

# Plant Science Days, Rovaniemi 14-15 March 2023 – Abstracts

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## SESSION 1 - Arctic and plant life

### **Aerobic anoxygenic phototrophic bacteria are ubiquitous in boreal and arctic plant microbiomes**

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In addition to plants, algae and cyanobacteria, photosynthetic systems are widely present in evolutionary ancient anoxygenic photosynthetic bacteria, including aerobic anoxygenic phototrophic bacteria (AAPB). AAPB are abundant in aquatic ecosystems in all climates, but they have been also detected in terrestrial ecosystems, and recently, in metagenomes of several plant phyllosphere microbiomes. However, it is currently not known, how common AAPB are in plant microbiomes, what is the role of AAP (if any) in plant-microbe interactions.

We screened phyllospheres and endospheres of over 30 plant species for AAPB by near infrared fluorescence imaging of culturable bacteria. In collaboration with seven high schools in Finland, we sampled in ten distinct locations across latitudinal gradient spanning from hemiboreal to oroarctic climate zones in Finland, and additional locations in Svalbard and Greenland.

We show, that AAPB are consistently present in plant microbiomes in these climates, as they were detected in all sampled locations, and in virtually all plant species studied. Most of the characterized isolates represent alphaproteobacterial genera *Sphingomonas* and *Methylobacterium*, but AAPB representing betaproteobacteria as well as several novel proteobacterial lineages were also identified. *Methylobacterium* isolates were mostly present in the phyllosphere with weak host specificity, while *Sphingomonas* AAPB showed clear host plant specificity.

Intriguingly, the AAPB detected in this study represent bacterial taxa with plant growth promoting representatives, and several are part of their host plant core microbiota, prompting questions about putative role of AAP in plant-microbe interactions in cold climate ecosystems characterized by strong annual fluctuations of light and temperature.

# The snow must go on: effect of winter climate change on tree seedling growth and survival

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In northern boreal forests, increasing winter temperatures and wide temperature fluctuations are leading to more frequent rain-on-snow events and freeze-thaw cycles. This may be harmful or even lethal for tree seedlings that spend up to a half of the year under snow. We conducted a snow cover manipulation experiment in a natural forest to find out how changing snow conditions affect growth and survival of young Scots pine and Norway spruce seedlings. Three snow manipulation levels were applied: artificial ice encasement (IE), snow compaction (COMP) and complete snow removal (NoSNOW). All three treatments affected soil temperature, soil frost depth and subnivean gas concentrations compared to the ambient snow cover (AMB), leading to increased physical damage and mortality of the seedlings under a modified snow cover. Pine seedlings were analyzed for the expression of 28 genes related to circadian clock, aerobic and anaerobic energy metabolism, carbohydrate metabolism and stress protection. The results revealed that seedlings were exposed to different stresses in a complex way depending on the thickness and quality of the snow cover.

Our findings show that altered snow conditions and causes tree seedlings various abiotic stresses, whose effects extend from overwintering to the following growing season.

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# How do pendulous lichens grow in different continuous cover forests?

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Competition for land use between forestry and reindeer husbandry has been debated in northern Finland, particularly due to the possible adverse effects of forestry on the availability of forage resources for reindeer. To achieve a better reconciliation of these land uses, and to promote multiple forest use, we studied the effects of 37 variables, and compared two methods of continuous cover forestry (CCF) on pendulous lichen occurrence in Finnish Lapland.

The main findings of the study indicate that the number of years after cutting, and the trunk diameter increase the probability of pendulous lichen occurrence the most. Of the two CCF methods studied – small gap cutting and selection cutting – the former was slightly more successful in maintaining pendulous lichens, because it allows to keep at least parts of the forest intact for a longer period of time, which advances the growth of pendulous lichens. Furthermore, we compared our data with a reference material, and found that the CCF stands of the present study maintained more pendulous lichens than managed young stands, but less than managed mature stands. As an outcome we state that continuous cover forests do not manage pendulous lichen as such, but it needs the largest trees to be saved, and the cutting cycle to be kept as long as possible. In addition, lichen-rich areas should be excluded from logging.

## SESSION 2 - Plant soil microbe interactions

### **Mycorrhizas in cold climates**

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In northern regions, organisms must have mechanisms for surviving the winter and functioning in low-temperature conditions. The temperature relations of mycorrhizal fungi and mycorrhizas have been studied surprisingly little, and most studies on mycorrhizal functioning have been done at growing-season temperatures.

