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BACKGROUND

- **Germination is a fundamental trait both for seeds and for malting barley**

- **The traditional testing of germination ability is not always appropriate:**
  - Manual methods, often sent to a centralized laboratory, considered too costly and time consuming:
    - Repeated measurements may be necessary
    - Time often crucial factor

- **Quick methods, without claim on official status are used in practice:**
  - Mycotoxins are analysed using immunological methods.
  - Methods for seed purity are available, ex. www.cgrain.se, www.videometer.dk

INTRODUCTION

The project was financed by Swedish farmers' foundation for agricultural research 2016-2019 and focused on comparing different available quick methods that are candidates for being efficient for making quick and accurate enough estimation of germination ability:

- The VideometerLab 3
- Isothermal calorimetry
- GERMINATOR

Why chosen? Methods been reported to be successful in monitoring germination in seeds.

- Boelt, B., Shrestha, S., Salimi, Z., Jørgensen, J. R., Nicolaisen, M., & Carstensen, J. M. (2018). Multispectral imaging–a new tool in seed quality assessment?. *Seed Science Research*, *28*(3), 222-228.
- Calvet, E. and H. Prat (1963). Recent progress in microcalorimetry. Oxford, Pergamon Press.
- Joosen, R.V.L., Kodde, J., Willems, L.A., Ligterink, W., van der Plas, L.H. & Hillhorst, H.W.M. 2010. Germinator: A software package for high through-put scoring and curve fitting of *Arabidopsis* seed germination. Plant J. 62:148-159.

BARLEY GERMINATION

- Most important quality parameter on malting barley
- Checked on all intakes
- Long analysis time (3+2 days) gives slow reaction when outside specification
- Two different methods (EBC 3.5.2 & 3.6.1*) used depending on dormancy or not
- Results used both for quality assurance and depreciation
- Both methods depend on manually counting

* EBC = European Brewers Convention

VIKING MALT

SAMPLES

Samples from 2015:

- barley samples were divided using sample divider and sent for quick analyses and for reference analyses. Samples were natural samples supplied by The Swedish Board of Agriculture and Eurofins.

Samples from 2016:

- 40 wheat, oats and barley samples as above.

Samples from 2017:

- wheat and barley sample was artificially aged* at 60° C for 30, 60, 90 and 120 min.

* Møller, B., & Munck, L. (2002). Seed vigour in relation to heat sensitivity and heat resistance in barley evaluated by multivariate data analysis. *Journal of the Institute of Brewing, 108*(3), 286-293.

VIDEOMETER

- Mimic human vision

- Eliminate subjective assessment

- Non-homogeneous samples

- Focusing on certain areas of a sample

- When the shape, size and texture of the object is of special interest

- Documentation

- Twenty cereal seeds were placed in a petri dish on top of a blue filter paper and multispectral images were captured at intervals of 24 hours.
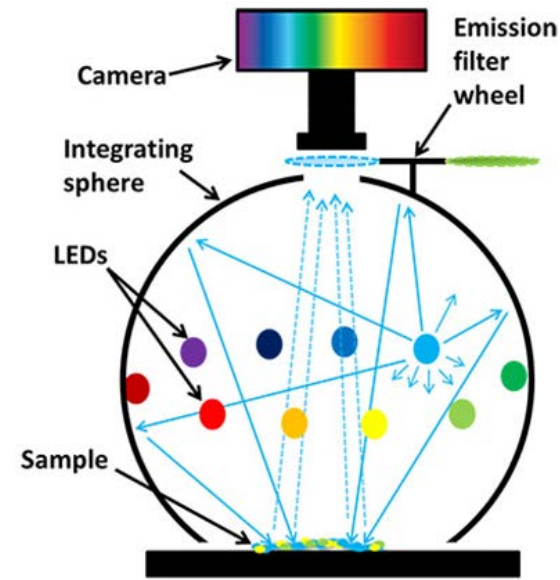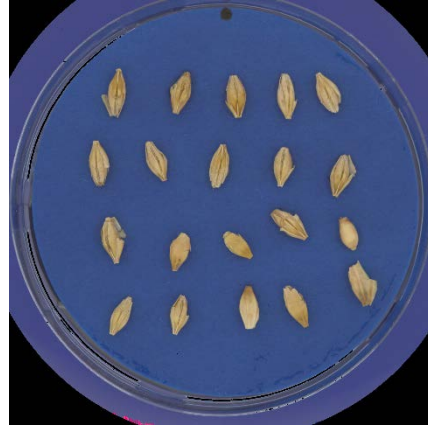
VideometerLab

