Forest trees under climate change – using Big data and hyperspectral imaging in genetic research

Elina Oksanen

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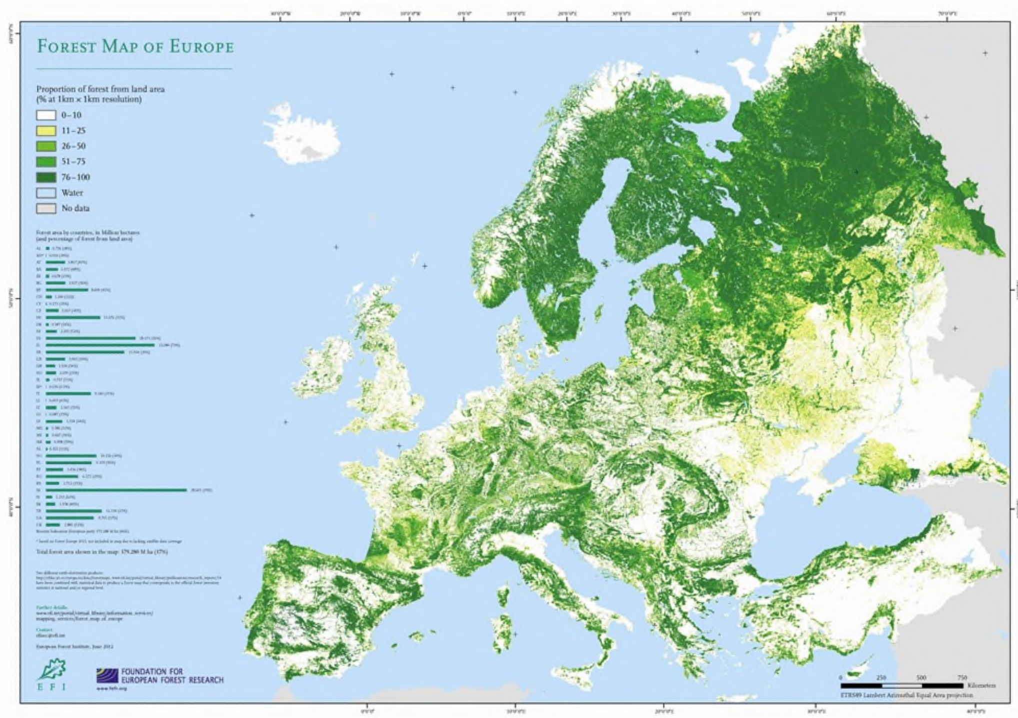

Birch as a new model system:

Past and present signatures of molecular adaptation using Big Data

Nature Genetics 49, 904–912 (2017)

nature genetics
OPEN

Genome sequencing and population genomic analyses provide insights into the adaptive landscape of silver birch

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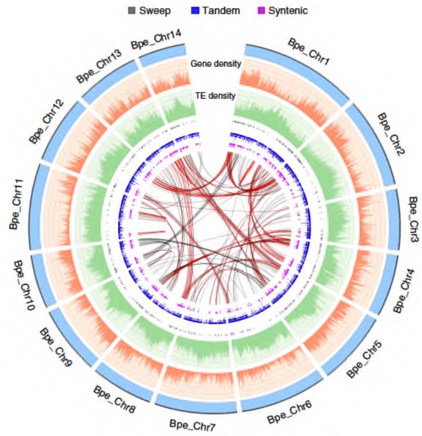
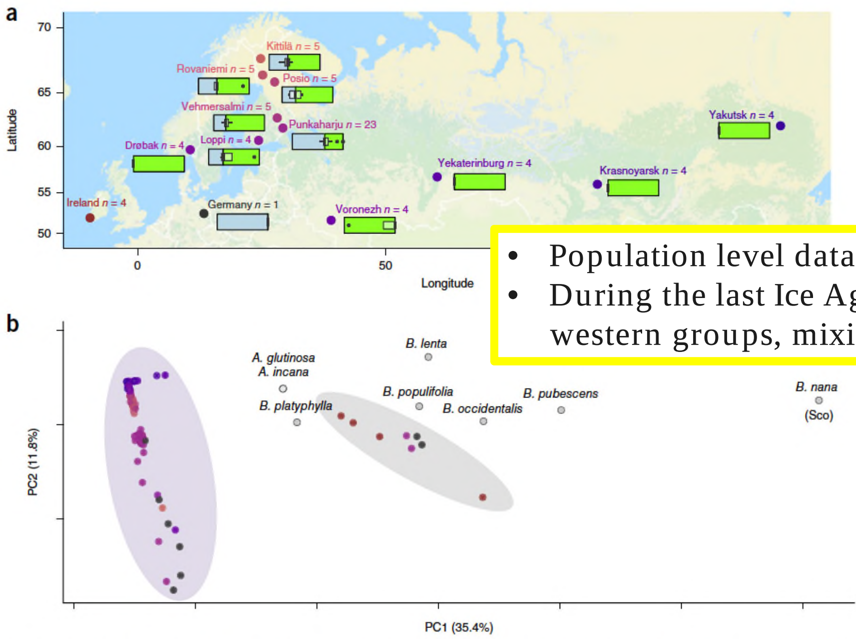




- Population level data (700 Gigabases)
- During the last Ice Age > eastern and western groups, mixing in Finland

Figure 2 Population genomics of silver birch and Betulaceae relatives. (**a**) Dispersion of 80 silver birches sampled from 12 sites across most of the geographic range of *B. pendula*. Populations are plotted with dots color-coded based on dispersion by latitude and longitude. ADMIXTURE analysis of SNP variation (superimposed with bar plots; center line, median; box, interquartile range; whiskers, 1.5× interquartile range; points, outliers) shows that Finland is a mixing zone between European (blue) and Asian (green) source populations. Samples from Ireland were highly admixed with other birch species and/or polyploid and were removed from the analysis. Source: OpenStreetMap contributors. (**b**) PCA shows clear separation between *B. pendula* populations and other sampled birch species (open circles). Eight *B. pendula* individuals (gray shading) were putative polyploids or interspecies admixed individuals. These included all Irish individuals and two individuals from Punkaharju, Finland. The main *B. pendula* population formed a cline along PC2 (purple shading). Fin: Finland, Sco: Scotland.

- Duplication of important genome parts
- Gene flow between the species still going on > Hybridizations

