As a result 36 % (4/11) of tested hop clones were found to be infected by ApMV and 2 of 3 plum cultivars were found to be infected by ACLSV. Previously ApMV and ACLSV have been observed from apple (Malus domestica L.) in Finland, but these were the first detections of ApMV on hops and ACLSV on plums in Finland. Plant viruses were indexed by ELISA also from a heavily symptomatic farmer clone of garlic (Allium sativum L.) which was brought from Canary Islands in the end of 1980's. In addition, viruses were tested from an asymptomatic old Finnish garlic clone. The farmer clone was infected with Onion yellow dwarf virus (OYDV), Shallot latent virus (SLV), Leek yellow stripe virus (LYSV) and Garlic virus B (GarV-B). The old Finnish clone was infected by OYDV, GarV-B and Garlic virus C (GarV-C). GarV-B and GarV-C were determined for the first time in Finland.

During the past few decades global trade of horticultural plants has expanded and the risk to get new alien pathogens has increased. The viruses detected in this study may have existed in Finland for a long time, but the importance of them and other still undetected pathogens may change when the climate will enhance the survival and dispersal of pathogens and their vectors. The advances in diagnostic methods will improve the detection of new pathogens, like in this study. These virus findings prove also that plant disease indexing in certified production is an effective way to prevent the spreading of new pathogens. Infected plants may easily contaminate valuable plant collections. Therefore, in the future, more attention should be paid to the health status of plants selected to the long term preservation.

Effects of a fungal symbiont (*Epichloë* endophyte) on performance of a subarctic grass (*Festuca rubra*)

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Mutualistic symbionts may play an important role in performance and survival of plants. Seed-borne systemic endophytic fungi of grasses (*Epichloë / Neotyphodium* fungi) are thought to be plant mutualists, because they have shown to improve their host's resistance against herbivores and other biotic and abiotic stresses. The interactions in plant–endophyte associations vary from mutualistic to parasitic with environmental conditions and genotypes of interacting species (Saikkonen et al. 2004).

The most highly mutualistic grass-endophyte symbioses have been found in agricultural system, but more variation in interaction is predicted and found in natural grass populations. Occurrence of *Epichloë* fescue endophyte in subarctic fine fescue populations differs among grass species and habitats (Wäli et al. 2007). In this study, the effects of habitat of origin and additional players on the interaction between a grass and its endophyte are explored. In addition, the endophyte effects on the performance of other species interacting with the host grass. The red fescue grass (*Festuca rubra*) and its *Epichloë festucae* symbiont originating from subarctic seminatural populations are used here as a study system.

Grasses originating from the different habitats differed in several traits. Also the endophyte infection affects differently the grasses from different habitats. Endophyte seems to strengthen the growth characters probably beneficial to grasses in its original habitat, e.g. higher investment on strong tillers and ramets in river bank originating red fescues and better tolerance/resistance of parasites and competition in meadow originating grasses.

These results suggest adaptation of grass-endophyte symbiosis to different habitats and underline the role of other interacting species in outcome of the symbiosis.





(16) Application of tobacco rattle virus based gene silencing in *Gerbera hybrida*

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Gerbera hybrida, belonging to the large sunflower family, has attracted considerable attention as a new model plant for flower development study because of its particular flower organization in the inflorescence. As a typical member of Asteraceae, gerbera tissues are rich in two glucosidic lactones gerberin and parasorboside, and many other secondary metabolites, which are important for the plants to protect against microbial attack and insect herbivores. During the recent years, high throughput genetic methods, such as EST sequencing and microarrays, have been adopted for identification of hundreds of candidate genes affecting gerbera flower development or secondary metabolism. In this highly heterozygous species, the functional studies need to be conducted through reverse genetic methods by producing transgenic lines. To facilitate these studies, our aim is to adapt virus-induced gene silencing (VIGS) method for gerbera. Tobacco rattle virus (TRV) has a very broad host range including gerbera. VIGS vectors based on TRV have been powerful in inducing gene silencing in many important plant species. Our initial studies show that the TRV-based vectors induce gene silencing also in gerbera leaf and flower tissues. Screening of 21 different gerbera genotypes showed large differences in VIGS response. Out of these varieties, 6 most sensitive gerbera genotypes were selected for further studies. Among different inoculation methods, vacuum inoculation induced most intensive silencing on gerbera leaf tissues, and stem wound scratching was more practical for inducing gene silencing on flowers. The silencing of gerbera phytoene desaturase gene (GPDS) and gerbera chalcone synthase (GCHS) gene induced typical gene knock-out symptoms on host gerbera plants.



Fig. 1. Symptoms when gerbera PDS (Right) and CHS gene (left two) were silenced.

(17) Virus-derived suppressors pf RNA silencing: effects in transgenic *N. tabacum* and *N. benthamiana* plants

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RNA silencing is a highly conserved gene regulation mechanism occurring in all eukaryotic species, and in plants it also functions as a major defence mechanism against viral pathogens. The viruses have to be able to counteract this defence mechanism by some means in order to infect their host plants, and to this end they express various suppressors of the silencing machinery. While these RNA silencing suppressors are essential factors in the virus-host interactions, they also interfere with the endogenous RNA silencing pathways, and indeed, many of the symptoms of virus diseases may be related to disturbance of silencing-mediated regulation of various host genes. Interestingly, the silencing suppressors encoded by various virus groups are very different from one another, and as they appear to be targeted at different steps of the silencing pathways, they can be used as tools to investigate the silencing machinery, and its interactions with viral pathogens.

In this work silencing suppressor genes derived from six different virus genera were transformed into Nicotiana benthamiana and N. tabacum plants. These suppressors were P1 of Rice yellow mottle virus (RYMV), P1 of Cocksfoot mottle virus, P19 of Tomato bushy stunt virus, P25 of Potato virus X, HcPro of Potato virus Y (strain N), 2b of Cucumber mosaic virus (strain Kin), and AC2 of African cassava mosaic virus (ACMV). HcPro caused the most severe phenotypes in both Nicotiana spp. AC2 also produced severe effects in N. tabacum but a much milder phenotype in N. benthamiana, although both HcPro and AC2 affected the leaf tissues of the two Nicotiana spp. in similar ways, causing hyperplasia and hypoplasia, respectively. P1-RYMV caused high lethality in the *N. benthamiana* plants but only mild effects in the *N. tabacum* plants. Phenotypic alterations produced by the other transgenes were minor in both species. Interestingly, the suppressors had very different effects on cruciferinfecting Tobamovirus (crTMV) infections in N. benthamiana plants. AC2 enhanced both spread and brightness of the crTMV-green fluorescent protein (GFP) lesions, whereas 2b and both P1 suppressors enhanced spread but not brightness of these lesions. P19 promoted spread of the infection into new foci within the infiltrated leaf, and also systemically in the plants, whereas HcPro and P25 suppressed the spread of crTMV-GFP lesions (Siddiqui et al. 2008. MPMI 21:178-187). Effects of these silencing suppressors on the transcriptome profiles of tobacco plants are currently being investigated.

(18) Effects of sugar beet chitinase IV on root-associated fungal community of transgenic silver birch (*Betula pendula*) in a field trial

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Heterogenous chitinases have been introduced in many plant species with the aim to increase the resistance of plants to fungal diseases. We studied the effects of the heterologous expression of sugar beet chitinase IV on the intensity of ectomycorrhizal (ECM) colonization and the structure of fungal communities in the field trial of fifteen transgenic and eight wild-type silver birch (*Betula pendula* Roth) genotypes. Fungal sequences were separated in denaturing gradient gel electrophoresis (DGGE) and identified by sequencing the ITS1 region to reveal the operational taxonomic units (OTUs).

ECM colonization was less intense in seven out of fifteen transgenic lines than in the corresponding non-transgenic control plants but the slight decrease in overall ECM colonization in transgenic lines could not be related to sugar beet chitinase IV expression or total endochitinase activity. One transgenic line showing fairly weak sugar beet chitinase IV expression without significantly increased total endochitinase activity differed significantly from the non-transgenic controls in the structure of fungal community. Five sequences belonging to three different fungal genera (Hebeloma, Inocybe, Laccaria) were indicative of wild-type genotypes, and one sequence (Lactarius) indicated one transgenic line. In cluster analysis, the nontransgenic control grouped together with the transgenic lines indicating that genotype was a more important factor determining the structure of fungal communities than the transgenic status of the plants. With the tested birch lines, no clear evidence for the effect of the heterologous expression of sugar beet chitinase IV on ECM colonization or the structure of fungal community was found.

(19) The effects of boron on the herbivory resistance of *Betula pendula* (Betulaceae)

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Boron (B) deficiency is the most common micronutrient deficiency in the world and causes severe financial losses both in forestry and agriculture. Boron deficiency has been reported to affect the phenolic metabolism of plants and thus it may play an important role in the defense against plant eating animals. However, the role of B in the plants' resistance against herbivores has not yet been studied.

In this study, we tested the effects of B nutrition 1) on the biochemical and mechanical defenses of birches and the growth of seedlings and 2) the resistance of the seedlings against *Epirrita autumnata*.

Boron fertilization improved birch resistance shown as a reduced pupal weight and immune defense of the herbivore but B fertilized trees suffered from heavier defoliation than unfertilized ones due to compensation feeding. Herbivory decreased the growth of seedlings and caused several biochemical changes in birch leaves. Polyphenoloxidases (PPOs) and peroxidases (PODs) and their substrates, chlorogenic acids, were induced by herbivory in B fertilized seedlings but not in unfertilized ones, suggesting that B deprivation may suppress defense signaling in plants.

Decreased pupal weights and increased consumption were most probably linked to the phenoloxidase-mediated production of reactive quinones

that decrease the nutritive value of plants. Herbivory induced also an enhanced production of hairs and resin glands in new stems, the latter being a defense against mammalian herbivores. In other words, birch seedlings responded to intense insect herbivory producing defenses both against insect and mammalian herbivores. Our study shows that B may have an important role in the resistance of plants against herbivores.



(20) Effects of different silencing suppressors on systemic spread of different GFP-tagged viruses

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In plants, RNA silencing mediates multiple defence mechanisms against invading genetic elements, such as transgenes, transposons and viral pathogens. To encounter this defence mechanism, plant viruses produce various silencing suppressor proteins. These are known to suppress different steps of the silencing machinery, and due to their various modes of action they can be used as probes to study specific silencing-related phenomena and host-virus interactions. We have produced a set of transgenic *Nicotiana tabacum* and *N. benthamiana* lines expressing different silencing suppressors (Siddiqui et al. 2008, MPMI 21:178-187). To study the effects of these silencing suppressors on spread of heterologous viruses, transgenic *N. benthamiana* plants were agro-infiltrated with GFP-tagged crucifer-strain of *Tobacco Mosaic Virus* (crTMV-GFP).

The local spread of the virus was clearly enhanced in the plants expressing the P1, 2b, AC2 and P19 silencing suppressors, but the virus could spread systemically into new leaves only in plants expressing the P19 protein, suggesting that only this silencing suppressor prevented the systemic spread of the silencing signal. Surprisingly, virus spread was not enhanced in the HcPro-expressing plants, and in P25-protein expressing plants the spread was strongly reduced (Siddiqui et al. 2008, MPMI 21:178-187). Epigenetic expression of Pectin Methyl Esterase (PME) enhance the silencing, and when coinfiltrated with GFP-crTMV, it strongly reduces the accumulation of this virus in wild type plants (Dorokhov et al. 2006, FEBS Lett 580:3872-3878). Interestingly, while P1, 2b, AC2 or P19 transgenes did not interfere with the PME-induced silencing-enhancement, in P25 and HcPro transgenic plants co-infiltration of the PME construct was unable to reduce spread of crTMV-GFP. Thus, P25 and HcPro silencing suppressors prevented the PME-induced silencing enhancement.

(21) HcPro silencing suppressor significantly alters gene expression profile in tobacco leaves and flowers

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RNA silencing is an evolutionary conserved mechanism to protect plant cells against invasive nucleic acids, such as viruses, transposons and transgenes. However, viruses have evolved to produce factors that are able to suppress RNA-silencing in plants. The action of these silencing suppressors may relate to the symptoms of the virus infections by changing the balance between microRNA (miRNA) and mRNA in the cells and on the other hand silencing suppressor may also change metabolic pathways in plant cells (e.g. overexpressed HcPro protein may function as cysteine endopeptidase and thioredoxin in the cells). Previously characterized transgenic tobacco plants containing an HcPro silencing suppressor under a constitutive CMV 35S promoter were used in this study (Siddiqui et al. 2008).

Over expression of this silencing suppressor induced clear phenotypic changes both in growth rate of plants and morphology of flowers (e.g. petals grown together, stamens transformed to petals). To find out the genetics behind these phenotypic chances in transgenic HcPro plants, we performed microarray analysis (Tobacco 4x44k, Agilent) of both leaves and flowers. The expression of genes was changed significantly (two-fold up or down regulated as cut of value) in leaves (843 genes) and in flowers (342 genes). 463 genes were up regulated and 380 genes were down regulated in transgenic HcPro leaves, whereas only 121 genes were up regulated and 221 were down regulated transgenic HcPro tobacco flowers.

Interestingly, expression of HcPro in tobacco plants induced many similar types of genes than previously have been found after infection of plants with ssRNA-viruses. A functional categorization of most up-and down regulated genes demonstrated that genes related to defense and stress response, cell wall modifying enzymes, protease inhibitors, transcriptional regulation, and photosynthesis were clearly over-represented.

The results also suggested that sugar metabolism was severely affected in HcPro expressing plants, as lots of genes previously characterized as stress and energy responsive were induced. Many of these genes are expressed in wild type plants only under severe nutrient or energy deprivation like in darkness. Energy deprivation may have also changed the cell wall synthesis due to highly energy demanding S-adenosyl-L-methionine (SAM) biosynthesis leading to decreased transmethylation capacity. Protein analysis by 2D-PAGE also indicated decreased translational activity of most proteins.

In conclusion, the expression of silencing suppressor HcPro is capable of changing gene expression in a way corresponding to poty virus infection in *Arabidopsis* plants. Ref: Siddiqui S. A. et al.: Mol Plant Microbe Interact. 2008 Feb; 21(2): 178–87.

(22) Aphid parasitism on chitinase transgenic silver birch (Betula pendula Roth)

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The study of the effects on natural enemies forms an important part of the environmental risk assessment of transgenic plants. Transgenic chitinases have been tested in the attempts to improve fungal and insect resistance in plants. Chitinases are enzymes able to hydrolyse chitin, the main component of cell walls of fungi and insect exosceleton. We studied if a transgenic chitinase has impact on the occurrence of natural enemies of herbivorous aphids.

Fifteen chitinase transgenic silver birch lines, their corresponding control and seven other wild-type genotypes were monitored in a field trial. Transgenic trees carried a sugar beet chitinase IV gene that was originally introduced in order to improve fungal disease resistance in birch. The trees were grouped based to their transgene expression level. The trees were monitored for three times during one season. Aphids (mainly Birch aphid *Euceraphis betulae* Koch) and parasitized aphids (mainly parasitized by braconid wasps) were visually estimated.

No difference in the proportion of parasitized aphids was found between the transgenic lines and their corresponding control or other wild type genotypes. The mean proportion of parasitized was lower in most of the transgenic lines than in the control being on the same level as at the other wild-type trees. The variation in the parasitism rate was higher among the transgenic lines than among the wild-type genotypes. No difference was found between horizontal branch thirds or vertical thirds of the tree crown in any of the studied trees. The transgene expression level did not explain parasitism rate.