- Up to 20 light emitting diodes in the range from UV, VIS to NIR acquired sequentially in 0.5-1.5 seconds depending on camera

- Reflected light is captured in raw images

- Information from each pixel, exposed to each wavelength is stored in data files

- Transformed images provide information about surface characters

VIDEOMETERLAB IMAGES OF GERMINATING BARLEY

	0 HR	24 HR	48 HR	76 HR

Germination set-up
20^0 C at 24hr dark /
Top of paper method
In rows and random

Images captured
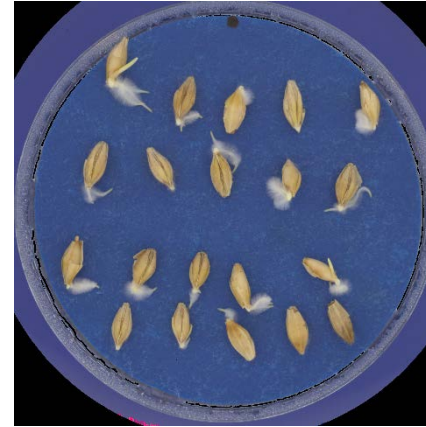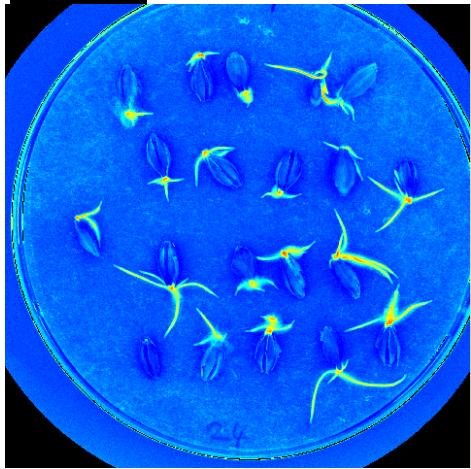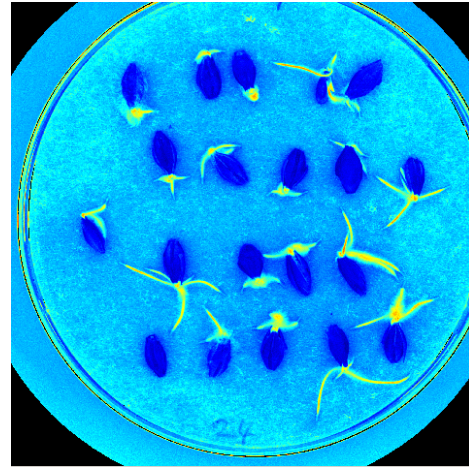At 24 hr interval
0 hr to 100 hr
By Videometerlab

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DANSEED SYMPOSIUM 2020 | JOHANNES RAVN JØRGENSEN
10 MARCH 2020 | ASSOCIATE PROFESSOR