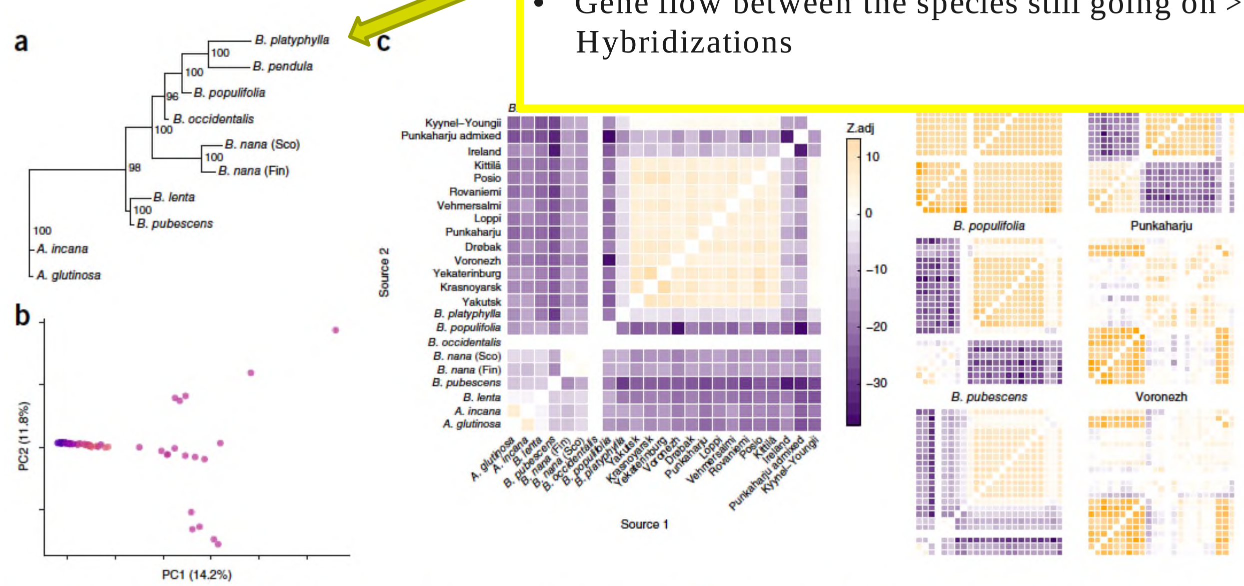
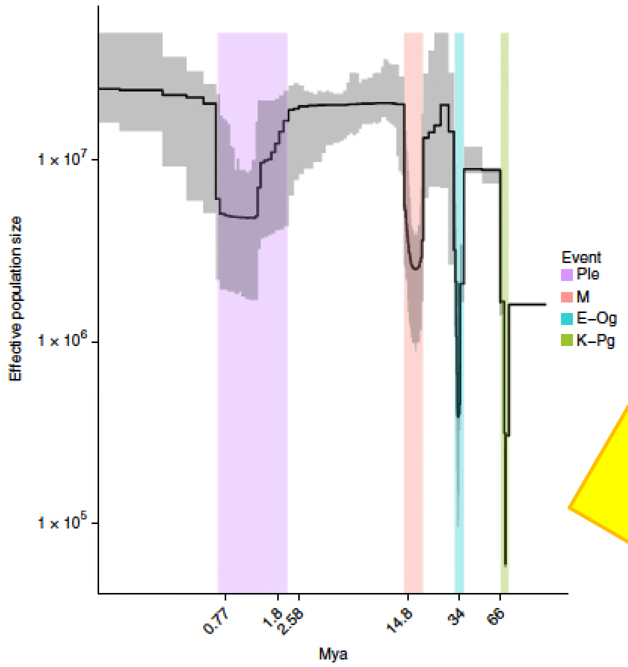


Figure 3 Phylogenetic splits and admixtures among Betulaceae. (**a**) A phylogeny for *Betula* species, estimated from SNPs in neutrally evolving sites and noncoding sites 2 kb upstream and downstream of genes. Fin, Finland; Sco, Scotland. (**b**) PCA plot of 60 *B. pendula* individuals with low levels of admixture and known location information. Individuals diverging along PC2 are all from Punkaharju, Finland, indicating possible admixture from unknown source populations. (**c**) Heat maps for three-population F_3 test statistics. Introgression is significant if the false discovery rate–adjusted *Z*-score (Z.adj; see **Supplementary Note**) is significantly negative (adjusted *Z*-score < –1.96, purple).

Deep-time evolution with population bottlenecs

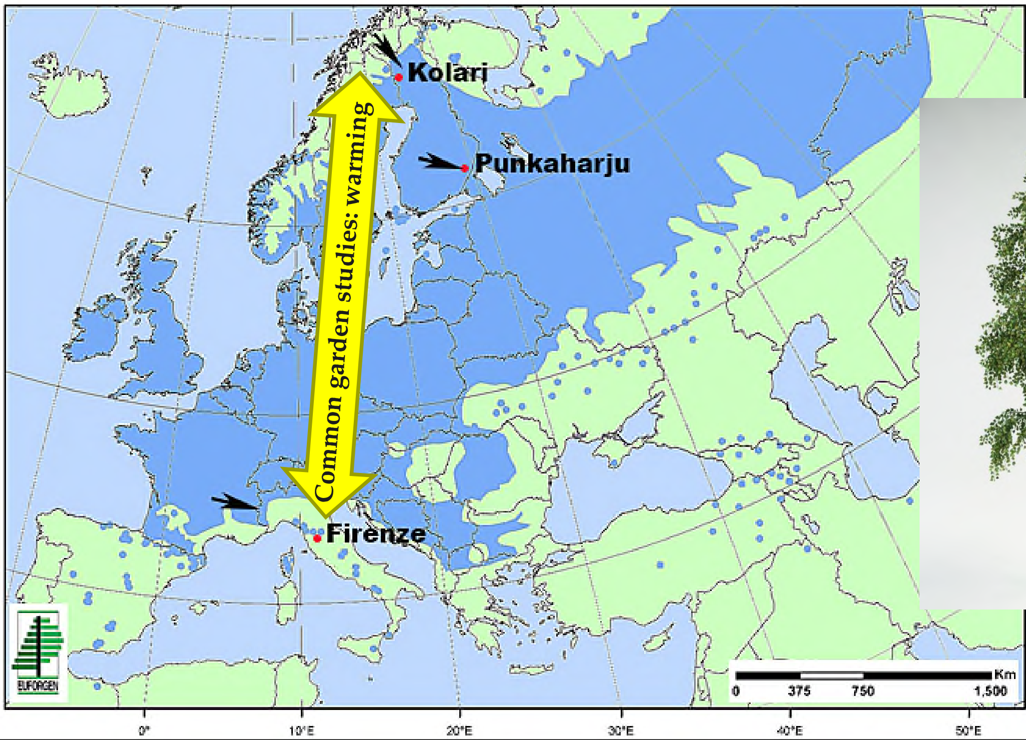


- 66, 34, 14.5 and 1 Mya
- Historical effective population size using reference genome, phylogenetic tree, mutation rate estimations and generation time

Candidate genes for development of birch phenotypes under changing environment > Breeding

- Candidate genes (900 genes) have been identified e.g. for
  - changing temperature and precipitation: cold and drought tolerance
  - light environment, photoperiodic control: growth cessation, development of cold acclimation, induction of senescence, flowering time
  - defence against fungal pathogens
  - wood formation
- Breeding can focus on these key genes when developing new birch lines for biotechnology purposes
  - further breeding is quite fast - birch can be made to flower within less than one year!

Betula pendula, silver birch: common garden experiments



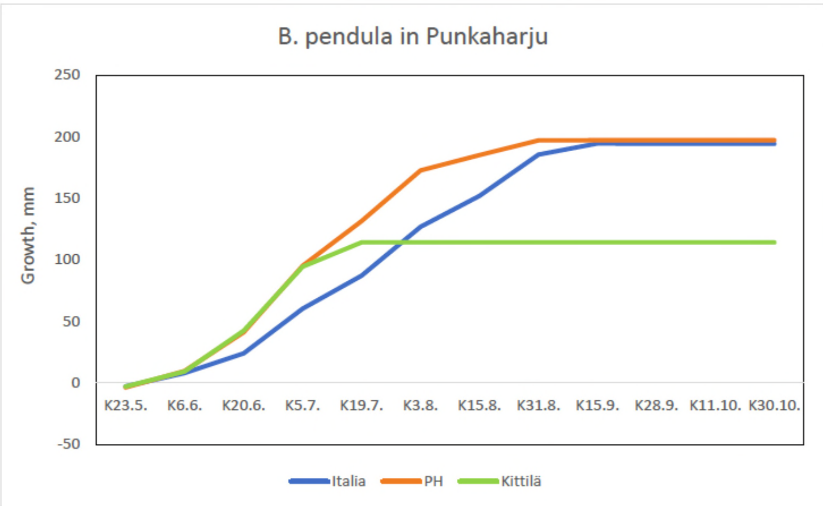
Limits for acclimation/adaptation in Italy (Firenze/Ugnano):