Changing environment (sessions 7 and 9)

ABSTRACTS OF

8 ORAL PRESENTATIONS

AND

13 NUMBERED POSTERS (23 - 35)

Climate change in the high latitudes: effects on the emissions of biogenic volatile organic compounds

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Biogenic volatile organic compound (BVOC) emissions from subarctic and boreal ecosystems are important for the atmospheric chemistry and their feedbacks to climate change are uncertain. We present data on effects of simulated climatic warming and enhanced UV-B radiation on the BVOC emissions from subarctic ecosystems, a tundra heath (warming responses) and a peatland (UV-B). We also show how vegetation changes and water table drawdown affect the BVOC emissions from boreal peatland microcosms maintained in growth chambers. BVOC emissions were measured using conventional chamber and GC-MS methods. The BVOCs presented are isoprene, monoterpenes, sesquiterpenes, other reactive VOCs (lifetime < 1 day) and other VOCs (lifetime > 1 day).

Warming on the subarctic heath doubled the emissions of isoprene, monoterpenes and sesquiterpenes. This warming response was higher than predicted by the current models and should be considered in the next modeling efforts. Enhanced UV-B radiation increased the isoprene emission from the subarctic peatland but did not affect emissions of the other groups of BVOCs. In the boreal peatland microcosms, the removal of vascular plants decreased the emissions of isoprene, that of monoterpenes in hummocks and that of sesquiterpenes in hollows. The water table drawdown applied decreased the emissions of isoprene, monoterpenes and other VOCs. These results demonstrate that climate change has significant effects on the BVOC emissions from the subarctic and boreal ecosystems following warming and the consequent vegetation and water table changes. These changes have implications for the biological interactions and the atmospheric chemistry via the impact of BVOCs on aerosol formation and the lifetime of greenhouse gases.

Changes in the vegetation and flora of rich fens around Kolatselkä village, southern Republic of Karelia, from 1942 to 2008

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Numerous Finnish war botanists worked in the area of present Republic of Karelia during the Second World War to study the possibilities for agriculture, especially during the summers of 1942 and 1943 under the leadership of professor Mauno J. Kotilainen. The materials were well documented, which makes it possible to compare the situation after almost 70 years. Botanists from Finland and Russia have made joint expeditions during the latest ten years especially to the north of Petrozavodsk.

To the south-west from Petrozavodsk, studies were made in Kolatselkä region during the wartime by Jukka Lounamaa and Harry Waris, altogether in almost 50 mires, mostly rich fens due to the calcareous bedrock.

Two Finnish – Russian botanical expeditions were organized in Kolatselkä during the summers 2007 and 2008. Our aim was to collect data on the vegetation and flora of rich fens to compare the situation with wartime, and further with the last rich fens of southern Finland.

Altogether 12 mires studied in the wartime have been later drained for agriculture or forestry, mainly to the west of the village. Other mires have remained in natural condition hydrologically. We have so far studied 12 previously studied mires and four which have not been visited earlier.

The main result of our study is that open rich fens in this region are cultural habitats of traditional agriculture. Haymaking and grazing have kept the fens open, and given space to numerous rare and threatened species. After the fall of socialistic system, changes in society and economical conditions have caused the reduction and aging of the human population in the village. As a consequence, the traditional agriculture has collapsed.

As a consequence, only the wettest fens have remained open, and their physiognomy and plant communities are similar to 1940s. In all lawn and hummock level fens trees and bushes are growing well now, causing increased evapotranspiration and shading, which has led to vegetation changes, and to the disappearance of numerous species, especially mosses of wet surfaces. The cover of true rich fen species has also reduced, and it seems that many of them will also disappear from many sites in near future.

The development is very similar to that in the rich fens of southern Finland, but due to the longer time since the cessation of the traditional use of rich fens, the situation in southern Finland, according to our unpublished data, is much worse than in Karelian Republic.

The time series of flowering and leaf bud burst of boreal trees (1846 to 2005) support the direct temperature observations of climatic warming

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The timing of spring phenological events of boreal trees, such as flowering and leaf bud burst, is controlled to a great extent by the ambient air temperature, and these events are already showing an advancement that can be attributed to climatic warming. In this paper we utilized this phenomenon to verify direct observations of climatic warming. We constructed eight phenological time series using observations covering 160 years of the leaf bud burst of two species and of the flowering of six species of native deciduous trees growing in Finland. To eliminate the effect of urbanization on local temperature, we rejected the observations made in densely populated areas. When analyzing the trends in the timing of leaf bud burst and flowering we found an advancement of these phenological events ranging from 3.3 to 11.0 days per century. We then converted the observed phenological trends into temperature trends by using Thermal Time -type models that link the timing of bud burst and flowering to the temperature conditions during bud development. Warming estimates derived from the phenological trends suggested that the mean spring temperature increase in Finland has been 1.8 °C per century, which is very close to the value of 1.5 °C per century indicated by long-term temperature records.

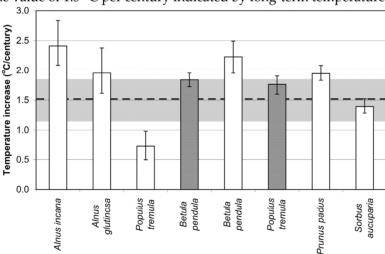


Fig. 1. Warming equivalents derived from phenology. White bars = flowering, grey bars = leaf unfolding. Dotted line indicates warming trend from direct temperature measurements of spring months. Grey band and error bars are 95 % conf. int.

Linkosalo, T., Häkkinen, R., Terhivuo, J., Tuomenvirta, H., Hari, P. 2009. The time series of flowering and leaf bud burst of boreal trees (1846-2005) support the direct temperature observation of climatic warming. Agricultural and Forest Meteorology, 149: 453–361.

Clear-cutting of forests influences concentrations of phenolic compounds and nitrogen in bilberry (*Vaccinium myrtillus*) leaves and shoots

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Bilberry (*Vaccinium myrtillus*) is a keystone species in boreal forests, hosting and providing food for many herbivores. At the same time, bilberry is sensitive to changes in abiotic factors, which may affect morphology, reproduction and chemical composition.

In the present study we chose five old-forest stands and five clear-cut stands in a sub-oceanic spruce forest in south central Norway. Within each stand we established two macro plots, of which one was fertilized and one not, and within the macro plots we established four micro plots with the following treatments 1) root cutting, 2) flower removal, 3) root cutting + flower removal and 4) control.

In the second growing season, biomass was estimated for each plot, and shoots and leaves were collected twice (early summer and autumn) for analysis of phenolic compounds. Concentrations of carbon and nitrogen were analysed from shoots sampled in the autumn.

Concentrations of catechin derivatives and condensed tannins were highest in autumn, while concentrations of phenolic acids and flavonols were highest in summer. All groups of phenolic compounds had highest concentrations on clear-cuts, and the concentrations of phenolic acids and flavonols were highest in non-fertilized plots. Removal of flowers and root-cutting had no effects on phenolics. Nitrogen concentrations in shoots were highest in old-growth forests and when flowers were removed (no berries produced). The amount of flowers was highest in old-growth forests, in non-fertilized plots and in plots with no root-cutting. Berry production (amount of berries and fresh weight of berries), on the other hand, was highest on clear-cuts and in fertilized plots, but also if no root-cutting was done.

The results indicate that forest practice might influence taste and nutritive value of bilberry plants for herbivores, as the defence (phenolic compounds) is higher and nitrogen content lower on clear-cuts. The berry production, on the other hand, may increase on clear-cuts. Fertilization, e.g. as nitrogen deposition, may decrease phenolic content and increase berry production.

Tropospheric ozone causes oxidative stress to northern deciduous trees (*Betula* and *Populus* species)

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Northern forests are subjected to a changing environment where one of the major threat is increasing load of oxidative stress, e.g. through rising tropospheric ozone levels and abrupt changes in water availability. The proportion of deciduous trees species, i.e. *Betula* and *Populus* sp. is expected to increase in northern latitudes due to warming climate. Unfortunately, we have growing evidence that the vitality and the carbon sink strength of birch and aspen trees are weakened under oxidative stress.

First, we present negative correlations of the main growth responses of birch (*Betula pendula*, *B. pubescens*) and aspen species (*Populus tremula* and *P. tremula* x tremuloides) with ozone load. Data are derived from 23 different laboratory, open-top chamber and free-air fumigation experiments, providing information from complementary methodological approaches. Impaired biomass accumulation due to ozone was most evident in roots, and growth reductions were accompanied by increased visible foliar injuries, C allocation towards defensive compounds (phenolics, in particular) reduced carbohydrate contents of leaves, disturbed photosynthesis and stomatal processes, changes in gene expressions and earlier leaf senescence. However, the genetic differences in ozone responses are large in both species. For hybrid aspen, some "ozone-thriving" genotypes were identified, which could be used as breeding material for improved ecological tolerance.

Secondly, our recent ozone x temperature experiments with birch suggest that apoplastic ascorbate, often regarded as the main ROS (reactive oxygen species) -scavencing pathway, plays only a transient role during the early ozone defence, whereas stomatal regulation and structural plasticity of leaves (i.e. development of thicker leaves with increased apoplastic volume) are more important long-term mechanisms under chronic ozone stress. Ozone detoxification capacity was, however, modified by elevating temperature in a complex manner. Therefore, our next challenge is to determine the ozone x temperature interactions. The results contribute to development of more accurate ozone risk assessment approaches, which are based on ozone flux into the leaves and an array of detoxification processes. These results can be also applied in the context of other oxidative stresses.

Physiological and growth responses of Norway spruce (*Picea abies*) to delayed soil thawing

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Snow is an important insulator of forest soils against frost in northern latitudes. The lack of this insulation layer may result to more severe soil freezing. If the snow cover diminished by climate change in future this might lead to colder soils in a warmer world.

We studied the effects of soil frost and its delayed thawing on the roots and shoots of 47-year-old Norway spruce in a snow manipulation experiment during two winters between 2005 and 2007. The treatments with three replicate plots were: 1) CTRL with natural snow accumulation and melting, 2) OPEN with artificial snow removal during the winter, and 3) FROST that was similar as OPEN but the ground was insulated with a layer of hay between plastic sheets in early spring to delay soil thawing. Physiology and growth of trees was monitored from April to September in 2006 and 2007.

Chlorophyll fluorescence, chlorophyll a and b content and apoplastic electrical resistance of the needles were affected by FROST in 2006 but not in 2007. Typical peak for starch content in needles in spring, trunk diameter growth (i.e. formation of new tracheids) and bud burst were all delayed by FROST in 2006. In that year cross-sectional area of new needles remained smaller in FROST than in other treatments. Growth of fine roots tended to increase by FROST and OPEN as compared with CTRL which suggests to some compensatory responses. Overall, the results indicate that Norway spruce responded in varying ways to delayed soil frost thawing.

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CO₂ exchange of a bioenergy crop on a drained peat soil

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Northern peatlands store vast amounts of carbon. In their natural state, they have been shown to be sinks for atmospheric CO₂ and sources of methane on a long term basis. However, when they are drained for forestry, agriculture or peat extraction, the thickness of their aerobic soil layer increases. This leads to an increase in the rate of organic matter decomposition. As a result, peatlands which were once a sink for carbon are turned into atmospheric carbon sources after drainage.

Irrespective of being cultivated with agricultural crops such as barley, wheat or potato or grasses, organic soils in the boreal region have been reported to be emitting large amounts of CO₂ into the atmosphere. Drainage of peatlands for peat extraction for energy is a common practice in the Fenno-Scandinavian region, Canada and Russia. These peatland areas abandoned after peat extraction are a threat to the environment. Significant CO₂ emissions have been reported also from afforested organic soils. Therefore, organic soils in general, have been included among the areas with high risk of significant soil carbon losses and hence have been recommended to be kept out of biomass production for bioenergy.

Recently, the cultivation of reed canary grass (RCG, *Phalaris arundinacea* L.), a bioenergy crop, on organic soils has been gaining importance in Finland. What impact does the RCG cultivation have on the carbon balance of peat extraction sites? To answer this question, we are measuring carbon exchange and supporting climatic data from RCG cultivation on a cutover peatland in eastern Finland using micrometeorological eddy covariance technique since March 2004. So far we have collected continuous carbon balance data for six years. These years are climatically different and have thus provided us an opportunity to examine the influence of changing climatic conditions on the RCG C balance. Also, starting from the fifth year of measurements, we have artificially elevated the water table level at the whole ecosystem scale by blocking the drainage ditches. We will present in this paper our observations on how the climate affects the C balance of such ecosystems and what role do bioenergy crops play in regulating the C balance on such peatland types.

Changes in carbon storage of Finnish forestry-drained peatlands based on reinvestigation of old peat inventory study sites

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Concern about climate change has focussed attention on the carbon balance of drained peatlands in Finland, because the total drained area of about 60 000 km² (mainly for forestry) constitutes one of the major potential sources of greenhouse gas emissions. Unfortunately, the carbon balances of various types of drained peatland forests are still poorly known. According to some earlier studies, the forestry drained peatlands have been considered a significant carbon sink, but more recent studies suggest that the carbon balance of drained peatland forests is on average negative (i.e. there is a net release of carbon). Credible data on the carbon balance of drained peatlads has been obtained through *in situ* gas exchange measurements of peatland soils. However, such measurement data is available only from a very few sites, and the results are greatly variable, leading to considerable uncertainties on the carbon balance estimates.

Our carbon storage approach to the carbon balance of drained peatlands is based on the very extensive peat resource inventory data archives of the Geological Survey of Finland. This data contains detailed information from several hundreds of peatland sites with accurate coring site documentation, including site coordinates. The peat core data of GSF provides accurate peat masses for the sites (bulk density, dry mass, ignition residue), from surface to the bottom. We selected about 50 sites in five areas in central Finland that were investigated by GSF some 20-30 years ago, representing different peatland vegetation types and fulfilling some unifying criteria (initial peat thickness about 2 m, and the GSF survey data collected soon after ditching). In most cases we managed to locate precisely the old coring sites via GPS, and have been able to correlate the old and modern stratigraphic inventories to assess the average annual post-drainage carbon balances for the sites. Some preliminary results will be presented. The general objective of the study is to produce a realistic estimate of carbon balance of drained peatlands in Finland and to find possible patterns for drainage-related carbon balances for peatland types of different trophic levels.

(23) How does the elevated temperature affect on phenolics in *Populus tremula x tremuloides*?

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Phenolics such as salicylates and tannins are the main secondary compounds in *Populus* species and their maintenance in tissues is costly. The aim of this study is to test how climate change factors may affect growth and different phenolic compounds of the hybrid aspen (*Populus tremula x tremuloides*) plantlets when the tannin metabolism is modified. The plant material consisted of three lines of the same clones (control, MYB 54 and MYB 61) of the hybrid aspen. MYB lines were over-expressed of their tannin metabolism with gene manipulation. Plantlets were grown at the ambient temperature and elevated temperature (+2 °C) chambers. The lower molecular weight phenolic compounds of the leaves were analyzed with HPLC and tannins were determined by acid-butanol assays.

Increasing temperature decreased several phenolic compounds. The line which contained more tannins and did grow intensively at the beginning of the experiment had less other phenolics. This indicates partly trade-off of carbon allocation between growth and phenolic metabolites and partly dilution effect. The possible role of tannins in seedling development will be discussed.