Ectomycorrhizas and ericoid mycorrhizas are the most common types in boreal forest vegetation, and arbuscular mycorrhizas are more common in warmer regions. We have hypothesized that the low-temperature tolerance in the different mycorrhiza types is a critical factor as such, although water availability and soil nutrient availability also change together with temperature.

We have shown that ectomycorrhizal fungi in pure culture can tolerate very low frost temperatures. However, comparisons between ectomycorrhizal and non-mycorrhizal Scots pine seedlings did not show any benefit in the tolerance of the host plant to a short exposure to frost. Recent results from a similar experiment on an arbuscular-mycorrhizal species showed some negative effect of mycorrhiza on the recovery of the plants from frost exposure (poster by V. Virjamo et al.).

Experiments comparing the formation of arbuscular and ectomycorrhizas showed also better performance of ectomycorrhizal fungi at low temperatures and better performance of arbuscular-mycorrhizal fungi at higher temperatures.

Longer-term studies are needed on the winter survival of mycorrhiza types and their functioning in soils with low temperature in the spring and during the growing season. The results so far suggest that temperature may be a direct factor in the performance of the different types.

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# Ecosystem-level carbon exchange and storage in the greening Arctic

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The consequences of warming-induced ‘greening’ on Arctic soil carbon storage are under intensive research, as the majority of ecosystem carbon is in the soil. The response of soil carbon release may depend on the shrub species that increases: deciduous shrubs such as *Betula nana* likely promotes a loss of soil carbon, whereas the opposite may be true for evergreen shrubs such as *Empetrum hermaphroditum*. We analyzed vegetation, ecosystem CO<sub>2</sub> exchange, soil organic matter stocks and microbial activities and biomass after 20 years of experimental warming in a tundra heath with increased deciduous shrubs, and in a mountain birch forest with increased evergreen shrubs.

In contrast to expectations, increase in shrubs was not cumulative in time. Shrubs were still more abundant in the warming treatment, but the total vascular plant abundance was lower than nine years earlier, likely because several moth and rodent outbreaks had taken place in the area. The only exception was formed by *Betula nana* that had increased in time. When expressed as per gram microbial biomass, microbial activities were higher under warming, but warming also led to a lower microbial biomass, which counteracted increased activities resulting in no effect on activities per gram soil, and no effect on soil organic matter storage. Microbial biomass nitrogen correlated negatively with evergreen shrub density at both sites, indicating that ‘shrubification’ may have intensified nutrient competition between plants and soil microorganisms. Also contrasting expectations, ecosystem respiration was lower under warming in both habitats, which likely resulted from lower soil temperatures due to a higher insulation of soil thicker vegetation. Put together, these studies show that although ‘greening’ increases microbial activities, several mechanisms may counteract this effect and contribute to the stabilization of ecosystem carbon under warming.



## Microbial responses to increased shrub growth in the greening Arctic

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Ongoing climate change affects Arctic tundra ecosystems most severely and is already altering vegetation and ecosystem structure. Besides increased temperatures, expansion and increased growth of shrub dominated vegetation (“Arctic greening”) is considered to affect soil microbial activities and it is of concern that tundra ecosystems are turning into C sources with increased decomposition rates and priming by the increased plant derived C. However, predicting the fate of organic C is complicated by our still very poor understanding of the soil microbial communities and how the environmental factors and changes in vegetation drive the community structure and function.

We characterized soil bacterial and fungal communities under long-term (ca. 20 years) experimental warming conducted in two tundra sites and a mountain birch forest located in Kilpisjärvi, Finland and Abisko, northern Sweden. Warming increased especially the dominance of *Empetrum nigrum* subsp. *hermaphroditum* in all experimental sites, *Betula nana* in the tundra site of Abisko and *Vaccinium myrtillus* in the Kilpisjärvi tundra site. Tundra and mountain birch forest sites were characterized by distinct bacterial and fungal communities but warming induced relatively similar changes in the microbial communities of all sites. Warmed plots were enriched especially with members of the Helotiales and Agaricales fungi as well as Actinobacteria and some Acidobacteria. The data indicated that warming increased nutrient limitations and this was associated with an increased dominance of versatile ericoid mycorrhizal and saprotrophic taxa that are able to decompose recalcitrant organic matter.