Growth, Chl content, herbivory and pathogen measurements

12 Nov 2018

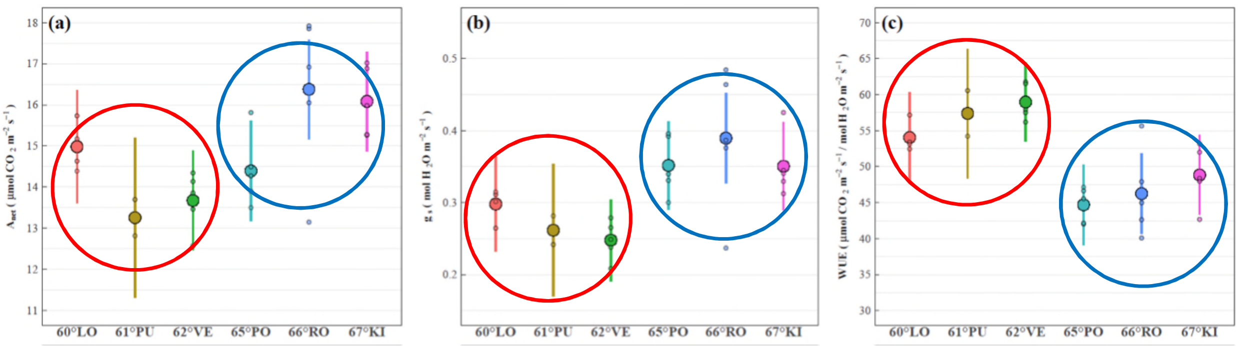
Consiglio Nazionale delle Ricerche
Istituto per la Protezione Sostenibile delle Piante

Movement of southern population to north (Punkaharju)



Luke
LUONNONVARAKESKUS

Photosynthesis Stomatal conductance Water use efficiency

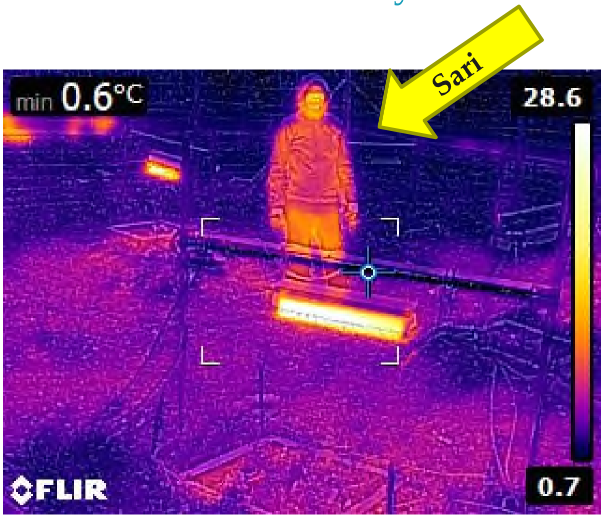


Monitoring the effects of warming on a subarctic treeline ecosystem

Figure 1. The warming experiment will be established near the Kevo Subarctic Research Station, located in Utsjoki at the northernmost tip of Finland (69°45' N, 27°01' E) right next to the Kevo Strict Nature Reserve.

- A heating system in Kevo

Monitoring the effects of warming on a subarctic treeline ecosystem in Kevo

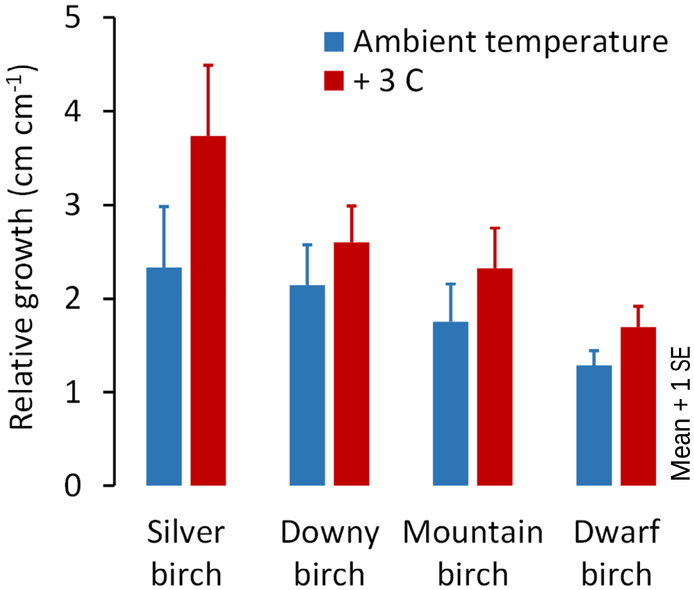


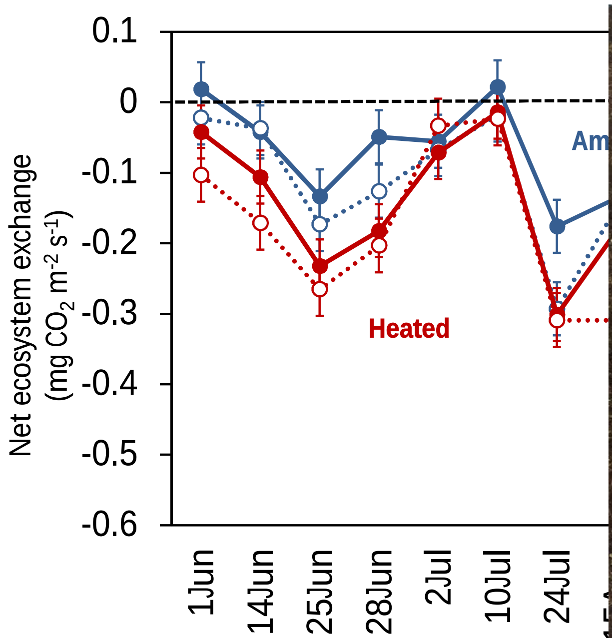
- Impact of warming (infra-red heaters) on plant growth, competition, nitrogen cycling and herbivory with four native Betula species (*Betula pendula*, *B. pubescens* (including the northern sp. *czerepanovii*) and *B. nana)*

Kevo warming experiment

- twenty field plots, with a **2×2 factorial set-up** consisting of warming and herbivory treatments (n = 5)
  - plots with **ambient vs. + 3 °C "leaf" temperature** (infrared heaters)
  - plots with **normal vs. reduced insect herbivory** (weekly insecticide sprayings)
- **cloned offspring of northern *Betula* populations** planted on each plot