(24) Simulating the effect of nitrogen dynamics to the growth of boreal forest under changing climate, using a combination of interacting process-based models

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The growth of most boreal forests is currently limited by the amount of available nitrogen. Plants take up N from the soil, then return it to the soil systems in form of litter that is decomposed in the soil and released again for the plants to use. Increased temperature and atmospheric CO₂ is expected to increase the growth potential of boreal forests, but this potential cannot be realized if there is lack of required nutrients. Increased temperatures will hasten the rate of decomposition of plant litter, but they may also reduce the soil moisture, which would then slow down the decomposition.

There are already process-based models that describe and predict both the stand growth and soil decomposition, but to grasp the interaction and dynamics of the whole system, both models need to be combined to be enumerated simultaneously. In this paper, we have combined a process based stand growth model PipeQual [1] with a soil decomposition model Romul [2,3] and a simple bucket model of soil moisture [4]. The model combination describes the dynamics of soil water, organic matter/carbon and nitrogen between external environment, the stand and the soil.

The combination model is currently being validated using empirical measurements of stand, soil organic matter and nitrogen content. Under current climatic conditions, the nitrogen released from the soil processes and needed for the stand growth are balanced, suggesting that the individual sub-models are in good agreement, and thus promising realistic predictions of the nitrogen dynamics under changing climatic conditions. The model combination has already been used to predict the development of forest ecosystem carbon balance under current and changed climatic conditions, with various management options. We will further utilise the model to predict the effect of new energy-related management options, like whole-tree harvest, to the nitrogen balance and hence growth potential of the boreal forests.

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(25) Effects of night-time temperature on growth, net carbon assimilation, and metabolism of *Betula pendula* and *Populus tremula*

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Global climate change scenarios predict the rising of temperatures in the near future due to changes in atmospheric gas balances. The effect of temperature on tree growth is complicated because it affects extensively most processes in the plants. In this study we focused on understanding the role of night-time temperatures on growth, photosynthesis and respiration of silver birch (*Betula pendula*) and European aspen (*Populus tremula*) clones, and thus on the potential of these two species to grow and sequestrate CO₂ from the atmosphere. Moreover, we were interested to see what kinds of metabolic differences rising night temperature might cause in these species.

Three different genotypes of both species were selected for the experiment. Micro-propagated plantlets of silver birch and aspen were grown at the same day temperature (22 °C), but at different night-time temperatures (6, 10, 14, 18, 22 °C) for six weeks. Gas exchange was measured, and at the end of the experiment leaf samples were collected for metabolic analyses. Polar and lipophilic metabolites were analysed with GC-MS and in addition, polar phenolics with HPLC-DAD.

Height increment was strongly dependent on the night-time temperature in both species. In spite of a remarkable increase in stem height by warmer night temperatures, the effect on total biomass was minor. Specific leaf area (SLA) increased with increasing temperature, as the number of leaves and total leaf area increased, but leaf biomass was similar in all treatments. Concentrations of many phenolic compounds were stable, but some phenolics increased or decreased according to night temperatures. Discrimination of genotypes from each other, based on metabolome analysis, was clear and some discrimination between treatments was also evident.

(26) Effect of drought on the expression of genes in polyamine metabolism in needle tissues of *Pinus* sylvestris seedlings

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Frequency of diverging temperature and drought stresses in boreal forests has been proposed to increase in future due to climate change. Polyamines (PAs), putrescine (Put), spermidine (Spd) and spermine (Spm), are ubiquitous compounds essential for cell growth and proliferation and they play a key role in stress tolerance (Alcazar et al. 2006, Peremarti et al. 2009). Spd acts indirectly during stress by maintaining high Spm levels while Spm is the direct effector of stress tolerance (Peremarti et al. 2009). Amine oxidases catalyze PA oxidation reaction where hydrogen peroxide, H₂O₂, is produced. H₂O₂ is involved in the expression of defence and detoxification genes, cell death and developmental programmed cell death (Cona et al. 2006). The general aim of this work is to study the metabolite and transcriptome level changes in PA-metabolism in Scots pine seedlings proned to drought stress.

Scots pine seedlings were subjected under drought stress by growing them in peat on three moisture levels for two months. Samples from the seedlings were collected three times during the experiment. Dry mass, number of root tips and mycorrhizae, polyamine concentrations (with HPLC) and expression of arginine decarboxylase (*ADC*), spermidine synthase (*SPDS*) diamine oxidase (*DAO*) and S-adenosylmethionine decarboxylase (*SAMDC*) were measured.

According to our results drought stress increased expression of *DAO* in Scots pine needle tissues. *SPDS* and *SAMDC* expression levels in the needles were more variable on the drought treated samples than on the well watered ones. *ADC* gene expression studies with melting point analysis suggested the presence of more than one *ADC* gene in Scots pine. In *Arabidopsis* there are two ADC genes; ADC1 and ADC2, from which ADC2 is responsive in abiotic stresses (Hummel *et al.* 2004, Soyka and Heyer 1999).

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(27) Foliar responses of European aspen and hybrid aspen to changing environment

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Plants are being exposed to various environmental changes such as increases in temperature, N deposition and O₃ concentration as well as longer and more severe drought periods. We studied key leaf traits that relate to acclimatization (specific leaf area, SLA; amount of epicuticular waxes) and nutrient balance (N concentration, C:N ratio) of European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x tremuloides) from three different settings:

- mature European aspens at three urban and three rural forest sites in the Helsinki Metropolitan Area in 2006–2008,
- 13 hybrid aspen clones (8- and 9-year-old) growing 10 and 25 m away from a motorway and at a control site in 2007,
- two European aspen and two hybrid aspen clones (5-month-old) grown in pots under elevated (1.5 x ambient O₃) and ambient O₃ in a free-air fumigation experiment in 2006.

The studied trees acclimatized to environmental changes brought about by urbanization and traffic pollution by producing leaves with higher amount of epicuticular waxes and hybrid aspen also by leaves with lower SLA. These xeromorphic adaptations may result from higher solar radiation and wind speed in fragmented urban forests and from pollutant-related malfunctioning of stomata that promote moisture stress. Interannual variation in climatic factors was manifested as variation in leaf traits between the study years, which further indicates the ability of aspen for phenotypic plasticity. Yet, elevated O₃ alone did not affect these leaf traits when the young trees were grown under optimal water and N supply. However, leaf N concentration decreased and C:N ratio increased in European aspen exposed to elevated O₃. Urban trees, on the other hand, had higher leaf N concentration and lower C:N ratio than rural ones, suggesting higher atmospheric NOx concentrations and/or soil N availability in the urban environment. Leaf N concentration correlated positively with wax amount in both field studies, which may mean that a better N status provides more assimilates to be used e.g. in wax production.

The results indicate that aspen, a pioneer species, can rather easily acclimatize to environmental changes by means of its leaves. SLA is often used as an indicator of environment quality, but the results suggest that the amount of epicuticular waxes can also be considered as one, perhaps more suitable indicator for aspen.

(28) Elevated CO₂ and temperature may influence the UV-B defense of silver birch (*Betula pendula*) seedlings

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Combined elevations of carbon dioxide (CO₂), temperature (T) and ultraviolet-B (UVB) radiation may have significant impact on plant growth and secondary chemistry, and the different factors may work in opposite directions or induce additive effects.

Seedlings of six silver birch (*Betula pendula*) clones were exposed to combinations of ambient or elevated levels of CO₂, T and UVB radiation in top-closed chambers for seven weeks. All measured growth parameters increased under elevated CO₂, and elevated T also increased seedling height and biomass. Elevated CO₂ and T combined resulted in the highest total biomass production, height and diameter growth. Enhanced UVB had no effect on any growth parameters, nor did it alter growth responses to CO₂ and/or T in combined treatments. Only leaf biomass differed between genotypes.

Elevated CO₂ increased the concentrations of condensed tannins and myricetins, while elevated T decreased myricetins, quercetins and chlorogenic acids. Enhanced UVB increased quercetins and kaempferols. Quercetin and chlorogenic acids had highest concentrations under enhanced UVB and CO₂ combined, but this effect disappeared in the triple treatment. There were no significant interactions between UVB, CO₂ and temperature on phenolic compounds, but almost all identified chemical compounds varied between genotypes.

Increased CO₂ may thus add to the UVB induced chemical protection of *B. pendula* seedlings under enhanced UVB, but this effect may be neutralized by elevated T. However, we found no signs that UVB would have negative effects on growth of young *B. pendula* seedlings, neither alone, nor if added to other climate stressors.

(29) Integrated effects of elevated ozone and temperature in European aspen (*Populus tremula*) and silver birch (*Betula pendula*)

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Northern trees are experiencing major changes in their environment due to global warming, increasing greenhouse gases and changes in precipitation. Therefore, an open-field experiment was established in University of Eastern Finland, Kuopio campus, to study impacts of combined action of warming and ozone on silver birch (*Betula pendula*) and European aspen (*Populus tremula*). Our exposure system consists of four elevated-ozone plots and four control (ambient air) plots. Each plot is divided into two infra-red heated sub-plots and two ambient-temperature sub-plots. There were four genotypes for both species planted in soil-submerged pots. The first exposure with birches and aspens started in June 2007 and continued until October 2008.

Exposure plants were measured for growth, senescence, gas exchange profiles along the plant axis, fluorescence, respiration, volatile organic compounds (VOCs), soil respiration, mycorrhizal infections, extramatrical mycelium, antioxidants, reducing potential, and changes in leaf anatomy, metabolites and VOC-related gene expression. In addition, C allocation was studied with stable isotope (13 C-CO₂) labelling methods.

Our results indicated that rising temperature increases the growth, photosynthesis and VOC emissions from leaves in both species, whereas stomatal conductance and antioxidative capacity were decreasing and senescence was delayed. The increase in the total leaf area had a remarkable influence on the whole-plant net C assimilation level, resulting in a considerable increase of net C assimilation in both species at elevated temperature. Elevated ozone impaired leaf growth in birch and photosynthesis in both species and declined the emissions of some VOCs in birch. However, there seems to be a large variation among the genotypes in their responses to warming and ozone with complicated interactions.

(30) Scots pine (*Pinus sylvestris*) saplings survived after six weeks waterlogging

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Flooded soil is low in oxygen, which disturbs root and rhizosphere function. Also, metabolism and growth of shoots can be severely affected. Increased flooding in winter and early spring is among the predictions of the future climate. The aim of this study was to evaluate the effects of flooding and soil frost on Scots pine saplings during their winter dormancy.

The study was performed in dasotrons that are large growth chambers. Four-year-old Scots pine saplings from a forest plantation site were replanted into root containers (0.48 m³) filled by mineral soil with a thin organic top layer. The experiment consisted of a 12 weeks growth season followed by a gradual change towards a six weeks dormancy phase. The FLOOD and FROST treatments took place during this simulated winter. In the FLOOD, the pots were filled with lake water (pH 5.2). The NONFLOOD pots did not receive additional water during the winter. In the FROST, soil temperature was decreased to -2 °C, but in the NONFROST soil was kept thawed (+2 °C). In the FLOOD+FROST, soil was frozen after water addition. Air temperature and light conditions were similar for all the treatments. At the end of the dormancy phase, excess water from the pots was removed through a bottom valve and frozen pots were thawed. After 4 weeks gradual change, a new 12 weeks growing season started. Soil (temperature, water and oxygen content) and air conditions (temperature, RH, photon flux density) were monitored throughout the experiment. Needles were sampled for assessment of dark-adapted chlorophyll fluorescence (F_v/F_m), chlorophyll, soluble sugar and starch concentration, water potential and electrical impedance spectroscopy (EIS). Trunk sap flow was logged continuously. At the end of the experiment, the biomass of the above-ground parts of the trees was measured.

The hypoxic root conditions during simulated winter were not lethal for pines. The physiological and growth responses of pines will be discussed.

(31) Responses of boreal dwarf shrubs to changing snow conditions – a snow manipulation experiment

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The depth and duration of snow and its physical properties are fundamental factors affecting the overwintering of northern plants. In the current study we manipulated snow cover on sample plots dominated by either lingonberry (*Vaccinium vitis-idaea*) or blueberry (*V. myrtillus*) in a boreal coniferous forest through the winter 2008-2009. The manipulations consisted of the following treatments: (i) partial removal of snow to 20 cm; (ii) addition of snow (the amount of snow added was equal to the amount of snow removed in the first treatment); (iii) increase of snow density (by compressing snow); (iv) control plots (natural snow cover).

Because snow is an efficient insulator, both the removal of snow and the increase of snow density increased the variability of temperature close to soil surface. Both of these treatments also increased the penetration of photosynthetically active radiation in the snow. Compared with the control plots, the water equivalent of snow was 52 % higher in the addition treatment and 44 % lower in the removal treatment.

Several ecophysiological traits related to overwintering success were assessed at the time of maximum snow depth in late March. Like in our previous studies, lingonberry retained its photosynthetic capacity through the winter. Photosynthetic light responses showed no major differences among the treatments. Despite similar light responses, net photosynthetic gain before snow melt was higher in the density increase and removal treatments where more photosynthetically active radiation was available. The highest electrolyte leakage percentages of the leaves of lingonberry and the shoot tips of blueberry were measured in the snow removal and increased density treatments, which indicates winter damage affecting the permeability of cell membranes. Lowest Fv/Fm ratios of chlorophyll fluorescence were measured in the same treatments, which suggests that a thin or dense snow cover may be harmful for dwarf shrubs.

Even if snow melted approximately 10 days later in the snow addition treatment compared with the snow removal treatment, no major differences were observed in the subsequent phenological development, i.e. timing of bud burst, leaf unfolding or flowering. However, the annual increment of shoot length of blueberry was almost 50 % higher in the snow addition and density increase treatments. Despite enhanced shoot growth, the number of leaves produced was not affected. The results of the present study show that snow conditions may not only affect the overwintering success of dwarf shrubs, but also influence growth during the following summer. Similar effects can be expected when snow conditions change along with global warming.

(32) Microscopic development of vegetative buds of Norway spruce (*Picea abies*) in relation to temperature and visible bud phenology

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Timing of bud development in ecodormancy has always been critical for the trees growing in boreal and temperate regions with seasonally alternating climates. The predicted global warming over the mid- and high latitudes of northern continents can make the boreal forests more sensitive to frost damage due to temperature fluctuations in early spring. At present, the initial beginning of vegetative bud development in phenological models, used to forecast the possible consequences due to the global climate warming, is fixed on theoretical assumptions or it is estimated using visible bud burst observations. Our study aims to clarify, if there are microscopic, "invisible" developmental phases in vegetative buds of Norway spruce (*Picea abies* L. Karst.) that could be described and used to evaluate and improve the phenological models. To achieve the aim, microscopic development of the buds of Norway spruce was followed during two consequent springs in relation to externally visible bud phenology and to accumulated temperature.

The results showed accumulation of lipids and starch grains in late winter and early spring. These compounds most probably function as energy reserves for early bud development. The development of conduction tissue began in early April and overlapped with the morphological changes in the primordial needles from rounded to pointed tips. These changes occurred before any externally visible changes in the bud scales in both springs, probably not depending on the accumulated temperature. Instead, the elongation of primordial shoots, which also began before externally visible changes, as well as the visible bud elongation seemed to be dependent on the accumulated temperatures. The results showed the importance of temperature in timing of early internal bud elongation. Although representing only two years, the results are promising in respect of evaluation and improvement of phenological models. Furthermore, the onset of cellular events and tissue development in the buds at very low temperature rises up the question about the regulation mechanism that induces the early bud development.