## **Frost tolerance and recovery of arbuscular mycorrhizal and non-mycorrhizal *Thuja occidentalis* (L.)**

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In the boreal forest zone, plant symbioses with ecto- and ericoid mycorrhizal fungi are more common than arbuscular mycorrhizal (AM) fungi and tolerance and recovery of AM in freezing conditions is poorly understood. Here, we studied *Thuja occidentalis* (L.), which forms obligately arbuscular mycorrhizas. Its natural range falls between 40°N and 50°N in North America.

AM and non-mycorrhizal seedlings were exposed to seven temperatures from +5°C to -45°C after which freezing damage (relative electron leakage of foliage) was recorded. Seedlings were then allowed to recover for two weeks in long day growth conditions where day temperature was either +10°C or +22°C. After this damage was assessed visually, and shoot and root biomass, nutrient concentrations, and mycorrhizal colonization was analyzed.

Whole-plant freezing tolerance (visual damage) of *T. occidentalis* seedlings was not affected by AM. Freezing tolerance of the foliage was -23°C but there was a decrease in the nutrient levels and root biomass already after treatment in milder temperatures (-12°C/-18°C) when compared to control (+5°C) treatment. AM had lower shoot biomass increment during recovery period than NM. AM had also higher P uptake than NM, but only after recovery period from control (+5°C) or -5°C treatment (frost treatment × mycorrhiza treatment interaction). These results suggest that function of AM fungi is highly susceptible to cold, possibly affecting their distribution in temperature-limited habitats.

## SESSION 3 - Practical applications from plants

### **Health from the forest: spotlight on arctic lingonberries**

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## **Harnessing the new faba bean reference genome for a sustainable northern protein crop**

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The substantial deficit in plant protein production in Europe is met currently by soy meal and beans primarily from South America, leading to reliance on both import of plant protein to Europe and access to mineral fertilizers by the soy producers. Having an average protein content of 29%, faba bean (*Vicia faba*) could substantially improve European protein sovereignty and security, both for food and for feed. It is a widely adapted protein crop, growing where soy cannot, making it well suited to Northern and most other parts of Europe as well as to regions worldwide where it is a traditional, staple food. The unprecedented challenges from biotic and abiotic stresses, worsened by climate change, will require genomics-enhanced tools for breeders to more efficiently develop sustainable, secure crops with the properties processors need. The 13 Gbp diploid genome of faba bean has until recently been recalcitrant to high-quality assembly, but recent advances have enabled our production of a highly intact reference genome. The genome is enabling us to address antinutrients, flavour, processing quality, and sustainability traits. We are now producing a pan-genome spanning the diversity space of the species to deliver the insight and tools needed for rapid improvement of faba bean. This PanFaba project will enable the high-resolution linkage of genotype to phenotype that is needed to improve faba bean as a protein crop and adapt it to likely future climatic conditions in Finland and elsewhere in Europe.

# Natural wax from arctic berries: Composition, morphology and effect of environmental factors

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**Keywords:** Bilberry, lingonberry, berry press cakes, cuticular wax, triterpenoids, glossy mutants

The outer surface of plants is covered by cuticular wax, which plays a role in non-stomatal water loss, protection from UV rays and plant defense. We have studied the chemical composition of cuticular wax in bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), bog bilberry (*Vaccinium uliginosum*) and crowberry (*Empetrum nigrum*) fruits. Triterpenoids, were found to be dominant compounds in bilberry and lingonberry cuticular wax. Supercritical Fluid Extraction (SFE) was used to extract wax from the industrial leftover of berry (bilberry and lingonberry) juice. Berry waxes show good *in vitro* Sun Protection Factors (SPFs) depicting high UV-B absorbing capacities. Glossy wax mutants and wildtype bilberry cuticular wax were studied through the course of fruit development. The wax load between mutant and wildtype bilberry was found to be almost similar, however the proportion of triterpenoids was higher; fatty acids, aldehydes and ketones, lower in mutant wax as compared to wildtype bilberry during development. While studying the effect of environmental factors on bilberry cuticular wax, we observed that the proportion of triterpenoids increases in as we move from northern latitudes to southern, and correlation analysis suggested temperature to be a major influence. The work aims at enhancing bioeconomy and circular economy, while exploiting potential of side streams of Nordic berry based industry for value added products.