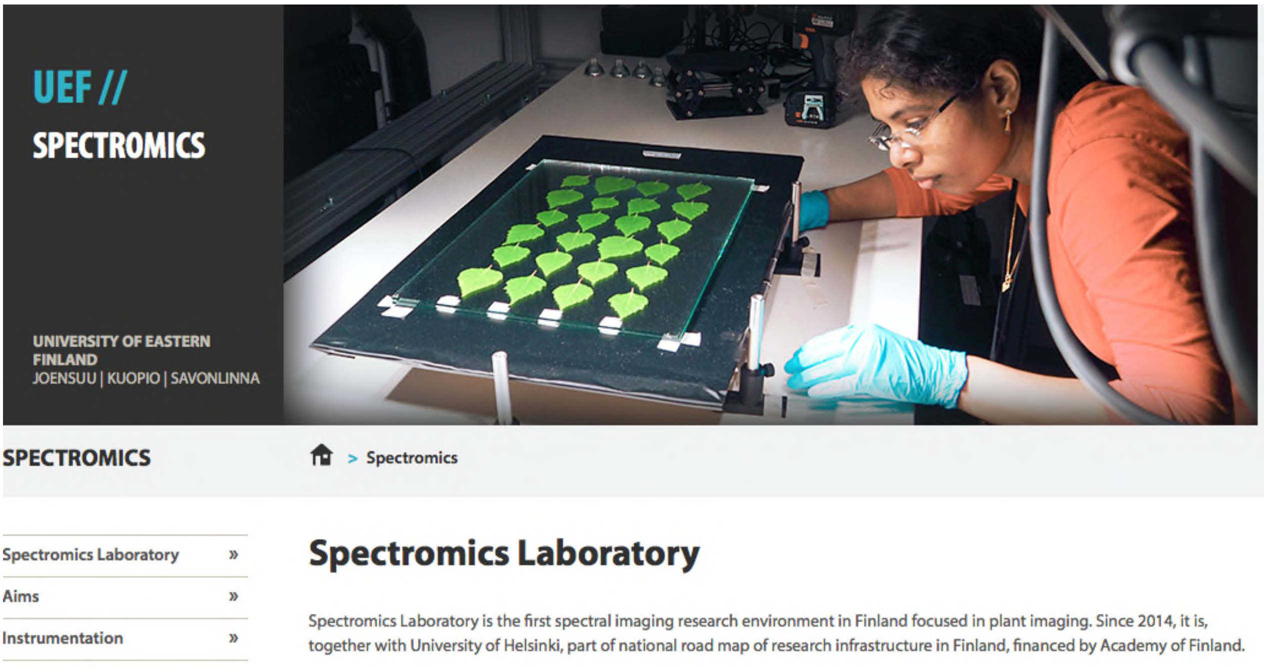
In field plots, all *Betula* species had positive responses to warming, with **growth rates increasing by 20–60%.**





Insect herbivory decreased ecosystem CO

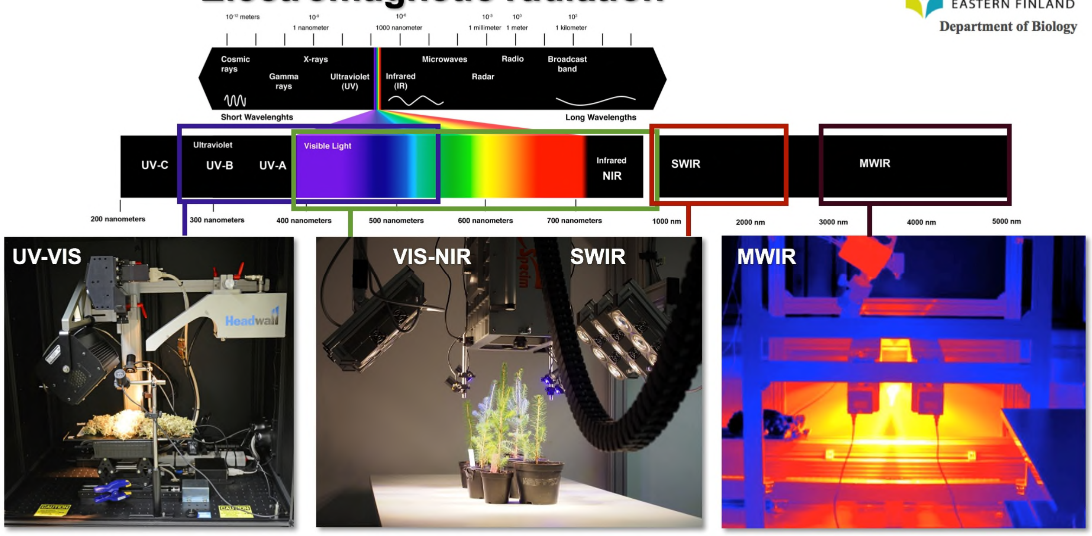
Hyperspectral imaging techniques
http://www.uef.fi/en/web/spectromics/



Hyperspectral imaging - spectromics

UNIVERSITY OF EASTERN FINLAND
Department of Biology

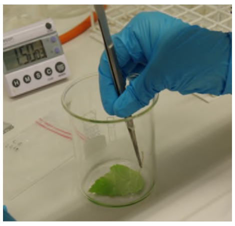
Electromagnetic radiation

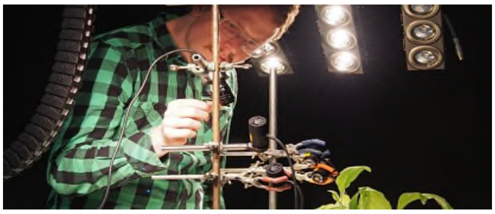


Imaging spectrographs of the UEF Spectromics Lab (www.spectromics.org)

Fluorescent stress compounds | Chl fluorescence | Water, nutrient status

Plant biology, field and laboratory experiments, environmental research

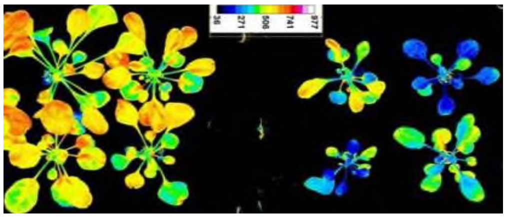




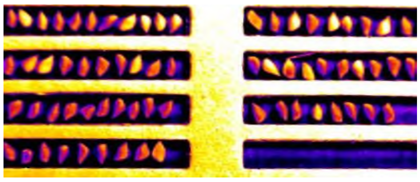
Plant stress treatments, acclimation to environmental change