(33) Microscopic structure of vegetative buds and needles and the growth of shoots from Norway spruce (*Picea abies*) exposed to delayed soil frost thawing

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Thinner snow covers and temporary melting of snow may be the consequences of climate change in the boreal areas. Lack of or very thin snow layer may result in more severe soil frost and thus increase freezing injuries of the roots. The aim was to study if different soil frost conditions affect the development of vegetative buds and needles and shoot elongation in a 48-year-old stand of Norway spruce (*Picea abies* L. Karst). The treatments were done during the years 2006 and 2007 as follows: 1) control (CTRL) with natural snow accumulation and melting, 2) OPEN with artificial snow removal during two consecutive winters, and 3) FROST treatment, where treatment OPEN was combined with an insulation (a layer of hay between plastic sheets) set on the ground surface in early spring to keep soil frozen until July.

Buds and needles were analysed under the microscope and the current-year shoot elongation was measured from the shoots developed during the summers 2006 and 2007. The shoots for analyses were cut from uppermost parts (about 15 m from the ground level) of the trees in March 2007 and 2008. There were only slight differences in the needle cross-sectional area and central cylinder area that were smaller in the FROST compared to the others. Instead, the shoot elongation was significantly reduced in the FROST compared to the others and more so in 2007 than in 2006. The number of healthy whorl buds and side buds was significantly reduced in the FROST compared to the others. Thus, it seems that the long lasting soil frost during growing season may affect the development of above ground organs, especially the length growth of the shoots and the development of healthy whorl and side buds.

(34) Can climate change increase carbon sequestration in boreal mires?

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Climate change poses threat on the carbon storage and sink of peat accumulating circum-boreal mires. Among the mires, the greatest amounts of carbon per unit area are stored by ombrotrophic mires, i.e. bogs that are dominated by *Sphagnum* mosses. Bogs show a climate-correlated distribution pattern across the hemi-boreal to south-boreal zones. Warming is expected to cause draw-down of water level in bogs, leading to increased decomposition and loss of carbon to the atmosphere, as indicated by many studies. North from the bog zone, however, similar changes of hydrological balance may have very different effect.

In the middle to north-boreal zones, mires are predominantly minerotrophic mires, i.e. fens that lack the thick *Sphagnum* peat layer characteristic of bogs. Hydrological surplus is greater in fens, resulting in richer runoff from mire basins. Therefore, increase of evaporation does not necessarily lower water table. Instead, the primary impact of increased water loss would be the cut down of runoff. Remarkably, runoff from mineral soil catchments of fens would be reduced simultaneously. Thus, the hydrological changes indicated by climate change models can be expected to reduce minerotrophy in fens, leading to changes in chemical balance.

Concerning mires, the generally expected northward movement of vegetation zones includes the development of northern fens into raised bogs, hence increase of peat accumulation. Which would come first, hydrological change or increase of *Sphagnum*, remains untested? How fast can mire vegetation and ecosystem balance change and is there potential for globally significant effect in the carbon cycle? It is argued here that changes in hydrological balance caused by climate change may trigger the development of boreal fens to bogs. Furthermore, certain remarkably nonlinear mechanisms that regulate the water chemical balance may lead to abrupt rather than slowly proceeding changes in vegetation and ecosystem balance. The hypothetical future carbon sink would be globally significant, while the scenario would also pose serious threat to biodiversity of fen biota.

(35) Invasion of *Elodea canadensis* in Kuusamo district, northeastern Finland – causes and consequences

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Canadian waterweed (*Elodea canadensis*, Hydrocharitaceae) is a submerged aquatic angiosperm native to North America. This strongly invasive species was introduced to Europe in 1836 and to Finland, to the Botanical Garden of the University of Helsinki in 1884. *Elodea* is common in shallow lakes or slowly flowing waters and characterized by a rapid growth and vegetative reproduction. Aggressive growth and mass occurrences may change the balance of lake and river ecosystems and even hamper the recreational use of infested water body.

Distribution range of *Elodea* covers South and Central Finland with separate plots at Lapland and Kuusamo district in Northern Finland. In Kuusamo *Elodea* was observed first time 1999 in Lake Joukamojärvi and in ten years it has invaded almost 50 kilometers upstream reaching for example to unique Lake Ala-Kitka. Dispersal of *Elodea* in northern lakes might be related to mild winters giving benefit to vegetative growth under ice. Water fowl and human related vectors such as transport of fishing gears and boat may play significant role *Elodea* s invasion in this area.

To better understand the success of *Elodea* in Kuusamo district, it is necessary to study factors affecting the distribution and wintering of this species. Dispersal factors and vectors are also investigated. In addition, we are going to survey effects of *Elodea* mass occurrence on threatened and rare macrophyte species of area by experimental plots. This study will help local authorities to select correct methods against this aquatic weed.

Omics (first part of session 10)

ABSTRACTS OF

2 ORAL PRESENTATIONS

AND

7 NUMBERED POSTERS (36 - 42)

Proteomics evaluation of food safety

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Placing on the market of new GM crops is preceded by a thorough safety assessment outlined in various international agreements. The starting point is a comparative compositional analysis in which the content of nutrients, anti-nutrients and toxins are compared between the GM crop and the conventional counterpart. The comparative analysis is based on the concept of substantial equivalence: if the composition of the GM plant does not differ substantially from the conventional counterpart with a history of safe use, it is considered as safe. However, this kind of targeted analysis has been criticized of being biased towards expected changes whereas possible unexpected changes might be left unnoticed.

EU FP6 project SAFE FOODS (http://www.safefoods.nl) was established to address the issue of how consumer confidence in consumer protection and risk analysis can be restored and strengthened. One part of the project was to evaluate the use of "omics" methods at transcriptome, proteome and metabolome levels in the food safety assessment. The power of "omics" methods lies in the high number of different compounds they are able to measure as they give a much wider picture of the food composition. Thus, "omics" methods have better chance of detecting also unintended effects.

In the comparative compositional analysis the knowledge of the natural variation in the contents of measured components is essential. Using the proteomics methodology developed in the Plant Biotechnology group at the University of Kuopio, we analyzed a range of maize kernel (*Zea mays*) and potato tuber (*Solanum tuberosum*) samples to discover the major sources of variation at the proteome level.

Field studies were set up to evaluate the effects of genotype, environment, agricultural regimen and genetic modification. Genotype was found to have the strongest effect, and major qualitative and quantitative differences were observed between different cultivars. Smaller differences were found between plants grown in different locations and growing season and under different agricultural regimen. However, the genetic modification did not seem to have detectable effect on the proteome. The results of the proteomics analyses were also supported by other "omics" methods.

Proteomics methodology in the food safety assessment has some deficiencies such as lengthy analyses and low percentages of identified proteins. However, even with these limitations, proteomic profiling together with targeted analyses can help to gain a deeper insight into the metabolism of the plant. Proteomics has clearly moved to the level of performance where it can reliably reveal even subtle differences in plant protein complement.

Proteomics of *Thlaspi caerulescens* accessions and inter-accession cross segregating for zinc accumulation

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Thlaspi caerulescens is one of the best models for studying metal hyperaccumulation in plants. In order to study the mechanisms underlying metal hyperaccumulation, we have used proteomic profiling to identify differences in protein intensities among three *T. caerulescens* accessions (La Calamine, LC; Lellingen, LE; Monte Prinzera, MP) with pronounced differences in tolerance, uptake and root to shoot translocation of Zn and Cd. Also five lines from an LC x LE cross, three with low and two with high Zn accumulation, were included to determine the co-segregation of protein expression with the level of Zn accumulation. Proteins were separated using two-dimensional electrophoresis and those showing differences among accessions, lines or metal exposures were subjected to mass spectrometric analysis for identification.

Crossing decreased the number of different proteins in the lines compared with the parents, particularly in the shoots, but the frequencies of Zn-responsive proteins were similar in the accessions and lines. This supports the finding that the Zn accumulation traits are mainly determined by the root. This study demonstrates that crossing accessions with contrasting Zn accumulation trait is a potent tool to investigate the mechanisms behind metal hyperaccumulation. The role of four tentatively identified root proteins that co-segregated with high or low Zn accumulation is discussed.

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(36) Biosynthesis of *Gerbera hybrida* flavonoids in multienzyme complexes

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The formation of protein complexes, metabolons, and the channeling of intermediates of secondary metabolism has been discussed for at least 30 years (Stafford 1974). Metabolons and channeling enable plants to perform a highly effective synthesis of specific natural products without or with reduced metabolic interference and avoiding accumulation of toxic intermediates (Winkel-Shirley 1999). In spite of a long tradition of the concept, precise examples of complete metabolons are still very scarce.

Our aim is to define falavone and anthocyanidin specific metabolons in the ornamental plant *Gerbera hybrida*. Our earlier data shows that specific genes are expressed early in development (*PAL* and *CHS3*) and others late (*PAL*, *CHS1* and *DFR*), possibly correlating with flavone and anthocyanin biosynthesis (Helariutta et al. 1995). Furthermore, transformation of the pelargonidin type *Gerbera* cultivar Terra Regina with a MYB-type regulatory gene induces cyaniding biosynthesis without interfering with the background pelargonidin biosynthesis (Laitinen et al. 2008). Using our large set of EST sequences and the expanding genomic sequence resource of *Gerbera*, we wish to establish correlations in expression and patterns of protein–protein interactions for the whole flavonoid metabolism isoenzyme complex in our model plant.

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(37) Gene expression responses of paper birch to elevated O₃ and CO₂ during leaf maturation and senescence

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Forest trees are exposed to increasing concentrations of O₃ and CO₂ simultaneously. Long-term field experiments are essential to study tree responses to these greenhouse gases because of differences between saplings and mature trees, and acute and long term exposure responses. The changes induced in the gene expression can be small in acclimated trees, but yet pivotal for their metabolism.

Gene expression responses of paper birch (*Betula papyrifera*) leaves to elevated O₃ and CO₂ concentrations were studied with microarray analyses during leaf maturation and senescence at Aspen FACE experiment, in Rhinelander, WI, USA (http://aspenface.mtu.edu/). In order to detect similar gene expression patterns within samplings and treatments, the microarray data was analyzed with multivariate methods; clustering with Self-Organizing Map, finding optimal cluster grouping by K-means clustering and visualizing the results with Sammon's mapping.

Most of the alterations in the gene expression were caused by O₃, alone and in combination with elevated CO₂ concentration. O₃ reduced photosynthesis and carbon assimilation and induced defensive reactions to oxidative stress resulting in earlier leaf senescence. Transport and proteolysis gene expressions were activated, indicating that at least some remobilization of nutrients for storage was completed. The effects of elevated [CO₂] reflected surplus of carbon that was directed to synthesis of secondary compounds. The combined CO₂+O₃ treatment resembled the O₃ treatment, indicating that elevated CO₂ is not able to totally alleviate the harmful effects of elevated O₃. Some specific gene expression changes in the combined elevated O₃+CO₂ treatment showed that experiments with O₃ or CO₂ exposure alone are not sufficient to predict plant responses to these gases together, and that field experiments with multiple variables are essential in order to understand responses to future environmental conditions.

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(38) Anthocyanin variation in Finnish northern crowberry (*Empetrum nigrum* ssp. *hermaphroditum*) populations

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Northern crowberry (*Empetrum nigrum* ssp. *hermaphroditum*) is the third most yielding berry in Finnish forests and annual yield has been estimated to be 250 million kilograms. Potential health promoting effects of crowberry may be related to the high number of different anthocyanins that have been identified from this northern crowberry. However, despite its rich anthocyanins concentrations and stable yields, this berry is exploited only in minor scale in food and beverages, which may be due to its "poisonous" reputation in some countries.

The anthocyanin content of crowberry has been determined in many different studies, but usually samples were collected only from one location/population. In wild blueberry, the anthocyanins content can be affected by different geographical growing areas and the genetic background. Variation within the population in the anthocyanin content may also be large, and should be take consideration for the phenolic studies. The aim of this study was to determine the anthocyanin variation in Finnish northern crowberry populations with a focus on variation within the population.

The anthocyanin contents were analyzed from four geographically different populations. Quantitative analyses were performed with HPLC equipped with a diode array detector and anthocyanins were identified using HPLC-MS/MS.

Eleven anthocyanins were identified and quantified. The same anthocyanin composition was detected in all populations, but the amounts of different anthocyanins varied significantly between and within population(s). The quantitative order of anthocyanins also differed between populations.

The results clearly indicate that genetic background affects to the anthocyanin contents between and inside population(s). The genetic background should be taken into account in the experimental layout when analyzing polyphenols from different berry populations

(39) Phenolics of winter-dormant Salix pyrolifolia

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Salix pyrolifolia is resistant to fungi, microbes and herbivores. There are only a few previous researches focusing on polyphenols, especially tannins, and salicylates of *S. pyrolifolia*. The aim of this study was to examine phenolics composition of *S. pyrolifolia*.

Polyphenols and salicylates were extracted from bark, wood and buds of winter-dormant branches of *S. pyrolifolia*. The extractions were made to three ages of *S. pyrolifolia*: a 20-year old tree, a 3-year old tree and saplings. The extractives were analyzed by RP-HPLC-MS to identify salicylates and other small molecular weigth phenolics and by acid-butanol test to discover condensed tannins. Also the extraction residues were analyzed by acid-butanol test to notice condensed tannins.

Recovery percentages were over 90 % for most of the examined compounds. The compound composition varied widely between studied organs and ages. There was also intra-organ variation. Concentration of the studied compounds and the number of compounds were highest in the buds and lowest in the wood. High content of phenolics may explain the resistance of the species in the nature.

(40) Red clover (*Trifolium pratense* L.): Identification and characterization of phenolic compounds and protective action against oxidative stress and inflammation

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Red clover (*Trifolium pratense* L.), a widely used feed crop, has recently received considerable interest as a valuable source of phenolic compounds with multiple potential protective functions. It is a rich source of isoflavonoids, plant secondary metabolites widely distributed in the Leguminosae family. Phenolic compounds in red clover were identified and characterized by high-performance liquid chromatography and mass spectrometry. A total of 31 and 28 phenolic compounds were tentatively identified in leaves and roots, respectively. Leaves were rich in glycoside malonates of biochanin A, formononetin and quercetin.

Quercetin (flavonol) and biochanin A (isoflavone) were selected for the evaluation of their ability to inhibit oxidative stress and inflammation using cell culture models (human ARPE-19 cells and N9 murine cells). Inflammation and oxidative stress responses were analyzed by measuring the expression of interleukin-6 (IL-6), IL-1 β , TNF- α (tumor necrosis factor alpha), GSTP1 (Glutathione S-transferase P1) and HO-1 (heme oxygenase 1). The expression of nuclear factor-erythroid 2-related factor-2 (Nrf2), a key transcription factor involved in defense against oxidative stress, was also analyzed.

Our results indicate that quercetin protected ARPE-19 cells from oxidative stress-induced cell death, whereas biochanin A had no statistically significant protective effect. Quercetin also effectively protected N9 cells from LPS-induced inflammation in a dose-dependent manner, measured as the levels of secreted IL-6, whereas the effects of biochanin A were minimal. Quercetin slightly reduced the expression of IL-6 mRNA in cells treated with LPS, whereas biochanin A showed no protective effects. Neither compound decreased the expression of TNF- α . Quercetin also reduced the gene expression of IL-6 and IL-1 β , and increased the expression of transcription factor Nrf2 and protective enzymes HO-1 and GSTP in cells treated with H₂O₂. Our results suggest that the flavonol quercetin possesses higher protective function against oxidative stress and inflammation related diseases than the isoflavone biochanin A.