## **Innoherb – Sundew and other high value plants for paludiculture and vegetation succession on Sphagnum moss biomass harvesting areas in Western Finland**

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Sphagnum moss is harvested mostly for growing media purposes from forestry drained, but low productive peatlands in Northern Pirkanmaa, Southern Ostro-Botnia and Northern Satakunta municipalities in Western Finland. Commercial scale harvesting has been practiced since year 2015 and the harvested area today is about 200 ha.

We have found that these areas are favorable for sundew (*Drosera rotundifolia*) propagation. The sundew seedbank in the area and the lack of competition by other mire plants and constant wet conditions, there is a timeframe of about 5 years for sundew to flourish. It's highly valued based on its several bioactive compounds. For other commercial herb like heather (*Calluna vulgaris*) these areas are also favorable, flowering parts of heather has versatile traditional uses and nowadays it is used also in cosmetics. These commercial plants have a large business potential. As these herbs may produce remarkable, much higher yields during 2-10 years after the *Sphagnum* moss biomass harvesting than on intact drained peatlands. In our project the idea was to find out novel value chains for underutilized herbs growing in low productive areas to be utilized in high value products like cosmetics.

In addition of the biomass production evaluation of these commercial herbs, we have done also explorative study on the raw material quality by using established antioxidative testing methods to control the different preservation options to keep the functional properties unchanged for different end products (e.g. antioxidants as preservatives or other functional ingredients).

In this presentation the results of this project are presented.

## SESSION 4 - Biodiversity

### **Plant biodiversity losses and mechanisms under nutrient enrichment and grazing**

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Plant biodiversity and its maintenance is widely recognized crucial for multiple ecosystem functions and services. Ecologists are expected to be able to inform societies about biodiversity trends and ways to mitigate losses. Global-local scales are providing rather mixed views drivers plant diversity trends. As many anthropogenic threats on plant diversity are intensifying simultaneously, including increases of nutrient deposition, climate changes, and land use, it is important to investigate mechanisms in experimental systems to provide better predictions. I will present results on the effects of nutrient additions and/or grazing exclusion and how these manipulations control community compositions and drive losses of species. A focal system is grasslands and their bryophytes in the coordinated distributed Nutrient Network experiment. Grassland bryophyte diversity decreased under N and NPK fertilization. Nutrient resource additions, particularly N, increased plant biomass production, and this reduced light availability near ground and consequently bryophyte diversity decreased. Vascular plant biomass consumption due to grazing biomass did not mitigate bryophyte loss. Nutrient addition effects on bryophytes were usually stronger than those on vascular plants. Inclusion of bryophytes may improve estimates on overall nutrient-driven plant loss rates in grasslands.

## Exploring adaptive traits and their genetic determination in the pre-breeding population of reconstructed garden strawberry

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Garden strawberry (*Fragaria* × *ananassa*) is the most important Nordic fruit crop in acreage and in value. This allo-octoploid ( $2n = 8x = 56$ ) species first emerged in the 18th century as the result of hybridization between two octoploid progenitor species, *F. virginiana* and *F. chiloensis* in Western Europe. Current-day strawberry cultivars are reported to have a diminished allelic diversity, and they are exposed to climatic changes which pose threats to fruit quality and annual yields. In the NORDFRUIT PPP project, a reconstructed strawberry F1 hybrid population was developed by crossing the pre-evaluated *F. chiloensis* or *F. virginiana* parents, in order to bring novel variation for strawberry breeding. This pre-breeding population was further evaluated in the BreedingValue Horizon 2020 project for multiple adaptive and horticultural traits in a field trial in Finland. It was also genotyped with the 50K SNP array. We found considerable variation in the overwintering percentage (2021-2022) and in male and female fertility (2020-2022), indicating the possibility to utilize this material in breeding. The GWAS analysis showed one strong signal on the chromosome 3C for the winter survival percentage. We also identified multiple GWAS peaks for male and female fertility on three different chromosomes of the octoploid strawberry. Our results provide basis for the efficient use of this pre-breeding population for introduction of several valuable traits in breeding. A core subset of 60 genetically diverse individuals was identified for future use and for genetic conservation purpose.