Imaging applications

Mapping photosynthesis

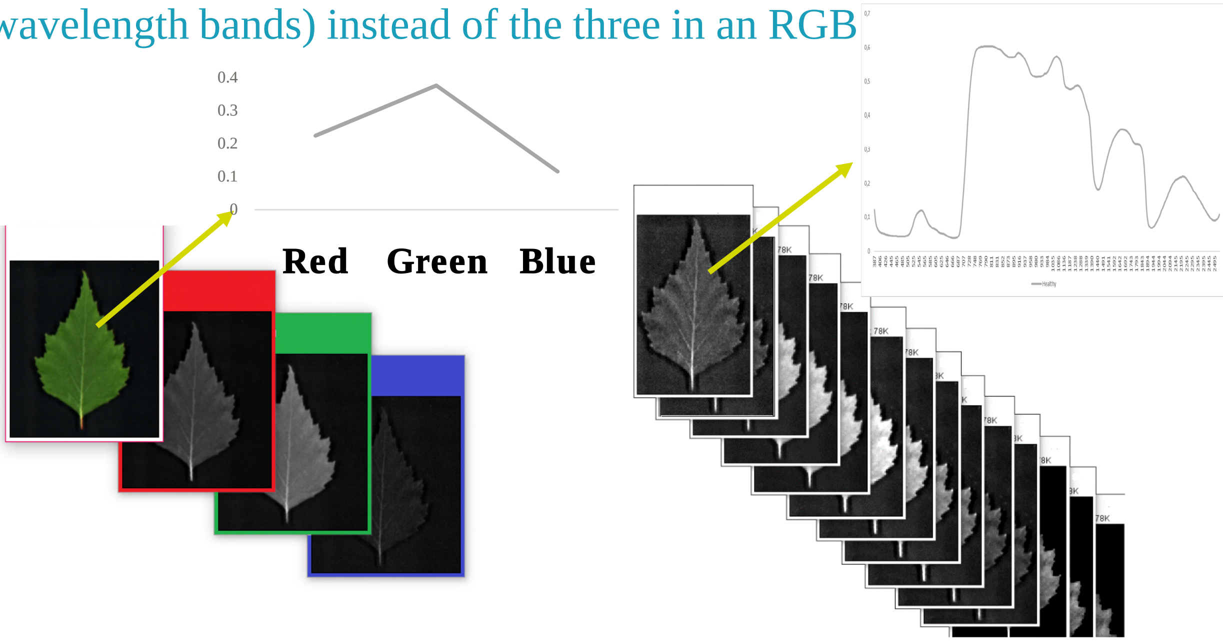


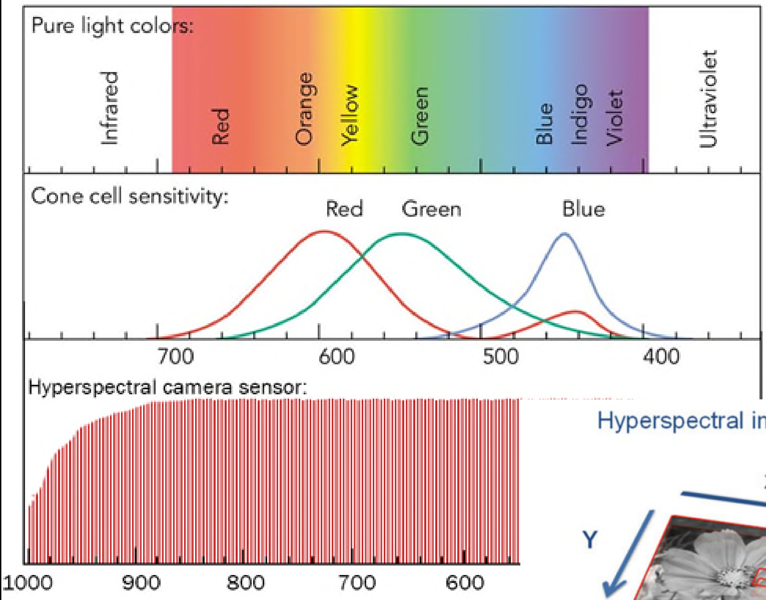
Heavy metal (Cd) accumulation, plant chemistry

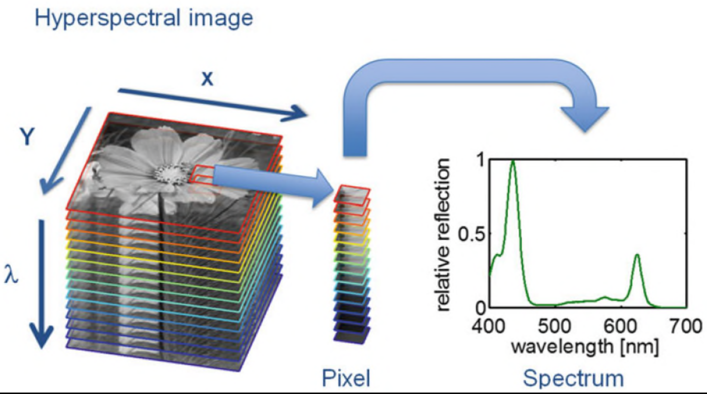


Seed quality screening

With spectral imaging, one can see hundreds of colours (different wavelength bands) instead of the three in an RGB



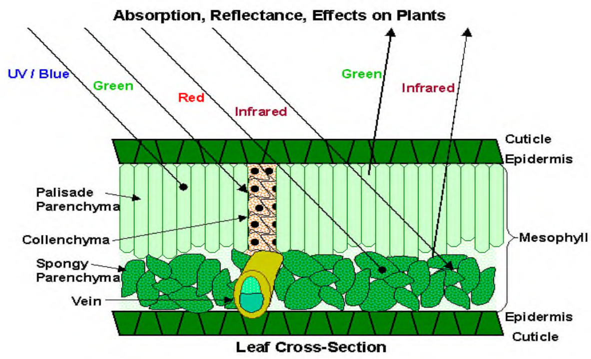




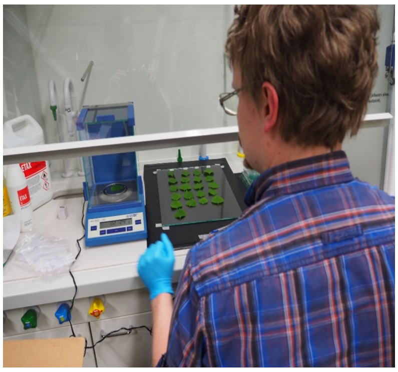
Each plant possesses its own spectral signature, which depends on the internal scattering of light and its chemical composition (such as chlorophyll, carotenoids, water, cellulose, proteins)

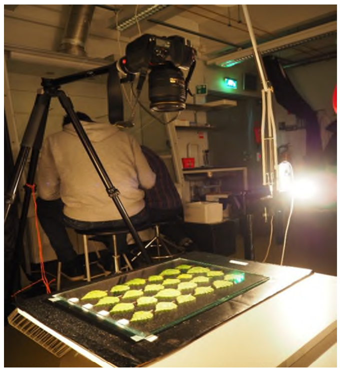
Table 1. Absorption features related to particular plant compounds (compiled from Elvidge, 1990).

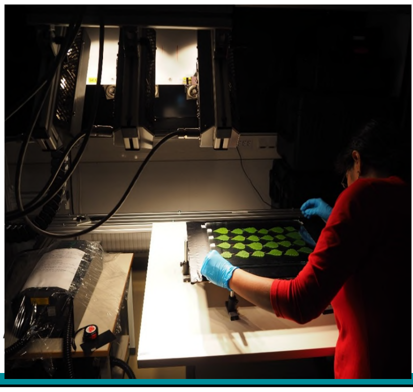
Wavelength (nm)	Absorbing compounds	Absorption mechanism
430	Chlorophyll a	Electron transition
460	Chlorophyll b	Electron transition
640	Chlorophyll b	Electron transition
660	Chlorophyll a	Electron transition
910	Protein	C-H Stretch, 3rd overtone
930	Oil	C-H stretch, 3rd overtone
970	Water, starch	O-H bend, 1st overtone
990	Starch	O-H stretch, 2nd overtone
1020	Protein	N-H stretch
1040	Oil	C-H stretch, C-H deformatio
1120	Lignin	C-H stretch, 2nd overtone
1200	Water, cellulose, starch, lignin	O-H bend, 1st overtone
1400	Water	O-H bend, 1st overtone
1420	Lignin	C-H stretch, C-H deformatio
1450	Starch, sugar, water, lignin	O-H stretch, 1st overtone, C-
1490	Cellulose, sugar	O-H stretch, 1st overtone
1510	Protein, Nitrogen	N-H stretch, 1st overtone
1530	Starch	O-H stretch, 1st overtone
1540	Starch, cellulose	O-H stretch, 1st overtone
1580	Starch, sugar	O-H stretch, 1st overtone
1690	Lignin, starch, protein	C-H stretch, 1st overtone
1730	Protein	C-H stretch
1736	Cellulose	O-H stretch
1780	Cellulose, sugar, starch	C-H stretch, 1st overtone, O-H stretch, H-O-H deformation
1820	Cellulose	O-H stretch, C-O stretch

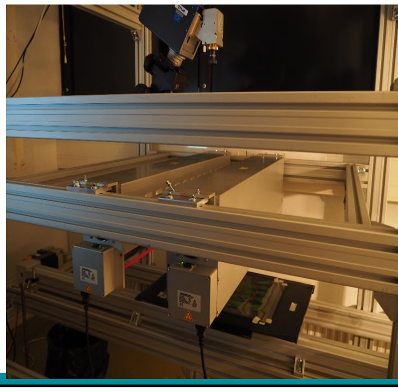


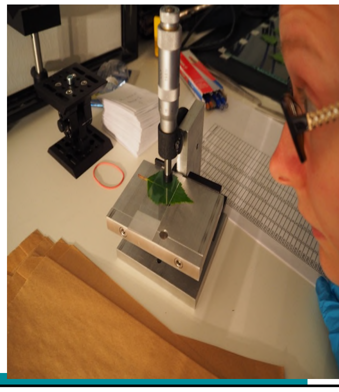
J. Agr. Sci. Tech. (2012) Vol. 14: 135-147