(41) Gerbera as a model for Asteraceae: evolution of the capitulum, and new genomic resources

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The ornamental plant Gerbera hybrida has grown into a powerful model for the sunflower family based on an effective transformation system and growing genomic resources. As a typical member of Asteraceae, Gerbera bears different floral morphs in its inflorescence, with different symmetry, sex and sometimes color. Compared to model species with simple flowers and inflorescences (e.g., Arabidopsis, Petunia and Antirrhinum), this more elaborate developmental program requires more complex regulation, possibly with extra tiers or levels. Weighed against Arabidopsis, Gerbera appears to have a larger number of regulatory genes in some key protein families, such as TCP and certain MADS domain transcription factors. APETALA1/ SQUAMOSA/FRUITFULL-like MADS domain proteins (referred to as GSQUAs for the Gerbera homologs) have been suggested to function as mediators of higher order complex formation, acting as "bridge proteins" in the formation of protein quartets. Based on pairwise assays, interaction capacity of GSQUA proteins appears to be limited. However, when assayed for higher order complex formation, together with the Gerbera B function dimer, or when combined with a Gerbera SEP-like GRCD protein and with a C function GAGA protein, all GSQUA proteins show activity. While GSQUA proteins do not interact with each other in pairwise assays, addition of a GRCD protein stabilizes some triad complexes containing two GSQUA proteins. It is tempting to speculate that gene duplication and divergence in the SQUA/AP1/FUL gene lineage has participated, together with the unique diversity in TCP family transcription factors, to help shape the complex Asteraceae inflorescence.

Gerbera genomics started some years ago with the sequencing 17,000 randomly selected clones from different cDNA libraries. DNA microarrays based on this set of ESTs have uncovered, e.g., gene sets that are regulated specifically in radial dimension of the capitulum, possibly presenting key genes in flower morph determination. Our EST collection has recently been upgraded using pyrosequencing (300,000 sequences) and the *Gerbera* nuclear genome is represented by a 5x BAC library.

(42) Optimizing the methods for quantitative analysis of alkaloids in Norway spruce (*Picea abies*) needles

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There are several potentially bioactive piperidine alkaloids in Norway spruce (*Picea abies*) needles. The solid-phase partitioning method (SPP) is regularly used with *Pinus* and *Picea* species for extraction of these alkaloids. It has earlier been optimized for *Pinus ponderosa* but the procedure should be optimized separately for each species because alkaloids are usually detected in low amounts and in very variable quality in tissues. Our aim was to test the whole SPP method for extraction of alkaloids in *P. abies* needles. The method developed was also tested for bark and wood parts of *P. abies* young branches.

There are several factors that could influence on the efficiency of the alkaloid extraction from needles. No difference on the (+)-6-epidihydropinidine yields could be seen when using methanol instead of hydrochloric acid as initial extraction solvent. Similarly, dichloromethane and chloroform were equally effective solvents in the elution phase. However, by splitting the initial extraction and elution treatments on smaller parts it was possible to improve the (+)-6-epidihydropinidine yield from $77.5 \pm 4.0 \%$ to 92.2± 1.4 %. We tested the actual effectiveness of the procedure with rasemic (±)-epidihydropinidine. Optimized method yielded 88.5 ± 2.3 % (S.E.) recovery showing that the method is suitable for the main alkaloid component of *P. abies*. Using the rasemic (±)-epidihydropinidine as reference, we were able to detect 493±29 μg/g, FW (S.E.) of the (+)-6-epidihydropinidine from the needles, $359\pm29 \mu g/g$, FW (S.E.) of the (+)-6-epidihydropinidine from the bark and 62±8 μg/g, FW (S.E.) of the (+)-6-epidihydropinidine from the wood part of adult *P. abies*. To our knowledge, this is the first time that piperidine alkaloids of *P. abies* are quantitatively analyzed. In addition, small amounts of (-)-pinidinol and isomers of pinidine were detected from needle samples.

Based on these results the (+)-6-epidihydropinidine yields of needle, bark and wood parts of *P. abies* can be quantitatively extracted with the SPP method. Results can be optimized with some minor changes during the procedure and the major compound, (+)-6-epidihydropinidine, can be collected from samples with reasonable yields.

Genetics and evolution (latter part of session 10)

ABSTRACTS OF

2 ORAL PRESENTATIONS

AND

6 NUMBERED POSTERS (43 - 48)

Variation in telomeric repeats in forest trees Scots pine (*Pinus sylverstris*) and silver birch (*Betula pendula*)

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The shortening of telomeres, the specific structures of repeated DNA sequence at the end of the eukaryotic chromosomes, has been connected with ageing and loss of cell replication or regeneration capacity. There is, however, only limited information available on telomeres of long-living trees. Physiological ageing, on the other hand, represents a serious problem for vegetative propagation of conifers, while in deciduous tree species ageing has no such a strict influence on regeneration ability. The aim of this study was, for the first time, to determine the variation in telomeric repeats in Nordic forest trees, and to compare potential differences in a conifer, Scots pine (*Pinus sylvestris* L.) and in a deciduous species, silver birch (*Betula pendula* Roth).

In Scots pine, different tissues of individuals ranging from immature embryos to 200-year-old trees were examined. In birch, samples from germinating seeds to 80-year-old trees were compared with tissue cultured materials of the same genotypes. In both species, the length of telomeric repeats in the extracted total genomic DNA was determined by Southern hybridisation using both more meristematic samples (embryos, buds or cambium) and somatic tissues (needles or leaves).

In Scots pine samples, the size of telomeric repeats detected was variable, ranging from 0.9 kb up to 26 kb. Based on exonuclease treatment, high molecular weight repeats seem to be genuine telomeres at the ends of the chromosomes, while the low molecular weight signals probably originate at interstitial or centromeric positions. A decline in the maximum length of the telomeric repeats was observed with increasing level of tissue differentiation: meristematic cambium had the longest repeats, followed by immature and germinating embryos, elongating buds with tiny meristems and, finally, full-sized needles without meristems having the shortest repeats. Also the position of the tissue within tree (stem base versus top) affected the length of telomeric repeats in the most of the trees. The telomeric repeats also varied between tree individuals, most of the variation being found in the high molecular weight telomeric signals (true telomeres).

In silver birch, the size of telomeric repeats was between 3.2 and 33 kb, and no such abundant interstitial or centromeric repeats as in Scots pine were observed. There was remarkable variation among individuals, and these genotypic differences were rather consistent across the different tissues (leaves or cambium of 15–80-year-old trees) and growth conditions (*in vitro* tissue cultured or outdoors) assayed. The *in vitro* cultures had slightly shorter telomeres than outdoor materials, and there were also differences among tissue types sampled, callus having shorter repeats than *in vitro* shoots.

Phylogenetic systematics and biogeographical history of the Pantropical fern family Lindsaeaceae

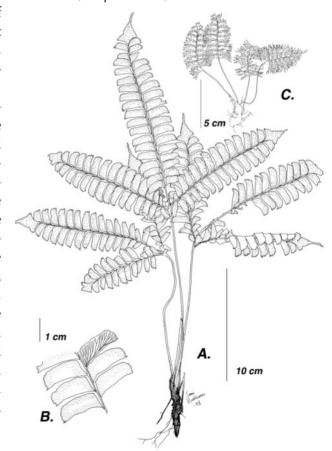
<u>Samuli Lehtonen</u>, Hanna Tuomisto, Germinal Rouhan & Maarten J. M. Christenhusz samile • utu.fi, University of Turku, Finland

The classification and generic delimitation within the Pantropical fern family Lindsaeaceae have been uncertain, and so far based on morphological charaters only. We have studied the evolutionary history of the Lindsaeaceae by simultaneously optimizing 55 morphological characters, two plastid genes, and three plastid noncoding intergenic spacers. Our dataset included all genera associated with Lindsaeaceae, except monotypic *Xyropteris*, and 175 taxa representing ca. 73 % of the accepted species.

The phylogenetic relationships of the lindsaeoid ferns with two enigmatic genera that have recently been included in the Lindsaeaceae, *Cystodium* and *Lonchitis*, remain ambiguous. Within the monophyletic lindsaeoids we found six clades that we recognise as genera: *Sphenomeris*, *Odontosoria*, *Osmolindsaea*, *Nesolindsaea*, *Tapeinidium*, and *Lindsaea*. Previous

subgeneric classification of the genus *Lindsaea* was not supported by our analysis, and is consequently rejected.

Our phylogenetic results indicate extensive convergent morphological evolution in many unrelated lineages. Phylogenetic patterns within the Lindsaea seem to correlate with the geographical history, indicating that the genus may be used in biogeographical analyses. Almost complete absence of otherwise Pantropical Lindsaea in continental Africa is striking, as well as apparently rapid and recent adaptive radiation of the genus in Amazonia.

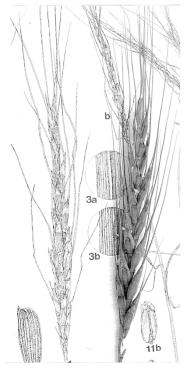


(43) An ægilopoid mutant of the anticipated phenotype of *Triticum* B-genome originator from a three-way cross of *Dasypyrum villosum*, tetraploid and hexaploid wheat

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The F₁ hybrid, Triticum turgidum conv. durum (AB, 4x) X Dasypyrum villosum (Haynaldia villosa) (2x) was pollinated with common wheat (Triticum vulgare cv. 'Ruso') (ABD, 6x). I started the crosses in 1967. The cross with the common wheat gave a single seed, the offspring of which was selected until F6 when stable lines were selected. In F10 generation, a dicoccoid mutant was found, which segregated non-mutants and dicoccoids in 1:3 (χ^2 = 1.2, P > 0.2) in F₁₁, and an ægilopoid mutant from the dicoccoid in F12. The ægilopoid has relatively slender stems, narrow leaves, somewhat cylindrical, thin spikes, narrow glumes, short awns (Fig.), dehiscing anthers, poor seed-set with small grains and is truebreeding self-pollinator. The rachis abscises with the segments as extensions of each spikelet being different from that of the spelt wheat. In appearance and dimensions, the ægilopoid resembles Ægilops speltoides var. ligustica (Fig.) having the S genome, related to the B genome. Occurrences of back-mutations from ægilopoid to dicoccoid and from dicoccoid to original type were observed. The ægilopoid has genes for the high-molar-mass storage proteins of the common wheat, absent from the *durum* parent, and has therefore genes of the D genome



expressed. Using mainly as the seed parent, the ægilopoid gave sterile hybrids with Æ. speltoides var. ligustica, intermedium and var. speltoides (S genome) and \mathcal{E} . longissima (S¹). The Ph meiotic paring controlling allele in the S genome probably causes high hybrid sterility. Weakly fertile hybrids were obtained with the ægilopoid and Triticum turgidum ssp. dicoccoides and ssp. dicoccon (AB). So far, hybrids were not obtained with tried strains of Ægilops bicornis (Sb), Æ. searsii (Ss), Triticum monococcum ssp. boeoticum, T. monococcum and T. monococcum var. sinskajæ (A).

Fig. Spike of Ægilops speltoides var. ligustica (on the left) as the drawing in the same scale as that of the ægilopoid at anthesis having the same dimensions and features. Enlarged glume and mature kernel (11b) of var. ligustica in the background drawing from M. W. van Slageren, 1995: Wild wheats. – Wageningen Agric. Univ. Paper 94-7. (The drawing printed with permission from Backhuys Publishers B.V.)

(44) Mesophyll-specificity of key C₄ genes is regulated via cis-elements present in 5' and 3' untranslated regions

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The C₄ photosynthetic cycle increases the efficiency of photosynthesis by concentrating CO₂ around RuBisCO and reducing the rate of photorespiration. This is achieved by spatially separating the initial fixation of carbon, the decarboxylation reaction and the regeneration of the substrate between mesophyll (M) and bundle sheath (BS) cells. Hence, the C₄ cycle requires accumulation of key C₄ gene products in either M or BS cells. We are interested in the evolution of the regulation of this cell specificity and use *Cleome gynandra*, the closest known C₄ relative of *Arabidopsis thaliana* (C₃) as a model.

We isolated genes encoding pyruvate, orthophosphate dikinase (*PPDK*) and a plasma membrane bound carbonic anhydrase (*CA4*) from *C. gynandra* using degenerate PCR and rapid amplification of cDNA ends (RACE). Quantitative PCR (qPCR) showed that transcripts of *PPDK* and *CA4* are 230-fold and 15-fold higher respectively in *C. gynandra* than a closely related C₃ species *C. spinosa*. Furthermore, laser capture microdissection (LCM) allowed preparation of M and BS-specific samples from *C. gynandra*, and qPCR showed that both *PPDK* and *CA4* transcripts accumulate preferentially in M cells. Subcellular localisation of the gene products was investigated with translational fusions to gene encoding the green fluorescent protein. The PPDK and CA4 localised to chloroplasts and the plasma membrane, respectively, and the accumulation patterns are consistent with these proteins being recruited into the C₄ pathway.

We investigated regions of each gene that generate accumulation in M cells. This showed that untranslated regions (UTRs) of both PPDK and CA4 were sufficient for M specific accumulation. This M specificity was maintained even under expression from constitutive promoter CaMV35S, indicating that this regulation is likely post-transcriptional. To study the evolution of this regulation, the CgPPDK and CgCA4 UTRs were tested in the closely related C_3 plant A. thaliana, and equivalent regions of the AtPPDK gene were tested in C. gynandra. We also have shown that the promoter activity of CgPPDK is 20 times higher than that of AtPPDK, explaining the increased transcript abundance. These results will be discussed in the light of evolution of cell type-specific regulation.

(45) Studying natural flowering time variation in *Arabidopsis lyrata*

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It is important for plants to synchronize flowering with the favorable seasons. Many genetic pathways mediate and combine signals adjusting flowering time: in *Arabidopsis thaliana* the photoperiod, vernalization and autonomous pathways are well known. Which pathways and loci affect flowering time differences between locally adapted populations is an interesting evolutionary question.

Here we study flowering differences between four *A. lyrata* populations from different latitudes and examine the expression of candidate genes for flowering time variation. Traits related to flowering were studied in three photoperiods after vernalization. Different populations responded differentially to the studied photoperiods. Southern populations were able to flower in the shortest photoperiod, where as the longest photoperiod was long enough to induce flowering in all studied populations. However, the shortest photoperiod induced flower bud formation in all populations indicating that the plants responded to the vernalization treatment, but the photoperiod was too short for flowering. As variation in gene expression can contribute to local adaptation, expression data of flowering time pathway genes will be compared with the phenotype data on flowering.

(46) The evolutionary strategies in leafy liverworts: do such strategies exist?

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Reproduction is one of the major functions facilitating the species evolution, with subsequent adaptation and speciation. Therefore, the environmental pressures in different environments are expected to force the evolutionary processes to develop into differing response modes called strategies. If this hypothesis is true, the strategies should be found to connect related species in similar environments, but also as similar responses in species not necessarily closely related to each other. We have previously presented preliminary results indicating correlations between certain reproductive morphological characteristics and environmental factors such as geographic distribution area in among species in four genera in Lophoziaceae s.lato (Laaka-Lindberg 2008).

We have made more thorough analysis with 8 leafy liverwort genera with altogether 96 species. The reproductive characteristics were grouped as asexual, female and male functions. The information has been extracted from literature and used mainly as classified variables, since quantitative data is available only on very few liverwort species so far. The reproductive capacities were then compared with the species ecological characters including frequency of occurrences, geographic distribution, competitive ability and substrate specificity.