## Finding a good mine tailing cover for Arctic conditions

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In Finland, the environmental risks of mining closures are usually managed by covering them with a layer of forest till soil (FTS). Water and nutrient retention, soil microbial activity and plant growth of the cover are suggested to be enhanced by organic amendments like biochar (BC) and composted sewage sludge (CSS). Furthermore, the right choice of plant species reduces oxygen flow to heavy metal containing mine waste. However, there is a lack of knowledge how this kind of structure acts in the northern latitudes.

In Biopeitto (“Bio-Cover”) –projects, we developed a bio-cover containing till, BC, and CSS to fasten the revegetation process and prevent water and oxygen leaching. Several studies were conducted in laboratory, greenhouse, field, and pilot scale. Three types of cover materials are tested: 1) FTS, 2) FTS + CSS, and 3) FTS + CSS + BC.

Field studies were established in 2018-2020 in the Rautuvaara closed mine tailings. The lysimeter experiment involves 24 lysimeters with or without grass species. Vegetation experiment consists of seeds and seedlings of 8 native arctic meadow species placed in 16 plots. Additionally, waste producers, landscape practitioners, and mining companies were connected for a pilot chain to produce a bio-cover from local organic wastes for Kevitsa mine.

We monitored: i) vegetation success; ii) heavy metal bioaccumulation; iii) quality and quantity of leachates. Furthermore, temperature and oxygen content of cover structures are measured continuously. The preliminary data showed that BC increases plant biomass and reduces water leaching.

The studies were implemented in ERDF-funded projects.

## Session 5 - New methods: monitoring from cell level to remote sensing

### **All the light we cannot see – hyperspectral imaging in plant biology and environmental research**

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Hyperspectral imaging or imaging spectroscopy is a non-invasive optical sensing technique that is widely used in various disciplines in scientific research. In plant and environmental research, the main application areas are remote sensing of vegetation, plant phenotyping, stress symptom characterization, estimation of chlorophyll, nutrient or water content, and evaluation of plant health status. The presentation gives an overview of various spectral imaging applications in plant sciences and on its potential as a tool to explore and quantify alterations in plant optical properties related to biologically meaningful factors.

# Leaf chlorophyll content of European aspen and its assessment by airborne hyperspectral imaging

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The ongoing biodiversity decline underlines the need to monitor terrestrial biodiversity to support the planning of land-use, and conservation. Remote sensing tools suitable for covering large land areas in a quantifiable, reproducible, and comparable manner to monitor biodiversity indicators in both spatial and temporal dimensions are needed. European aspen (*Populus tremula*) is a foundation species and a good biodiversity indicator in boreal forests. The aim of this study was to evaluate the applicability of aerial hyperspectral imaging in the estimation of foliar chlorophyll content in individual aspen trees in an 83 km<sup>2</sup> boreal forest area containing both protected and non-protected forests in southern Finland. We analyzed the chlorophyll concentration in the upper canopy leaves by traditional laboratory methods and compared it with the upper canopy spectral reflectance of aspen trees. We found out that 1) chlorophyll content had a high variability among aspen trees within the species, 2) the accuracy of foliar chlorophyll content estimation in aspen by airborne hyperspectral imaging was reasonably good, 3) the most informative spectral range in chlorophyll content estimation in aspen was the red edge. It can be concluded that there is within species variation in the spectral reflectance features of forest canopy. Thus, all spectral variation is not related to among-species variation, which needs to be considered when spectral diversity is used as an indicator of species diversity.

## Remote sensing the habitats of Northern Lapland

Anna Tammilehto<sup>1</sup>, Pekka Härmä<sup>2</sup>, Minna Kallio<sup>2</sup>, Markus Törmä<sup>2</sup>, Mika Heikkinen<sup>2</sup>, Mikko Impiö<sup>2</sup>, Kristin Böttcher<sup>2</sup>, Mikko Kervinen<sup>2</sup>, Tytti Jussila<sup>2</sup>, Saku Anttila<sup>2</sup>, Seppo Tuominen<sup>2</sup>, Katariina Mäkelä<sup>2</sup>, Aira Kokko<sup>2</sup>, Elisa Pääkkö<sup>1</sup> and Arto Saikkonen<sup>1</sup>

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Nearly 40 % of the fell habitats are estimated to be threatened (2018) and the conservation status of mountain birch forests and mountain heaths protected under the EU Habitats Directive is estimated to be unfavourable in the alpine zone (2019). The most significant factors affecting the state of the fell habitats are climate change, reindeer summer grazing and their combined effects. To conserve these habitats, up-to-date data on the habitats is the first requirement. However, collecting field data from the vast and often difficult-to-reach fell area is expensive and it is not possible to produce up-to-date habitat data for all areas.