Combining spectral and chemical data:

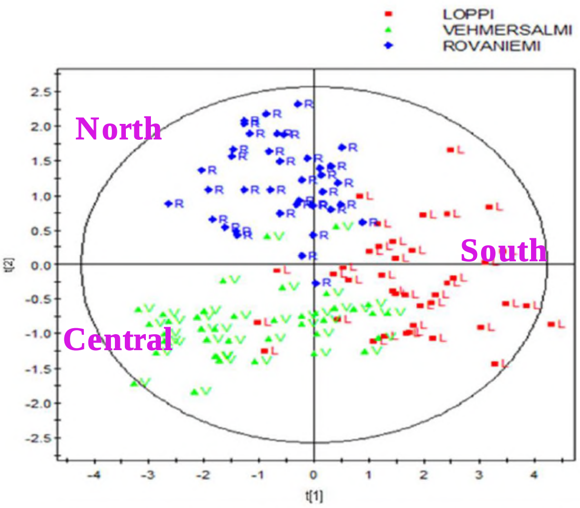
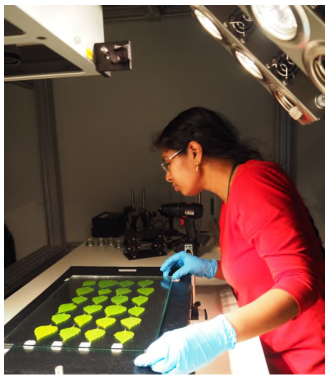


Figure 24. Score plot for OPLS DA of different provenances indicating the significant difference. Analysed with SIMCA-t[1] = 0.139628 and t [2] = 0.0518827

Spectral signatures- identification of different birch genotypes according to leaf chemistry or other leaf traits (thickness, water content etc)



Developing method for seed quality screening

- Availability of high quality of seeds is essential for forestry, agriculture and horticulture
- High quality seeds
  - lower nursery costs
  - improve plant quality
  - increase success in forest regeneration methods

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Thermal and hyperspectral imaging for Norway spruce (*Picea abies*) seeds screening

CrossMark

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Materials

Norway spruce seeds (*Picea abies*) divided in three classes based on X-ray image:

I. Filled/viable seeds
II. Empty seeds
III. Infected seeds with larvae (*Megastigmus sp.*)

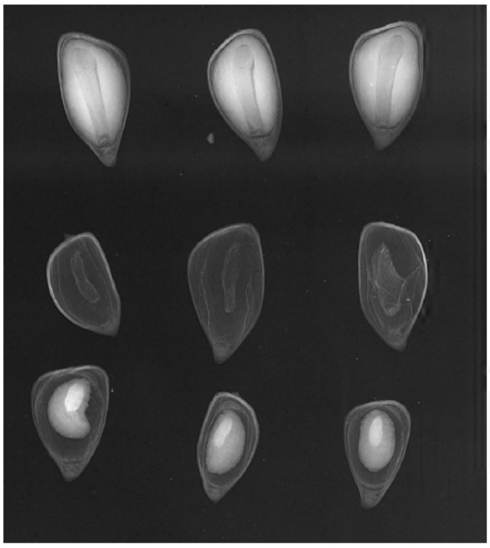
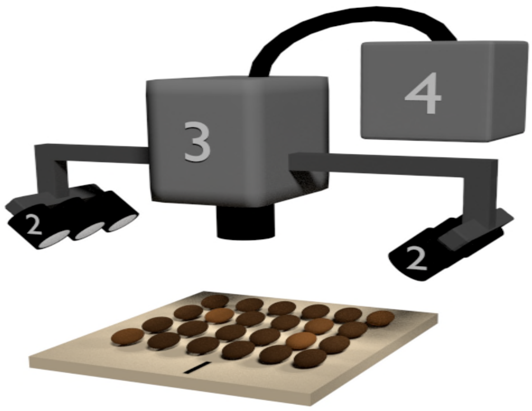
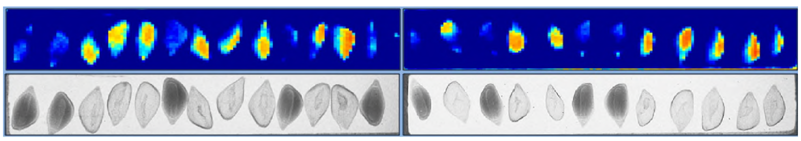


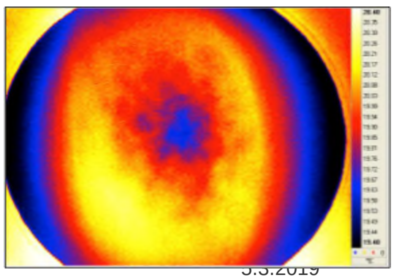
Fig. 1. X-ray images of filled/viable seeds (first row), empty seeds (second row) and infested seeds (third row). Photo copyright: Natural Resources Institute Finland/Seed Laboratory.

Thermal imaging

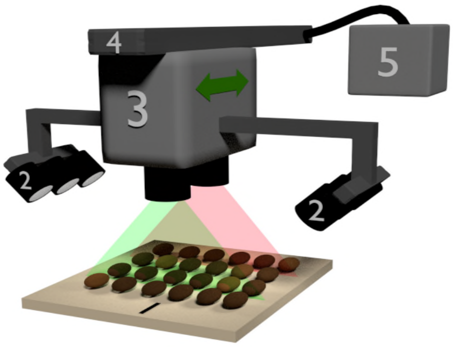


- Dark room conditions
- 3s light pulse with two 150W halogen lamps
- Thermal decay with FLIR SC7600 (3000 – 5000 nm)
- 200 seeds at the same time – fast technique





Hyperspectral imaging



- Pushbroom system designed by Specim
- VNIR camera combined with imaging spectrograph covering 400 – 1000 nm
- SWIR camera with imaging spectrograph covering 1000 – 2500 nm
- Six 35W halogen lamps for illumination

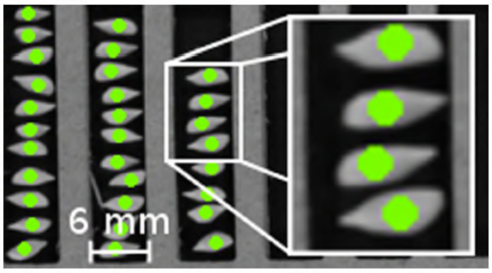


Fig. 4. Illustration of data selection of seeds where spectral data inside green circle is selected for further analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