The analysis revealed differences in substrate specificity among species representing different asexual characters and additionally also differing sporophyte frequency. Such characteristics as gemma frequency and amount of gemma production differed in groups presenting different competitive abilities. In our study, the asexual reproductive characteristics seemed to have highest impact on strategic differentiation among the species, and the between genera differences in these characteristics were also most prevalent. Different reproductive input was also found between differing female characters, but practically no differences in male characters were found among genera and strategic groups. The combinations of reproductive characters specified as reproductive strategies could be clearly separated in cluster analysis, but these strategies did not seem to have any obvious phylogenetic connection.

(47) Genetic architecture of adaptive differentiation in natural populations of a perennial plant *Arabidopsis lyrata* (Brassicaceae)

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We examined the genetic basis of adaptation in natural populations of a perennial model plant *Arabidopsis lyrata*. The close relatedness to *Arabidopsis thaliana*, and recently published genome sequence for *A. lyrata* enables the use of molecular resources available for this purpose. The populations were from two contrasting environments (Norway and North Carolina, USA).

Several quantitative traits have diverged since these populations were separated. The main goal of this study was to determine whether these differences are caused by adaptation or genetic drift. The fitness differences were examined in a reciprocal transplant field experiment in the original sites of the populations. We used an F2 cross between the two parent populations to examine the genetic basis. Using single nucleotide polymorphisms covering the genome at low density, we performed interval mapping using R/qtl software. Cytoplasmic effects were also examined.

We found that genetic differences are mostly caused by local adaptation, as the quantitative trait loci (QTL) showed that genotypes with alleles from the local population often enhanced fitness in the local environment. Also, we found evidence of contribution of cytoplasmic genes on fitness.

(48) Characterization of CYCLOIDEA-like gene family in *Gerbera hybrida*

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The sunflower family of plants (Asteraceae) is characterized by complex inflorescences that consist of morphologically and functionally different types of flowers. We are using *Gerbera hybrida* as our model to explore the molecular basis of flower type differentiation along the radial axis of the inflorescence. Our recent studies gave the first molecular evidence that ancient, plant-specific TCP domain (CYC/TB1-like) transcription factors are involved in this process (Broholm et al. 2008).

Interestingly, both in gerbera and sunflower, the *CYC/TB1*-like gene family shows extensive expansion. While *Arabidopsis* has only three of these genes, we have identified ten in gerbera. Recent analysis of nearly 1 million ESTs representing various species in Asteraceae revealed that the expansion of the TCP family dates back to a single polyploidization event near the origin of the Asteraceae family and to independent genome duplications in the tribes Heliantheae (sunflower) and Mutisieae (gerbera) (Barker et al. 2008). Therefore, we suspect that these genes have diverged and obtained novel functions contributing to the unique characteristics of the Asteraceae inflorescence, as already shown for *GhCYC2*.

We are currently focusing on analyzing this large gene family and the regulatory network connected to them. Expression analysis using qPCR indicates distinct expression domains for CYC2 and CYC3 subclade members in developing ray and disc flowers, respectively. Yeast two-hybrid assays show that TCP factors make homo- and heterodimers among themselves. However, we have also identified interactions with novel transcriptional regulators. In addition to functional studies using transgenic plants we are performing promoter analyses to identify upstream regulators of GhCYC genes. The nature of the signals that specify TCP gene expression to developing ray and disc flowers is currently not known. The latest results of these ongoing studies will be presented.

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Ecophysiology, whole-plant physiology and plant development (session 11)

ABSTRACTS OF

3 ORAL PRESENTATIONS

AND

13 NUMBERED POSTERS (49 - 61)

Storage protein types as indicators of ecophysiological adaptation of the plant tissues

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The storage tissues of plants show wide variation of proteins which are species-specific and highly genotypically controlled. These facts suggest that the variation observed in nature is adaptive and has been subjected to long-term selection. With the methods applied or developed by me, storage protein were fractionated into two classes, soluble and residual from ground dry, or homogenized wet tissues. The soluble fraction: extracted repeatedly with Trisbuffered (40 mM, pH 8.0) 2-propanol (50 %, v/v) including 2-mercaptoethanol (0.5 %, v/v) which was clarified by centrifugation. The proteins in the supernatant were precipitated with 2.5 volumes of methanol (Ahokas 1994, 2002). The residual fraction is the protein in the twice extracted pellet. After thorough evaporation of the 2-mercaptoethanol in vacuum, the dry protein was dissolved and quantified with direct spectrophotometric method based on the strong UV absorption of the peptide bonds (Ahokas 1978).

In general, the residual proteins vary less in their patterns on SDS-PAG electrophoresis than the soluble proteins within a given species. The ratio of the fractions vary widely in a given storage tissue and is highly characteristic to a species. The moisture conditions must be selective on the storage proteins of a given species. Concerning dry storage tissues in moist environment, the proteins need to have enough hydrophobicity which permits evaporation of water and crystallization or pleating of the proteins. As examples, barleys (Hordeum vulgare, H. vulgare ssp. spontaneum) originate from arid or semiarid, oats (Avena sativa, A. fatua) from mesic and millets (Panicum miliaceum, Echinochloa crus-galli) from moist environments. The internal moisture of the storage tissue is also reflected in the storage protein types, e.g. in bulbs of chive (Allium schoenoprasum) while its grains are rather adapted to mesic environment, probably temporary immersion in rain water streams on rocks or in sea water. The fractions can be taken as suggestive for the environment of origin, e.g. that of Bromus secalinus which is unknown. Its grain protein types suggest B. secalinus to have originated from arid environments. The fractionation method is of interest at searching for plant protein sources for humans having the coeliac trait.

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Ahokas, H. 1994: Searching for DNA introgressed from wheat and for wheat-like grain proteins in a rice X wheat hybridization derivative. – Euphytica 72: 177–182.

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Non-destructive root evaluation method – application and limitations of electrical resistance technique

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It has been reported that root electrical measurements is a non-destructive technique to estimate root morphology. However, affecting components between electrodes strongly affect the successful application of electrical measurement application.

We evaluated the effects of stem on the root electrical measurements. Electrical resistances of roots and stems of hydroponically raised willows (*Salix schwerinii*) were studied and related with root morphology. Willow cuttings with and without roots were set in a constant electric field (effective voltage of 0.1 V, sine-AC, 128 Hz) in a hydroponic solution.

The results showed that the resistance decreased in relation to an increase in the contact surface area of the roots with the solution. However, the resistance depended strongly on the contact area of the stem with the solution, thus causing bias in the evaluation of root surface area. This work is a new contribution for the understanding of current pathways in the root system as exposed to an external electric field and for developing a non-destructive method to study plant roots accordingly.

Effect of seed origin and latitudinal seed transfer on timing of height growth cessation and field performance of silver birch (*Betula pendula* Roth)

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Height growth cessation, which is the first visible component of the hardening process of trees in the autumn, is in the first hand regulated by photoperiod. Birch populations from different latitudes have adapted to differing photoperiodic regimes and have different critical night lengths inducing growth cessation and bud set. Latitudinal seed transfer entails a transfer to different photoperiodic regime and has an effect on the timing of height growth cessation and field performance of birch.

The effect of seed origin latitude and sowing time on timing of height growth cessation of first-year silver birch seedlings was studied in a greenhouse experiment with seven seed origins (lat. 58° – 67° N). The timing of height growth cessation depended systematically on latitude of seed origin and sowing date. The more northern the seed origin, the earlier the growth cessation and the shorter the growth period. Later sowing dates delayed growth cessation but also shortened the growth period.

Variation in critical night length (CNL) for 50 % bud set within two latitudinally distant silver birch stands (60° and 67° N) was studied in three phytotron experiments. The mean CNL of the southern stand was longer, 6.3 ± 0.2 h (95 % confidence interval), than that of the northern stand, 3.1 ± 0.3 h. Within-stand variance of the CNL was higher in the northern (0.484 h²) than in the southern stand (0.150 h²).

Browsing by moose on 5–11-year-old silver birch saplings from latitudinally different seed origins (53° – 67° N) was studied in a field experiment in southern Finland. Browsing by moose decreased with increasing latitude of seed origin and sapling height. Origins transferred from more southern latitudes were more heavily browsed than the more northern native ones.

Yield and stem quality of 22-year-old silver birch trees of Baltic, Finnish and Russian origin (54° – 63° N) and the effect of latitudinal seed transfers were studied in two provenance trials at Tuusula (lat. 60° 21' N) Viitasaari (lat. 63° 11' N). Latitudinal seed transfer distance had a significant effect on survival, stem volume/ha and proportion of trees with a stem defect (forked stem or vertical branch). The relationship of both survival and stem volume/ha to the latitudinal seed transfer distance was curvilinear. Volume was increased by transferring seed from no more than 2 degrees of latitude from the south. A longer transfer from the south, and transfer from the north, decreased the yield. The proportion of trees with a stem defect increased linearly in relation to the latitudinal seed transfer distance from the south. At Viitasaari, central Finland, highest volume per unit area was produced by southern Finnish seed origins. Estonian and north Latvian stand seed origins, and the southern Finnish plus tree origins, were the most productive ones at Tuusula, southernmost Finland.

(49) Comparison of carbon metabolism and transport in flax (*Linum usitatissimum* L.) plants fed with nitrates through the transpiration water stream or roots

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For many plants nitrate is the most important source of nitrogen in soil. Despite the acknowledged nutritional and signaling function of this element in plants, the mechanism of its action is still vague. Increased nitrate nutrition was shown to inhibit photo-assimilate export from photosynthesizing organs, to enhance sucrose hydrolysis in the apoplast, and to increase the shoot to root ratio.

To compare the action of direct nitrate inflow into shoot with its uptake from roots on carbon metabolism and transport along the plant we fed nitrates or reduced nitrogen (urea) through the transpiration water stream into flax (*Linum usitatissimum* L.) shoots for 4 h or we fertilized plants with nitrates in the evening previous to the day of the experiment. The middle shoot part was exposed to ¹⁴CO₂ for 3 min in 1 hour after beginning of solution feeding. Samples were taken immediately after ¹⁴CO₂ assimilation or 3 h later.

Nitrate feeding both through roots and directly to shoot resulted in decreased assimilate export compared to water or urea feeding. However, carbon metabolism was different between direct feeding and fertilization through soil. Under direct nitrate feeding 14C-sucrose was firstly hydrolyzed but later accumulated in leaves on the background of its decreased export, compared to control. Neither water nor urea had such effects. Leaf ultrastructure under nitrate feeding into apoplast was similar to that of leaves with cold-girdled petioles (Gamalei, Pakhomova, 2000). When nitrates were fed through roots there was no 14C-sucrose accumulation in 3 h after 14CO2 assimilation, though initial sucrose hydrolysis was also higher than in control. We guess that when nitrate appears in the shoot apoplast it induces a suppression of assimilate translocation, sucrose accumulation in the apoplast with its consequent hydrolysis therein. Hexoses act as signaling molecules to render leaf metabolism more sink-like, which in the future results in active growth of the plant upper part. When plants were fed through roots they had enough time to switch their metabolism from source to sink and hexoses were not accumulated as sucrose but were metabolized in leaves.

(50) Thylakoid protein phosphorylation optimises electron transfer under fluctuating light

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In higher plant chloroplasts photosystem II (PSII) and its light harvesting antenna (LHCII) are reversibly phosphorylated by STN7 and STN8 kinases dependent pathways according to light quantity and quality. The aim of the study is to elucidate the role of PSII-LHCII phosphorylation in regulation of photosynthetic electron transfer chain (ETC) reactions, and its interactions with non-photochemical quenching (NPQ) and cyclic electron flow around photosystem I (PSI). As tools of investigation we used mutant *Arabidopsis thaliana* plants, impaired in short-term acclimation mechanisms, grown under different white light intensities and under fluctuating light.

Chlorophyll a fluorescence measurements reveal that plants lacking the STN7 kinase have a much higher reduction level of ETC compared to wild-type. Similar measurements were carried out on leaves from plants grown under continuous moderate light: all the different mutants show long-term compensation of the missing regulatory mechanism.

From these results we can conclude that LHCII phosphorylation, catalyzed by STN7 kinase, is required to equally distribute the excitation energy between PSII and PSI under fluctuating light condition and consequently to maintain redox balance. Furthermore, it also became clear that the induction of NPQ diminishes the importance of the STN7 kinase.

(51) The influence of KNO_3 (0,5 %) on photosynthesis and assimilate transport in symplastic plant *Chamerion angustifolium* (L.) Holub.

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It was known, that high nitrogen feeding represses assimilate efflux from leaves. But nobody knows how it occurs. In apoplastic plants, one of explanations is the probable increase of acid cell wall invertase activity in apoplast by nitrate that destroys sucrose into fructose and glucose. These sugars cannot go to phloem and they come back to mesophyll cell. Would the assimilate transport be repressed in symplastic plant, where the stage of assimilate crossing the apoplast is absent or rare?

To study the influence of nitrate on distribution of 14 C in symplastic and apoplastic plants under N-conditions we chose *Chamerion angustifolium* L.Holub as a plant with symplastic loading of phloem and *Linum usitatissimum* as that with apoplastic one. KNO₃ (0.5 %) was inserted into a cut shoot of plants with the transpiration stream of water.

Analysis of ¹⁴C content after ¹⁴CO₂ assimilation by a part of mature leaf showed high inhibition of 14CO2 fixation under nitrogen treatment in both plant. Injection of KNO3 (0.5 %) into apoplast repressed assimilate efflux from leaves both in flax and Chamerion. Analysis of distribution of labeled product of photosynthesis among plant organs in 3 hours after assimilation of ¹⁴CO₂ showed that assimilate export intensity of Chamerion leaves was 7 time lower than that of donor flax leaves. In both plants nitrates stimulate translocation of labeled assimilates in upper shoot part. It was 20 % more labeled assimilate in nitrate plant than in control one. Possibly, labeled nutrients go to the upper shoot part along with the transpiration stream after their diffusion into shoot apoplast, so this data indicate elevation of permeability of phloem tubes for transport product of photosynthesis. It could be a result of decreasing transport speed of phloem or reduction in sugar return into phloem. Main radioactivity in flax was in sucrose both in control and nitrate treatment. This in Chamerion was in sucrose; oligosaccharides and start blot (unidentified composition(s) in chromatography paper). Maximal repression of incorporation of 14C was into oligosaccharide group under nitrate treatment. So, nitrate causes repression of assimilate efflux and this process does not depend on how sugars are transported from mesophyll cells to phloem. We expect that there is much similarity between nitrate ion action mechanisms in apoplastic and symplastic plants. Possibly it occurred through pore plugging of phloem by callose or P-protein.

(52) Frost hardiness of mycorrhizal and non-mycorrhizal Scots pine (*Pinus sylvestris* L.) roots and shoots in two fertilization treatments

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There are many studies about mycorrhizal associations but only a few about their survival and functioning at low temperatures. In this study, the aim was to compare the frost hardiness of roots and shoots of mycorrhizal (*Hebeloma sp.*) and non-mycorrhizal Scots pine (*Pinus sylvestris* L.) seedlings with two fertilization treatments and two hardening treatments.

Three hundred mycorrhizal and non-mycorrhizal seedlings were cultivated in growth chambers in four blocks for 16 weeks first nine weeks in long day and high temperature (LD/HT) with low fertilization (LF), then three weeks in LD/HT with either low (LF) or high fertilization (HF). After this, half of the plants in each treatment combination remained in the LD/HT and half were moved to short day and low temperature (SD/LT) conditions to cold acclimate. The frost hardiness of the roots and shoots was assessed with electrolyte leakage tests after 16 weeks cultivation. From each block three parallel root and shoot samples of the eight treatments were prepared for freezing tests at 6–7 frost exposure temperatures and assessment of frost hardiness by electrolyte leakage test. Analysis of variance was used to compare the frost hardiness, dry weights and nutrient concentrations of the roots, stems and needles.