Satellite-based remote sensing makes it possible to cover large areas with frequent observations. The advantage of this method is also the huge amount of data, which enables the use of machine learning techniques and cost efficiency. However, the habitat data produced by remote sensing is of a different nature compared to the data collected from the habitats in the field. Also, the resolution of the satellite-based remote sensing methods is often too low to detect minor habitats.

The three-year (2020-2023) project ‘Remote sensing the habitats of Northern Lapland’, has produced new habitat data for the conservation and wilderness areas of Northern Lapland. The production of the new habitat data in the project is based on combining remote sensing and field observations. The new data has been produced by extensively training a dataset containing various satellite and laser scanning data with field data from more than 4,000 observation points.

## Image-based time series analysis for fungal disease progression and severity in plant tissues

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Image-based symptom scoring of plant diseases is a powerful tool to associate disease resistance to plant genotypes. We have utilized two pathosystems to develop image-based disease scoring methods. In the first one, *Arabidopsis* and the fungal pathogen *Botrytis cinerea* were used to assess severity and symptom progression of the infection over time in dissected leaves. A pixel classification strategy using color hue values from Red-Green-Blue (RGB) images and the random forest algorithm were used to establish the leaf responses. The method allowed assigning leaf tissues to necrotic, chlorotic, and healthy at different levels in the wild type, sensitive and resistant genetic backgrounds. In the second pathosystem, *Fusarium* head blight was used to screen oat genotypes for their responses on two *Fusarium* species, *F. langsethiae* and *F. culmorum*. Dissected spikelets of several oat genotypes, with different resistance profiles, were subjected to inoculation by the two *Fusarium* spp. and the progression of the infections were analysed by chlorophyll fluorescence of each pixel in the spikelets. The recorded values were i) the change in photosynthetically active area of the spikelet as percentage of its initial size, and ii) the mean of  $F_v/F_m$  values of all fluorescent pixels per spikelet. The disease progression was successfully monitored, and different stages of the infection could be defined along the time series. The data confirmed the differential rate of disease progression by the two FHB causal agents. In addition, oat varieties with variable responses to the infections were indicated.

## Session 6 - Plant stress and function

### **Environmental sensing and anticipatory acclimation: an information-based framework**

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Fitness in wild species and yield in crops depends largely on responses taking place throughout the life of the plants. Many of these responses take time to be expressed and are costly or impossible to reverse. For example, plant morphology and anatomy can be modified after differentiation only by replacement of whole organs in most cases. Therefore, if the consequences of a developmental 'decision' can be determined by future events, any information about timing and likelihood of those future events at the time of making the decision facilitates a favourable decision. For example, timely cold hardening of boreal trees in advance of winter and timely greening of the African forest in advance of the rain season are anticipatory responses.

The genotype functions as a plastic template of possible phenotypes. The realized phenotype depends on acquired resources and the sensed cues and signals. All organisms use sensing to acquire information from the correlations existing among variables in their environment. Using this information, organisms can acclimate pre-emptively to conditions that they are likely to experience in the near or even far future. This has been variously called *biological forecasting*, *anticipation* or *future perception*.

I will argue that to fully understand the role of plants' sensory systems in anticipatory acclimation we must rely on a theoretical framework that links environmental cues, the information that different cues carry, the properties of plants' sensory systems and the functional role of plant responses.

## **Birch is a model system for acclimation and adaptation of northern forest ecosystems to rapidly changing environment**

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Northern forest ecosystems are exposed to rapid environmental change, i.e., climate warming, extended growing seasons, increasing greenhouse gases, and changes in precipitation and water availability, accompanied by increasing pressure of herbivores and pathogens. Silver birch (*Betula pendula* Roth) is an important deciduous trees species in the boreal zone, with extensive distribution across Eurasia. Silver birch is an excellent model system for the adaptation of northern trees to climate change due to recent advances in genomics, high genetic variation, and intensive studies with different abiotic and biotic stress factors. The aim of this presentation is to show the current understanding about the responses and acclimation mechanisms of birch to changing environment, based on Fennoscandian studies. Several complementary experiments in laboratory, semi-field and natural field conditions have shown that warming climate and increasing CO<sub>2</sub> is expected to increase the growth and biomass of birch, but the risk of herbivore damage will increase with negative impact on carbon sink strength. Deleterious impacts of high humidity, soil drought and increasing ozone conditions have been clearly demonstrated. All these environmental changes have led to metabolic shifts or changes in carbon/nutrient balance which may have further ecological impacts. However, high plasticity and genotypic variation predict excellent acclimation capacity of birches in rapidly changing environment and a rich genetic pool for sustainable forestry. Because the trees and forest ecosystems are exposed to multiple environmental factors simultaneously, it is necessary to continue research with multiple-stress interaction studies.