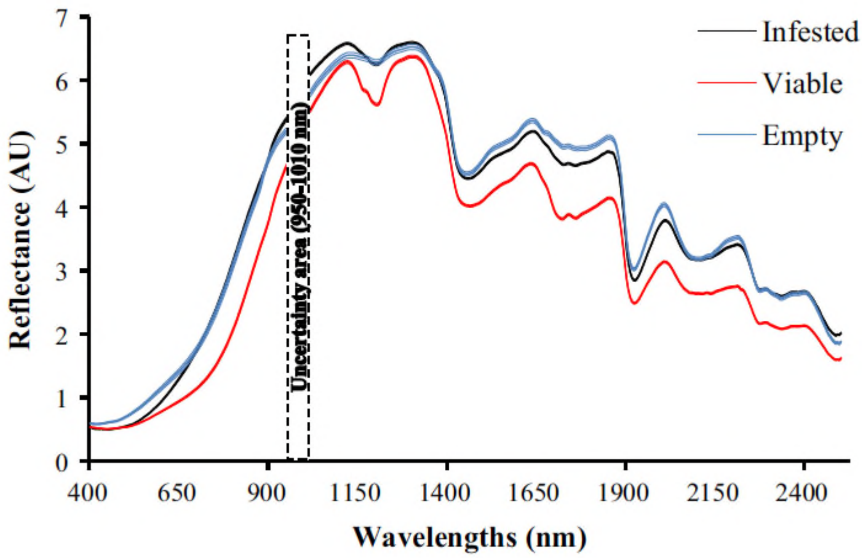


Fig. 6. The average reflectance spectra of all the seeds within each of the three classes of seeds ± SE (viable in red, empty in blue, infested in black) in the spectral range of 400–2500 nm. The uncertainty area is due to the reduced sensitivity at the extremity of the spectral range of the cameras. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Outcome

- Thermal measurements: It is possible to separate the three classes with 99% accuracy (using 30 features)
  - o but challenging for high-throughput production line
- Hyperspectral imaging: The maximum reflectance at 1310 nm, 1710 nm and 1985 nm (SWIR), with 94% classification accuracy
  - o more convenient for industrial production line (a conveyor belt)
- Automatic seed detection and feature extraction would be necessary
- Differences between the seed classes due to composition of the endosperm (content of oils, proteins, carbohydrates and water)
  - 1710 nm band: methyl/methylene groups, associated with fatty acids, chitin
  - 1985 nm band: proteins, asymmetric combination of N-H, influenced by water

Other potential applications

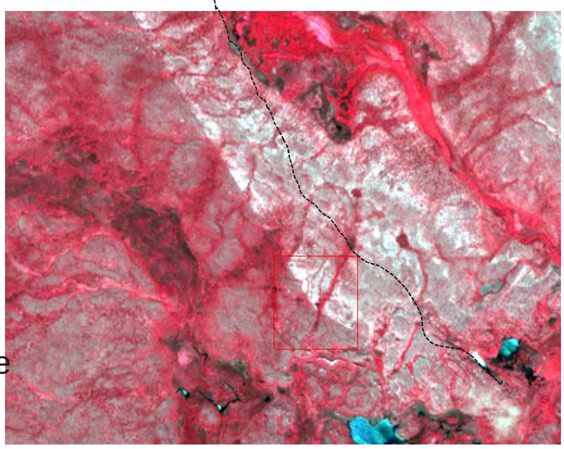
- Agriculture: Nutrient imbalance, fertilization maps, precision farming
- Early detection of pathogen/pest infections and damage *in situ*
- Identification of species (e.g. lichens) – environmental monitoring, maintanance of biodiversity
- Phytoremediation
- More specific phenotyping > Plant breeding
- Biomedical applications: dental diseases, cancer tissue etc.

Environmental monitoring

winter pasture with lichen

Finland

summer pasture without lichen



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Imaging lichen water content with visible to mid-wave infrared (400–5500 nm) spectroscopy

Check for updates

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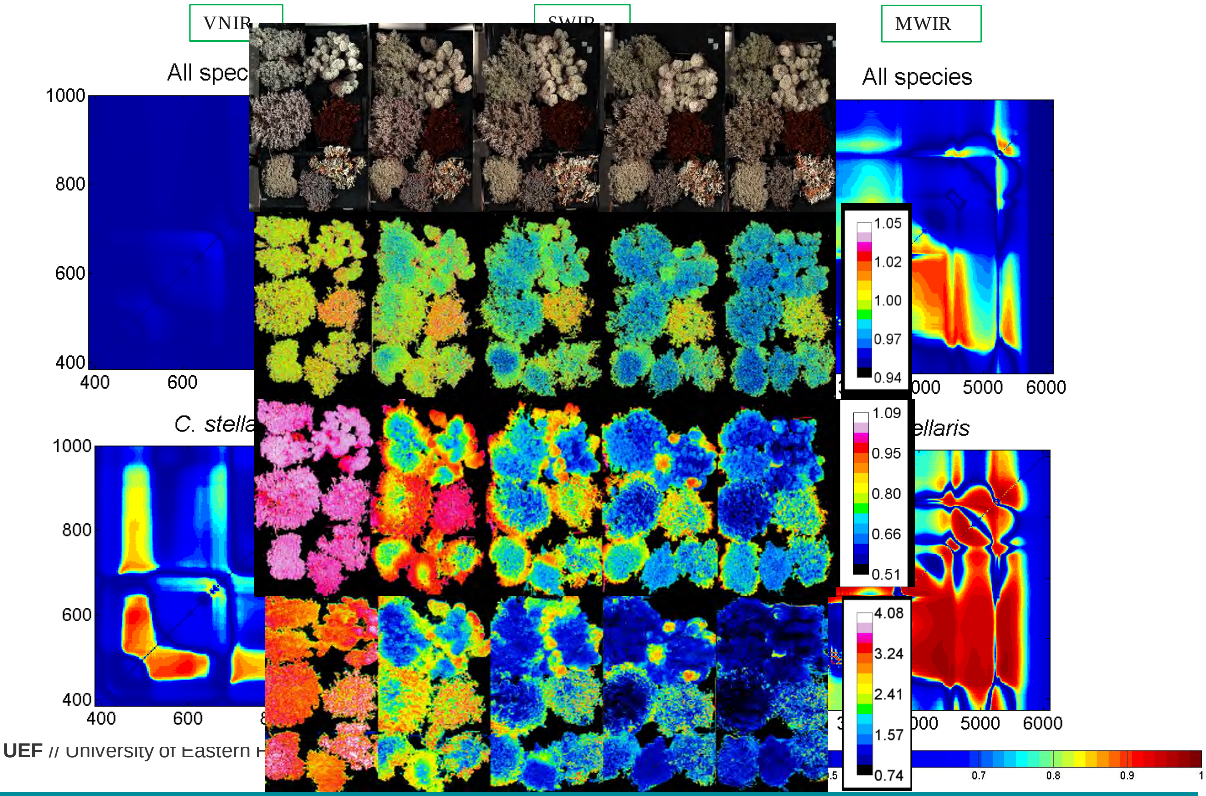


Separation of lichen species with HSI cameras

- *Cladonia arbuscula* (Valkoporonjäkälä)
- *Cladonia rangiferina* (Harmaaporonjäkälä)
- *Cladonia unicialis* (Okatorvijäkälä)
- *Cladonia stellaris* (Palleroporonjäkälä)
- *Cetraria islandica* (Isohirvenjäkälä)
- *Stereocaulon sp.* (Tinajäkälä)
- *Flavocetraria nivalis* (Lapalumijäkälä)