Mycorrhizal roots were slightly more frost hardy than non-mycorrhizal roots (P=0.055) but no difference was found in needles. The needles hardened significantly in the cold acclimation treatment but roots did not. The roots without the hardening treatment were more frost hardy than after the hardening treatment. The fertilization treatments did not affect the frost hardiness of roots or shoots. The interaction between mycorrhizal and hardening treatment was close to significant for roots (P=0.051). Mycorrhizal treatment improved the frost hardiness of the roots without the hardening treatment but not with the hardening treatment. The frost hardiness of mycorrhizal and non-mycorrhizal roots did not differ at the high fertilization level but at low fertilization level, mycorrhizal roots were more frost hardy than non-mycorrhizal ones (P=0.047). More information about the functioning of mycorrhizas at low temperatures is needed especially about different mycorrhizal forming fungi.

(53) Characterisation of a laccase in the lignin-forming Norway spruce (*Picea abies*) tissue culture

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We use extracellular lignin-forming tissue culture of Norway spruce (*Picea abies*, Simola et al. 1992) as a model for lignin biosynthesis. Our aim has been to characterise the oxidative enzymes that are responsible for activation of coniferyl alcohol, a precursor of lignin, to radicals for lignin polymerization. Numerous peroxidase isoenzymes together with one laccase (oxidase) are present in the culture medium of spruce cells (Kärkönen et al. 2002). Further isoenzymes of peroxidases and one for laccase are found bound to the extracellular lignin (Koutaniemi et al. 2007, Koutaniemi 2007). As hydrogen peroxide removal hindered the formation of extracellular lignin, peroxidases seem to have a major role in the activation of coniferyl alcohol for lignin polymerisation in this spruce tissue culture.

Soluble laccase with a pI of 8.4 was purified from the culture medium of spruce cells. The laccase had a high affinity towards coniferyl alcohol with an apparent K_m value of 3.5 μ M. Interestingly, the K_m value is lower than those estimated for the soluble peroxidases from the culture medium (Koutaniemi et al. 2005). The laccase is able to form dehydrogenation polymer (artificial lignin) from coniferyl alcohol *in vitro*. Cloning of the laccase genes is currently going on.

Removal of hydrogen peroxide from the culture medium was shown to lead to accumulation of dilignols in both cells and in the culture medium. Does this mean that laccases have a role in formation of dilignols? This is currently being studied.

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(54) Short day treatment and growth regulators as means of advancing winter dormancy of *Rhododendron x fraseri* and *Diervilla lonicera* – preliminary results

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Plant cold storage is a common practise in plant nurseries. In Finland, the plants grown outdoors are transferred to cold stores in October for overwintering. In some plant species (e.g. *Diervilla* spp), there is a problem of the plants not being in dormant stage when removed to storage. If dormancy related physiological changes are not completed, starch reserves may be inadequate to withstand several months in 0–2 °C. In addition, intact leaves pose a problem in storage conditions.

The purpose of this study was to screen methods of inducing dormancy before mid-October. Similar experiments with *Diervilla lonicera* and azalea *Rhododendron* x *fraseri* was set up in midsummer 2009. Both species were given short day (8 h) treatment for 6 weeks beginning from July 29th (shortday1) or August 10th (shortday2). Alternatively, the plants were given single treatment in August 21st with growth regulators mepiquat chloride+etefon (MepEt) or prohexadione calcium (ProCa). Control plants were not treated. The length of main stems was measured and leaf colour and formation of terminal bud was observed every 10 days until transfer to cold storage in late October. Starch content of roots and sugar content of stems were measured four times in the autumn. The experiment continues in spring 2010 by measuring the success of cold storage.

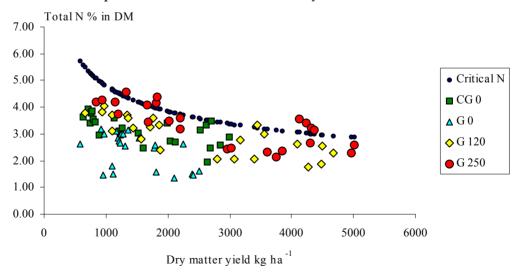
The growth of R. fraseri control plants ceased at the end of August. The accumulation of starch started early and autumn colours appeared first in azaleas treated with short day from late July. Other treatments did not differ from the control until late October. ProCa did not have significant effect on the growth of azalea, autumn colouration or accumulation of starch in roots but lower levels of sugars were found from stems at the time of uplifting. Plants treated with MepEt had more clear autumn colours than the control plants in late October but other parameters were not affected. Azalea leaves remained intact until uplifting but were easily removable soon after transfer to storage. The growth of *D. lonicera* continued until late September. Short day treatments effectively stopped growth soon after onset. However, none of the treatment had any effect on the autumn colours before uplifting in late October. Only after one week in cold storage, shortday1 and shortday2 treated plants had clear autumn coloration. Shortday1 treatment advanced the accumulation of starch in to the roots but the effect disappeared before transferring to cold store. ProCa treated plants had lowest starch and sugar content at the time of transfer. Shortening the day to 8 hours beginning from late July was the most effective way to advance the winter dormancy in azaleas but no treatment was effective enough for D. lonicera. The potential of growth regulators should be investigated more closely.

(55) Evaluation of N nutrition in white clover grass pastures by critical N curve

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In order to evaluate nitrogen nutrition of white clover lamb pastures, four treatments were established in a randomised complete block design on organic soil. Pastures were grass (G) (a mixture of *Phleum pratense* L., *Lolium perenne* L. and *Festuca pratensis* Huds.) with annual nitrogen (N) rates of 0 (G0), 120 (G120) and 250 (G250) kg ha⁻¹ and a white clover (*Trifolium repens* L.) grass without N fertilisation (CG0) containing 25 % clover in the seed mixture. The plots were fertilised with 36 kg ha⁻¹ P and 42 kg ha⁻¹ K in May. Besides dry matter production (DM) over three years, contents of total N and nitrate of herbage were analysed. The data was analysed statistically by the GLM Procedure of the SAS Institute.

Critical N curve for C₃ crops indicates total N concentration in plant DM in relation to DM production under non limiting N supply for crops. So far N concentration of herbage DM is under the curve, N is limiting plant growth and increasing N fertilisation may increase DM production. According to this measure N availability was limiting DM production in most pastures (Figure). Total N of herbage was high in consideration of animal need. In the grass pastures with the highest N rate, very high nitrate N contents (>10 000 mg kg⁻¹ DM) were recorded. In the same pastures, high N status in relation to DM production was indicated also by the critical N curve.



Critical N curve for grass and white clover pastures.

(56) Regulation of cell wall and lignin formation in timothy (*Phleum pratense*)

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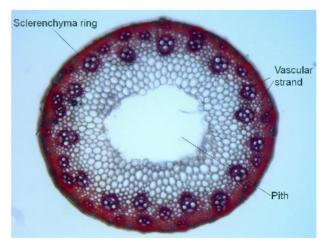
Milk and beef production contributes over a half of annual gross returns of farmers in Finland (1400 M $\mbox{e/year}$) and milk and beef processing is responsible for 80 % of the total number of employment in the food processing industry. Grass silage is the most important source of metabolised energy in milk and beef production.

Lignin content in grasses increases with maturity, as the need for structural strength increases especially during stem elongation. Cells of the outer parts of grass stem get heavily lignified during stem development. Accumulation of lignin in the matrix of cell wall polysaccharides makes them inaccessible to rumenal enzymes that would normally digest them. The digestibility of grass material in rumen is the factor that has the strongest economical effect on milk and beef production. In order to achieve high milk production with low feed costs the improvement of forage digestibility without decreasing the yield has a major importance. MTT Agrifood Research Finland, University of Helsinki and University of Oulu have established a research collaboration to study factors that affect digestibility of timothy.

Two cDNA libraries were prepared from the stems of timothy (*Phleum pratense*) at two developmental stages. The libraries are being utilized for mining of the candidate genes with special attention to transcription factors known to be involved in secondary cell wall and lignin biosynthesis. The timing and spatial expression of the selected genes will be studied in timothy clones of different digestibility. Lignin analyses will give information about the amount and type of lignin in different clones and in different regions of the stem. Our

aim is to find specific regulatory factors that could be used for targeted reduction of lignin content in the sclerenchymatous ring present in the outer parts of timothy stem (Figure).

Figure. Fully developed timothy stem with highly lignified sclerenchymatous ring in the outer cell layers of the cortex (L. Sarelainen, Univ. of Helsinki).



(57) Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in *Betula pendula* leaves

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The effects of solar UV-A and UV-B radiation on phenylpropanoid gene expression and phenolic accumulation in *Betula pendula* leaves were studied in a field test during summer 2007. Plants were grown in the field under six radiation conditions (UV-B 100 %, UV-A 100 %; UV-B 0 %, UV-A 100 %; UV-B 0 %, UV-A 50 %; and UV-B 0 %, UV-B 50 %), all created by using plastic films that exclude the UV spectrum. Young and unfolded leaves were sampled after 30 days of initiation of the experiment. A total of twenty-three individual compounds were identified by HPLC-MS.

There was an overall treatment effect on the accumulation of six flavonoids (P < 0.03). The individual effects of UV-A and UV-B on these compounds were analyzed (P < 0.10). UV-A regulated the accumulation of quercetin-3-galactoside (P < 0.07), quercetin-3-arabinopyranoside (P < 0.08), both compounds were also affected by UV-B (P < 0.06, P < 0.09 respectively). Myricetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-rhamnoside, and kaempferol-3-rhamnoside were affected by UV-B (P < 0.005). Transcript accumulation of nine phenylpropanoid genes and three transcription factors involved in plant response to UV was analysed by RT-PCR. PAL and HYH expression was significantly lower under UV exclusion. There were strong positive correlations between PAL expression and accumulation of four flavonoids under the UV treatments.

(58) Phenolic metabolism in a lignin-forming Norway spruce (*Picea abies*) tissue culture after apoplastic H₂O₂ removal

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Apoplastic hydrogen peroxide is required for extracellular lignin production in a tissue culture of Norway spruce (*Picea* abies) as removal of hydrogen peroxide by potassium iodide (KI; I⁻ being the active component) repressed the synthesis of extracellular lignin (Kärkönen et al. 2002). This suggests that peroxidases are involved in activation of monolignols for lignin polymerization. Phenolics were characterised from spruce cells and culture medium in cultures treated with KI. Interestingly, instead of monolignols, precursors of lignin, dilignols were identified both inside the cells and in the culture medium. This leads to a question whether laccases, O₂-utilising oxidases, are involved in formation of dilignols.

In order to find alternative ways for hydrogen peroxide removal the effect of catalase and catalysts, PtO₂ and MnO₂, was investigated. However, even though the tested compounds removed hydrogen peroxide efficiently *in vitro*, the water-soluble KI was the most efficient hydrogen peroxide scavenger *in vivo* with spruce cells.

Spruce suspension cultures produce extracellular lignin within five days after transfer of cells into liquid cultures and thereafter die. Cells with 5 mM KI supplementation, however, start to divide. This makes it possible to cultivate spruce cells in liquid cultures. Cell mass increases 4.5-fold between five to thirty five days of culturing. Interestingly, removal of KI after three week -treatment restores extracellular lignin formation.

We are currently measuring phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) activities to see whether removal of hydrogen peroxide has an effect on monolignol biosynthetic pathway. PAL is the first enzyme, and CAD is the last enzyme in a string of enzymes producing monolignols from phenylalanine. If changes occur in enzyme activities we will continue with transcriptomic analysis and look for the regulators of the lignin biosynthesis pathway.

Kärkönen A, Koutaniemi S, Mustonen M, Syrjänen K, Brunow G, Kilpeläinen I, Teeri TH, Simola LK (2002). Physiol Plant 114: 343–353

(59) Betula pendula Roth and Populus tremula L. show intraspecific variation in their response to soil moisture

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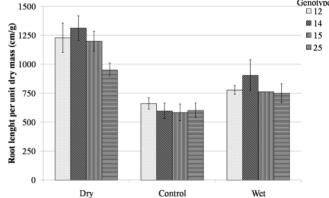
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Soil moisture is recognized to be the main determinant limiting plant growth and predicted climate change is expected to markedly change precipitation patterns. Trees combine life-history traits and levels of genetic diversity within populations that might allow them to adapt relatively quickly to environmental changes, provided sufficient gene flow can be retained.

Very little work on intraspecific variation in adaptability of *Betula pendula* (birch) or *Populus tremula* (aspen) – two important species in the boreal region – to soil moisture content has been carried out. Available studies found intraspecific differences in many parameters affecting drought tolerance in genotypes either selected from different geographic regions or for superior performance in certain traits. Results from selected trees may not reflect the magnitude of genetic variation within populations, which is so important for the influence of predicted climate change on populations of trees in nature. We examined intraspecific variation of birch and aspen to soil moisture using plant material cloned from naturally regenerated populations.

Our genotypes used the same mechanisms to cope with drought – i.e. decrease of average leaf size, lower stomatal conductance resulting in differences in gas exchange parameters – and were thus able to maintain relatively high leaf water potentials, protecting the photosystem. Interestingly, the genotypes showing the highest growth rates in the control treatment also performed best under dry conditions.

Our results show that the best performing genotypes (i.e. genotype 25 in birch) formed least root mass per unit root length under adverse conditions. This indicates investments in short lived fine roots, allowing these genotypes to access the remaining water more effectively. Gene ex-



pression study showed that drought treatment induced ACC oxidase at the beginning of the experiment, while prolonged wet conditions led to upregulation of LEA5, RD22 and ADH1, indicating oxidative stress and hypoxia.

We conclude that birch and aspen plantlets cloned from naturally regenerated individuals show intraspecific variation, which can thus be expected in nature.

(60) The effects of soil drought and needle age on fine particle capture efficiency of Norway spruce (*Picea abies*)

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Fine particles have adverse influence on air quality and human health. Road traffic, power plants and wood combustion produce most of the anthropogenic particulate pollution in Finland. Trees can capture considerable amount of particles from the air and particle capture efficiency can vary between tree species. However, the capacity of the naturally occurring northern tree species to capture fine particles is poorly understood. In addition, the environmental conditions, e.g. water availability, may affect the particle capture capacity by controlling stomatal function and wax structure.

The capture efficiency of fine particles to the tree foliage were examined in wind tunnel experiments with 20 three-year-old Norway spruce (Picea abies) saplings. Ten replicate saplings were allocated both to well watered and drought stressed treatments and five replicates from both groups were exposed to fine particles in wind tunnel and five were left as untreated controls. Stomatal conductance was measured before and after every exposure to control changes in stomatal function. Air flow of 3 m/s was used in the exposure time of two hours. Fine particles of mass mean diameter 0.7 µm (± 3.0 µm SD) were introduced to the trees within the air flow in a mass concentration of 933 µg/m³ (± 114 µg/m³ SD). After the wind tunnel exposure few needles from current and last year shoots were collected for scanning electron microscopical analysis. Then pieces of current and previous year shoots were collected separately and salt particles were flushed out with distilled water. The mass of particles captured on the trees was analysed from the water with an ion chromatograph. Captured particle amount was calculated per leaf surface area (mg/m²).

Results from the wind tunnel experiments showed that soil water content clearly affects particle capturing efficiency of Norway spruce. Spruces with less water in soil matrix captured more fine particles than well watered replicates.