## Chloroplast acetyltransferases in regulation of photosynthesis: New players in the game

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Acetylation is one of the most common chemical modifications found on a variety of molecules ranging from metabolites to proteins. Recent development of enrichment techniques and mass spectrometry has revealed that numerous nuclear- and plastid-encoded chloroplast proteins are acetylated, including various proteins involved in photosynthetic reactions. We have characterized a chloroplasts-localized family of GNAT acetyltransferases, which possess dual protein acetylation activity, i.e. they catalyze acetylation of the N-terminal amino acid as well as acetylation of the integral lysine residues of the protein. Our results show that each GNAT enzyme has distinct specificity in terms of favored substrates, and that they play unique roles in the accumulation of specific plant metabolites, e.g. ascorbate and oxylipins. Depletion of GNAT2 has marked effects on the acetylation level of chloroplast proteins, organization of thylakoid protein complexes and photosynthetic properties of plants. Specifically, formation of the Photosystem I-LHCII complex is prevented in the *gnat2* knock-out plants, which results in impaired balancing of the light energy between the photosystems (state transitions). Moreover, loss of GNAT2 severely disturbs light-dependent dynamics of thylakoid stacking. Altogether, our results indicate that chloroplast acetyltransferases are new and important regulators of photosynthetic light harvesting with a marked impact on the growth and metabolism of plants.

Koskela et al. 2018 Plant Cell 30, 1695-1709; Koskela et al. 2020 Photosynth Res, 145, 15-30; Bienvenut et al. 2020 Mol Syst Biol 16:e9464; Rantala et al. 2022 Plant Cell Physiol 63,1205-1214; Ivanauskaite et al. 2023 Manuscript.



# Effects of poty-potexvirus synergism on growth, photosynthesis and metabolite status of *Nicotiana benthamiana*

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Poty- and potexviruses are single-stranded RNA viruses, which damage cultivated crops worldwide. Mixed infections are particularly harmful due to the phenomenon of viral synergism where one pathogen enhances the impact of another and accentuates symptom severity. While single infections by potato virus X (PVX), the type member of genus *Potexvirus*, and potato virus A (PVA), a potyvirus, can be practically symptomless, in a co-infection PVA synergistically enhances PVX.

We combined image-based phenotyping with metabolite profiling of single and mixed PVA and PVX infections to compare their effects on growth, photosynthesis and metabolites of *Nicotiana benthamiana*.

Viral synergism was evident in symptom severity and impaired growth. Indicative of stress, the co-infection increased leaf temperature. Plant vitality declined and photosynthetic performance decreased during co-infection. In contrast, singly infected plants were able to maintain photosynthetic activity.

The host's metabolic response differed significantly between single and mixed infections. Over 200 metabolites accumulated differentially in the co-infection: half of them were unique and only 12% shared with both single infections. Changes in the levels of methionine cycle intermediates and a low S-adenosylmethionine/S-adenosylhomocysteine ratio suggested a decline in the methylation potential of co-infected plants. A decreased ratio between reduced glutathione, an important scavenger of reactive oxygen species, and its oxidized form, indicated that severe oxidative stress developed during co-infection.

Based on the results, infection-associated oxidative stress is successfully controlled in the single infections but not in the synergistic co-infection where activated defense pathways are not sufficient to counter the impact of the infections on plant growth.