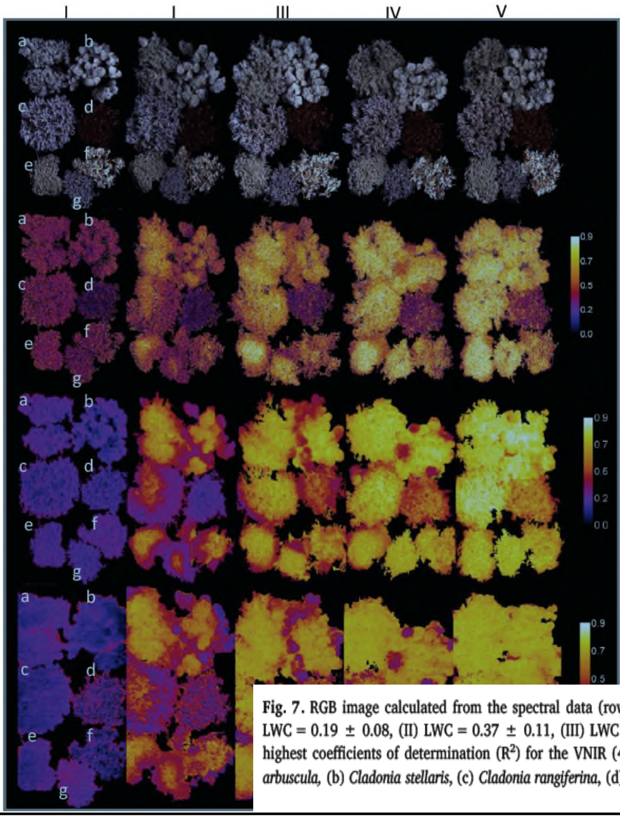
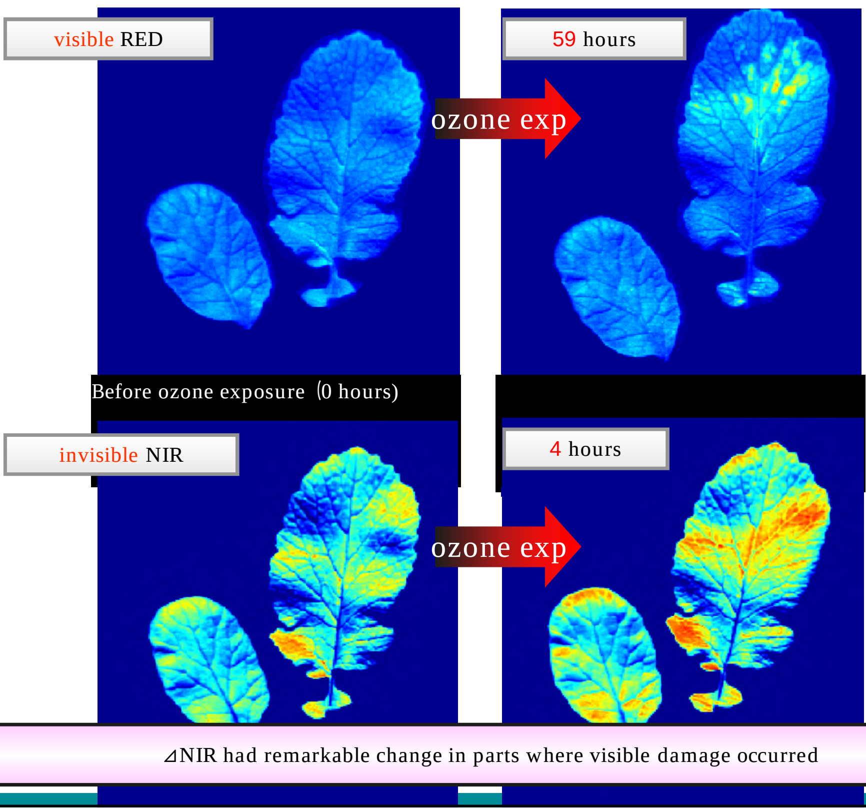


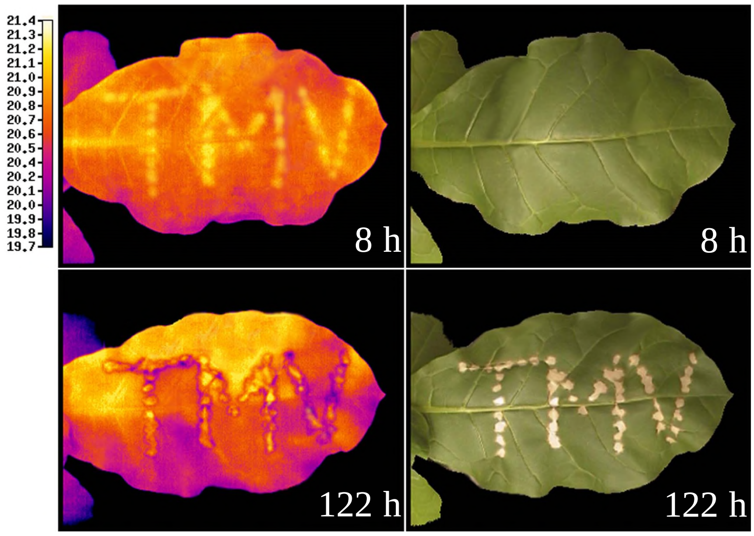
Fig. 7. RGB image calculated from the spectral data (row 1) and false colour heat maps of a representative sample of the ascending water content series. (I) LWC = 0.19 ± 0.08, (II) LWC = 0.37 ± 0.11, (III) LWC = 0.57 ± 0.08, (IV) LWC = 0.66 ± 0.06, (V) LWC 0.7 ± 0.05, calculated from the SR indices with highest coefficients of determination (R^2) for the VNIR (400–1000 nm, row 2), SWIR (1000–2500 nm, row 3) and MWIR (2500–5500 nm, row 4). (a) *Cladonia arbuscula*, (b) *Cladonia stellaris*, (c) *Cladonia rangiferina*, (d) *Cetraria islandica*, (e) *Cladonia uncialis*, (f) *Flavocetraria nivalis* and (g) *Stereocaulon* sp.



UEF /

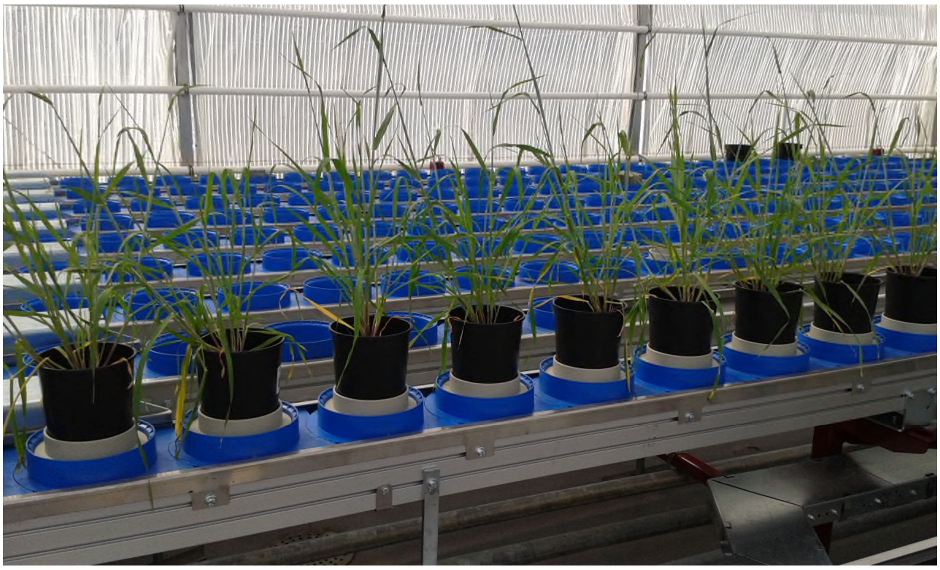
⊿NIR had remarkable change in parts where visible damage occurred

Thermal imaging for early detection of TMV infection in tobacco



- The plant was infected and kept at 32°C for 29 hours to allow the spread of TMV
- The plant was then shifted back to 21°C
- Two and a half hours after the temperature shift a thermal effect emerged.

Plant phenotyping (NaPPI project, Univ Helsinki)



http://blogs.helsinki.fi/nappi-blog/