(61) Exploring the Monolignol transport mechanisms in Norway spruce

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Lignin is an important part of the cell wall of trees forming c. 25 % of xylem cell wall dry weight. While the biosynthesis of monolignols is relatively well known, the transport of monolignols into the apoplastic space and their polymerisation are known to a far lesser extent.

Our aim is to understand the cell and molecular biology of the lignification process by studying the transport mechanisms of monolignols from the living cells to the cell wall space. There are three possibilities for this transport: Golgi-vesicle mediated transport, transport through ABC-type plasma membrane proteins or other channel proteins, or through diffusion based on the hydrophobic-hydrophilic properties of the monolignols. The Golgi-mediated transport has been shown plausibly not to take place in a coniferous species. We are now studying the ABC-transporters on the plasma membrane (ATP-binding cassette transporters) using ¹⁴C-labelled phenylalanine and transporter inhibitors in a Norway spruce (*Picea abies*) tissue culture line.

The results indicate that the ABC-transport inhibitors vanadate and reversin do not inhibit the transport of monolignols into the tissue culture medium. As to any other channel proteins taking part in transport, we have done an extensive database search on genes related to transport phenomena and their expression levels in different tissues of conifers. As we have now indications that this transport may not be ABC-transporter driven, we have extended the study on other transporters on the plasma membrane. We have not excluded the possibility of diffusion through the plasma membrane due to the hydrophobic interactions with the monolignols or their glucosides. For this purpose we have collected differentiating xylem material of a Norway spruce clone to be used in the membrane transporter study on the protein level.

Potential of novel plant biology applications (session 12)

ABSTRACTS OF

3 ORAL PRESENTATIONS

AND

3 NUMBERED POSTERS (62 - 64)

Effect of all organic or field organic feeding on milk omega-3 and omega-6 milk fatty acid concentration

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Organic farming systems promote to forage rich legume containing diets, which are known to affect milk fatty acid (FA) composition. At Finnish organic farms bovine winter feeding is based on grass-clover silage and supplemented with some concentrates. Red clover(*Trifolium pratense*) is used as a primary legume owing to its high yield potential and winter hardiness. The aim of this farm survey was to study the impact of current Finnish winter feeding practices of all organic and only field organic farms on milk FA composition. In this paper omega-3 and omega-6 FA results are given. The study is a part of Nordic Core Organic PhytoMilk project, in Finland founded by the MMM.

The survey was conducted in Eastern Finland with 45 organic farms in the end of January 2008. Of all farms 23 practiced organic field farming and 22 were certified organic milk farms. During the farm visit diets were documented, basic feed samples taken and bulk-tank milk samples collected and frozen prior to FA analysis at Aarhus University (PhytoMilk-project partner). Effect of farm type on concentrate proportion, estimated silage clover proportion and on milk FA composition was studied using the independent sample t-test.

All organic farms had lower proportion of concentrates in their diets than field organic farms (0.29 vs 0.43, P<0.001), but the estimated clover proportion of silage was similar (0.35). The average FA concentration of milk was also similar in both farm groups (mean 3.4 g/100 g DM). Predominating omega-3 FA and omega-6 FA were C18:3 n-3 (ALA) and C18:2 n-6 (LA), respectively. All organic farms resulted to similar proportion of total omega-3 FA, except higher proportion of C20:5 n-3 (EPA) (P<0.05), than field organic farms, but to lower proportion of total omega-6 FA (P<0.05). Consequently all organic farms resulted to lower relation of omega-6/omega-3 compared to field organic farms (2.7 vs 3.3, P<0.05).

In conclusions, certified organic milk from all organic farms had favorable omega FA composition than milk from farms practicing only organic field farming and giving more concentrates. Relatively high milk ALA proportions suggested positive effect of red clover in both farm groups.

Cloudberry (*Rubus chamaemorus* L.) cultivar 'Nyby' – an example of domestication of wild plant species to cultivation

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Throughout history, human has domesticated wild plant species into cultivation. In Finland, berry species have been a fruitful target for this work. Mostly, domestication occurs by finding an interesting plant individual or population in the nature and transferring this finding to home-garden or field conditions. Because field conditions differ from the natural ecosystems, domestication is not always successful. In Norway, the domestication strategy of cloudberry was to cultivate plants in the bogs, in surroundings where cloudberries have adapted. Domestication was done by cultivation of natural vegetation or planting propagated wild or bred material to natural bog surroundings. Both female and male clones were selected for the purpose.

In Finland a hermaphrodite cloudberry clone was found in 1991 at Nyby, Petolahti in Maalahti, 40 km south of Vaasa. The original plant was cloned by micropropagation and the clone has been under observation and in field experiments at MTT Laukaa since 1992. The mutation has proved to be stable and the clone was named as a cultivar 'Nyby', after the place of the original location. This cultivar has been in field trials also at MTT Sotkamo. Cultivar 'Nyby' has been in commercial propagation since 2005.

Micropropagation of cloudberry was started in 1994. The critical stage in the protocol was survival of microplants in the greenhouse after transferring to the soil. In the first experiments the survival rate was less than 25 %. After modifications the micropropagation protocol has been successful and survival rate has varied from 88,5 % to 95 %. Micropropagated cloudberry plants had a well developed root and shoot system 3 months after transferring from the laboratory to the greenhouse

Cultivar 'Nyby' can be grown both in the greenhouses and in the open field. Because of self-pollination ability, about 90 % of the flowers were pollinated and produced a berry. In the greenhouse the plants flowered and produced the first berries by self pollination after one year. Neither hand pollination was done nor pollinating insects were brought into the greenhouse. In the field experiments about 80 % of flowers developed a 1st class berry. Flowering at Laukaa has started about the 10th of June and harvesting time was at the end of July. The berries ripened within 10 days (20.7.–30.7.2004). 'Nyby' has not been susceptible to late night frosts.

Berries in the greenhouse had 9.25 seeds on an average and in the field the average number of seeds per berry was 14.1. The berries were flat rounded, the mean berry size was 15 mm x 12 mm. The average berry weight was 1.15 g varying between 0.55 and 2.22 g. The average cropping was from 101.6 g/m 2 up to 334 g/ m 2 .

Ethylene stimulates cambial cell division and enhances wood formation in *Populus*

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Ethylene is an endogenous stimulator of cambial growth and has potential to modify fiber structure and chemistry. Under natural conditions, ethylene is induced by gravitational stress and it regulates the formation of tension wood, characterized by increased growth and modified wood chemistry. External application of ethylene also stimulates secondary cambial growth in *Populus* significantly (Love et al., 2009, PNAS 106, 5984). Ethylene responses are mediated through a large family of Ethylene Response Factors (ERFs). The diversity of ERFs can explain the multifunctional role of ethylene in plants, and thus are key players in understanding the mechanism of ethylene action in wood development.

We identified 173 ERFs in the black cottonwood (*P. trichocarpa*) genome. With primers specific for these ERFs we used real-time quantitative PCR to identify those induced by ethylene in poplar stem tissues and during tension wood formation.

Several ERFs in stems of *Populus* showed considerable and consistent increases in transcript abundance when treated with ethylene or its precursor 1-aminocyclopropane-1-carboxylic acid (ACC). A population of ERFs was identified to also be induced by gravitational stress in tension wood forming tissues. These ERFs were not induced in ethylene-insensitive poplars expressing the *etr1-1* mutant allele of *Arabidopsis* ethylene receptor ETR1, showing that ERF expression was mediated by ethylene through the ethylene receptors.

A subset of the *Populus* ERFs was selected for functional characterization of their role in regulation of cambial cell division and wood formation. These 20 ERFs were ectopically expressed under the control of cambium/xylem-specific promoter in hybrid aspen, *P. tremula* × *tremuloides*. Greenhouse experiments where the transgenic lines were grown for 14 weeks showed that secondary growth was significantly enhanced in several of these lines resulting in up to 30-50 % increase in stem volume. Taken together, the experiments with ERFs in the stem of poplar provide a demonstration for significance of ethylene in wood development.

(62) Plant metal uptake from smelter-polluted forest soil at a phytoremediated site

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We studied how the application of a mulch cover (household biocompost and woodchips) onto polluted forest soil affects the metal uptake of planted pine (*Pinus sylvestris* L.) and downy birch (*Betula pubescens* Ehrh.) seedlings.

Tree seedlings were planted on summer 1996 in mulch pockets on mulch-covered and uncovered plots. Seedlings were harvested after 10 years growth for biomass determination and element analysis by dry ashing followed by ICP-AES. Soil samples for chemical analysis were taken in August 2005. Total concentrations of organic soils were determined by dry ashing and those of mineral soil by *Aqua Regia* -extraction, while the exchangeable concentrations of both organic and mineral soil by extraction with BaCl₂ + EDTA, all followed by ICP-AES.

The mortality rate of *Pinus* seedlings was low, less than 12 % in both treatments. The mortality of *Betula* on the mulch-covered plots (48 %) was higher than on the uncovered plots (17 %). However, the biomass growth was higher on the mulch-covered plot, both in the case of pine and birch. The root metal concentrations of both tree species were lower in seedlings grown in mulch-covered plots compared to uncovered plots. There was considerable variation between the treatments also in the metal concentrations of the above-ground parts of the seedlings, but due to surface contamination caused by aerial metal-containing dust, the observed differences cannot be interpreted to be caused by different root metal uptake rates.

(63) Evaluation of maize and hemp cultivars as bioenergy crop

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We are cultivating annual and perennial crops as monoculture and as mixed culture to investigate plant biomass use for production of renewable energy sources in northern latitudes. Prerequisites for sustainable energy production are high biomass production and low inputs of fossil fuels in agricultural operations as well as synthetic fertilizers and pesticides.

In addition to biomass quantity, also its quality is significant factor since different bioenergy technologies have different quality requirements. Biological fermentation methods benefit from high concentrations of soluble sugars and starch, in lignocellulosic mass using methods different pre-treatments are needed to convert cellulose and hemicellulose fibres more approachable to hydrolysing enzymes. Biomass quality and its usefulness can vary depending on plant species, harvesting time, fertilizer use as well as weather and soil conditions. Similarly different plant parts can vary in their chemical composition due to divergent translocation of different elements and partitioning of soluble sugars and storage carbohydrates in growing plants.

We have grown different hemp (Cannabis sativa L.) and bioenergy maize (Zea mays L.) cultivars in 2007–2009 at Viikki Experimental Farm, University of Helsinki. Samples of 1 m⁻² per plot were harvested in the autumn during the growing season and in the following spring for quantity and quality of biomass produced. Maize yield varied highly year by year from 15 t DM/ha to near 30 t DM/ha whereas hemp was merely cultivar dependent as the yield between cultivars varied from 6 t DM/ha to 14 t DM/ha. The chemical composition (Al, B, C, Ca, Cl, Cu, Fe, K, Mg, Mn, N, Na, P, S, Si and Zn) of the samples was analysed from dried, ground subsamples. The quantity of inorganic elements varied markedly between the studied species and cultivars. Cl and K content was highest at the lowermost plant parts whereas N and P content was highest in the uppermost plant parts and ears. Even though, the losses in biomass in spring harvest are marked, the loss of water and inorganic elements (except for Al and Fe which increased) in the biomass is economically important especially in case the material is used for combustion since high alkali metal (K and Na) and silicon (Si) concentrations in biomass can cause slagging and corrosion in combusting process. Both crops are high in straw cellulose and low in lignin content, and thus they are suitable for biomethane or bioethanol uses. Studied crops did not lodge during autumn rains, and thus they are also suitable for combustion. Cooperative research group is analysing the processes of biomethane and bioethanol production. Economic aspects will be evaluated by cooperative partners at the University of Helsinki, Department of Economy.

(64) Application of DGT-method for determination of trace metal bioavailability in forest soil

Marita Turunen, Tiina M. Nieminen, Liisa Ukonmaanaho, Marjatta Kantola & Arja Tervahauta

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There are significant gaps in the knowledge on environmental chemistry of many nutrients and trace elements in forest soils. In particular, their bio-availability in the soil – plant system is not fully understood and cannot be accurately measured by current methodology in standard use. The aim of this project is to study the suitability of DGT techniques (Diffusive Gradients in Thin films) for estimating the bioavailable and/or mobile pool of elements in forest soil by comparing results obtained by DGT to those obtained by traditional soil extraction techniques and to the element uptake of test plants. The DGT technique (1) uses a chelating resin separated from the soil by a diffusive gel, so that metal uptake by the DGT device is controlled by diffusion. The chelating resin absorbs selectively the element under interest, e.g. chelex bounds many trace metals.

Mineral soil samples were taken from a phytoremediation site polluted by smelting activities and analyzed by wet digestion (HCl+ HNO₃) prior to analysis by ICP-AES for the total amounts of Cu, Ni and Zn. Plant available fraction was determined by 0.1 M BaCl₂-EDTA extraction. DGT analysis of metals was performed for the samples and compared to the 0.1 M BaCl₂-EDTA extraction. DGT devices for metals were inserted into samples for 24 hours in room temperature. Metals accumulated by DGT were eluted from the resin with 1 ml of 1 M HNO₃ for another 24 hours, then diluted to 10 ml and analyzed with ICP-AES. The DGT results were compared to metal concentrations in pine seedlings grown for three weeks in a growth chamber.

Cu concentrations obtained with the DGT method and in pine seedlings correlated well. Further work will be needed for the validation of the method in laboratory and with pot experiments.

1) Davison, W. & Zhang, H. 1994. *In situ* speciation measurements of trace components in natural waters using thin-film gels. Nature 367: 546–548.

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Additions and corrections to the printed version

These already have been corrected to the pdf version. In addition, numerous other tiny changes and corrections have been made.

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- p. 2 Other members: + Janne Räsänen, + Attendants: Eveliina Hiltunen, Jenna Lihavainen, Katri Nissinen, Ella Nukarinen, Jukka Vuorinen Scientific committee: + Prof. Heikki Roininen
 - + Symposium home page: http://www.uef.fi/kasvitieteenpaivat2010
 - + Distribution of the electronic version: http://epublications.uef.fi/
- p. 6 **Visit to Botania** was arranged at 17:00–18:15 in 2 groups.
- p. 22 tuuli.lukkala[at]jyu.fi → tuuli.lukkala[at]joensuu.fi
- p. 31 Photo and caption removed
- p. 76, all paragraphs: Sphagnum \rightarrow Sphagnum
- p. 121–124 Participants: + Jenna Lihavainen, Katri Nissinen, Iina Penttinen, Janne Räsänen, Tuula Toivanen

Accidentally changed symbols (expurgated from the electronic version):

$$p. \ 30 \ @ \rightarrow \gamma \ \ref{p.34} \ @ \rightarrow \alpha \qquad \qquad p. \ 36 \ @ \rightarrow \gamma$$



Group photo (© Varpu Heiskanen) More photos by Ilkka Porali http://ilkkap.kuvat.fi/kuvat/KTP 2010

ELINA OKSANEN & MARKKU A. HUTTUNEN (EDS) IX Finnish Symposium on Plant Science, May 17–19, 2010, Joensuu, Finland

This book compiles the abstracts of the IX Finnish Symposium on Plant Science (IX Kasvitieteen päivät), to be held on May 17–19, 2010, at the University of Eastern Finland, Joensuu campus.

The 37 oral and 64 poster presentations were placed under eight themes: Changing environment, Ecophysiology, whole-plant physiology and plant development, Stress and signaling, Omics, Genetics and evolution, Potential of novel plant biology applications, Ecosystems and biodiversity, and Plant interactions.



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