## Phytochemicals and pathogen resistance in kales (*Brassica oleracea* var. *acephal*)

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Glucosinolates play a central role in the defence strategy of Brassicaceae. They are responsible for the pungent or bitter taste of many species in this family, contributing to resistance against herbivores, insects, and pathogens. The challenge is to find a profile of glucosinolates adapted to plant resistance while considering the palatability of commercial crops. As a fast-growing leafy vegetable, and a close relative to the model plant *Arabidopsis thaliana*, kale (*Brassica oleracea* var. *acephal*) is a possible candidate for the application of basic research to crop plants, and to test pathogen resistance and the associated metabolic regulation in horticulture. The amount and composition of glucosinolates differs between kale varieties and can also be affected by environmental stressors. Cultivated kale varieties also differ in resistance against different type of pathogens. The differences in defence metabolite composition between wild and cultivated kale varieties during pathogen infection is yet to be further studied. In this research, we analyzed the pathogen resistance commercially available, landrace and wild varieties, which gives a spectrum of genetic and metabolic diversity. Our preliminary results have shown that the different varieties differ in resistance against grey mould (*Botrytis cinerea*) and Alternaria leaf spot (*Alternaria brassicicola*). Next these differences in pathogen resistance are associated with differences in glucosinolate composition and their associations with resistance and susceptibility phenotypes.

## Plant hormone signalling and RNA methylation in *Arabidopsis* development

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We study cell patterning events involved in plant vascular development, utilizing the *Arabidopsis* primary root as our model system. Earlier, we have shown that phytohormones cytokinin and auxin interactively specify vascular patterning during root development. To identify novel factors that may participate in the cytokinin and auxin signalling pathways, and modify vascular patterning, we perform genetic screens for misexpression of specific markers in the *Arabidopsis* primary root. One such marker for vascular patterning is *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), a spatially specific inhibitor of cytokinin signalling that is also regulated by auxin (Mähönen et al. 2006). Based on this approach, we have recently identified two loci that are involved in RNA methylation. Here, we introduce a mutant initially distinguished by an expanded *AHP6* expression domain in a cytokinin hyposensitive background. This novel mutant is also characterized by a distinct shoot developmental phenotype. We study how the RNA modification affects cytokinin responsiveness and development in the *Arabidopsis* root and shoot.

## A modern molecular biology laboratory in Jokioinen: Genome editing and beyond

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Over the last couple of years, genome editing has become a versatile tool for modification of plants with a wide range of potential applications. The development of plants with more favourable traits is important for global food security. Several gene editing methods have been developed since the 1990s, e.g. zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), but clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system has become the predominant gene editing methods for plant genomes. However, despite improvement in gene editing of plants, there are still challenges remained. Adding the advanced methods to a modern molecular biology lab is still a laborious task, and it needs a lot of effort, expertise and a well-equipped laboratory. However, the benefits of gene editing will be a faster breeding, high accuracy and efficiency, which are usually not associated with traditional breeding technologies. To master these techniques, the Jokioinen facilities are a (*well-equipped*) modern genomics laboratory with the necessary expertise to cover all gene editing workflows and even beyond. We use GBS – GenotypeBySequencing and other genotyping methods, gene-expression, genomics, cDNA by the use of modern equipment, e.g. qPCR, dPCR, Fluidigm genotyping, Illumina short read and Oxford Nanopore long read sequencers. In addition, the molecular genetics laboratory has also great expertise, experience and equipment for the successful plant transformations in house. We use barley and potato as model plants to establish CRISPR/Cas system in Jokioinen, but are not limited to only those in the future, thereby cooperation is more than welcome.

## Scots pine 4-coumarate-CoA-ligase utilizes cinnamic acid to synthesize pinosylvin

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Scots pine (*Pinus sylvestris* L.) accumulates stilbenes in the form of pinosylvin and its monomethyl ether, which are derivatives of cinnamic acid. However, the enzyme that catalyses the reaction from cinnamic acid into the intermediate cinnamoyl-CoA, the substrate of stilbene synthase, has been unknown. Plant 4-coumarate-CoA-ligases (4CLs) activate hydroxycinnamates with varying efficiencies, but are typically inactive with cinnamate – only few exceptions are known. The objective of this study is to discover such an enzyme in the Scots pine stilbene biosynthesis pathway. By using our transcriptomic data, we identified three 4CL-like genes that are co-expressed with Scots pine stilbene synthase. The enzymes encoded by these genes were produced in *Nicotiana benthamiana* leaves using *Agrobacterium* mediated gene transfer and transient expression, and subsequently purified using their N-terminal His-tags. We demonstrate that two out of the three Scots pine 4CL-like enzymes that we identified are capable of utilizing cinnamic acid as their substrate, despite still having higher preference to 4-coumaric acid.