SOLIDUM PETIT IN PROFUNDIS · UNIVERSITAS ARHUSIENSIS
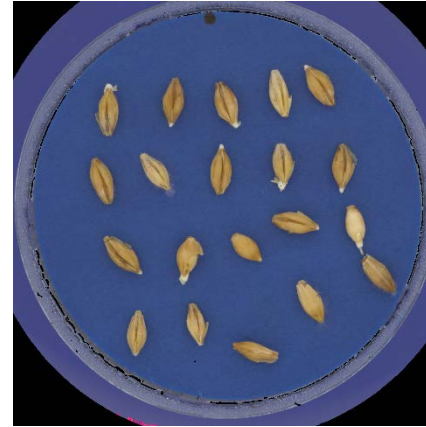
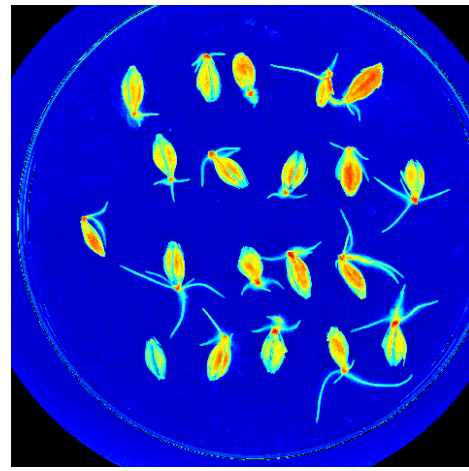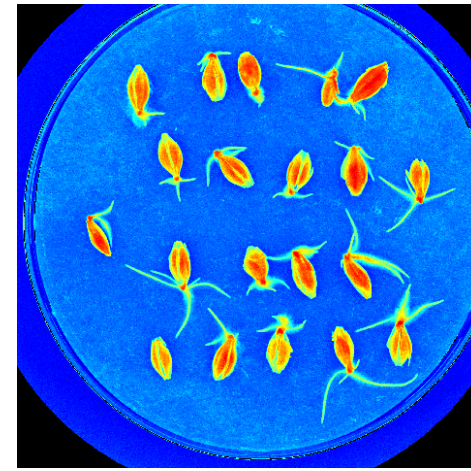# MULTISPECTRAL IMAGES AT 48 HR GERMINATION

375 nm UVA

405 nm violet
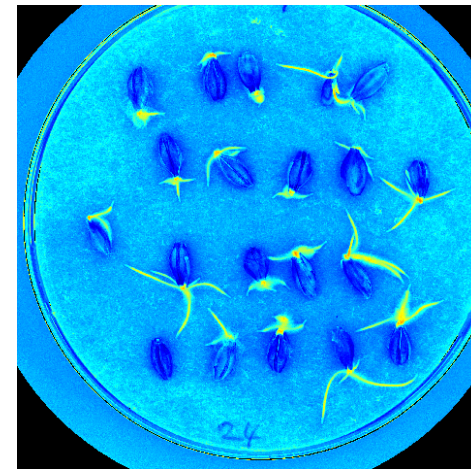
470 nm blue
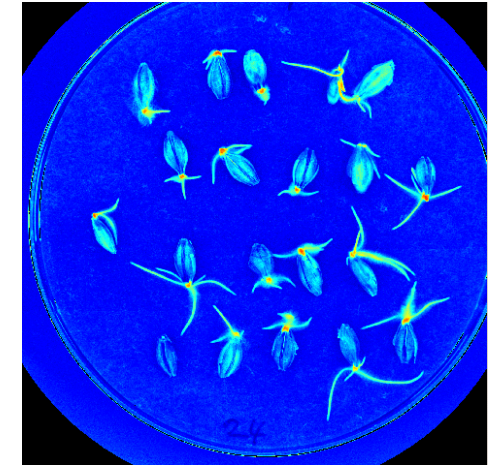
525 nm green
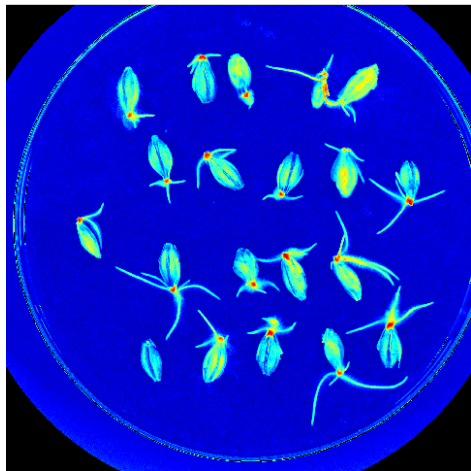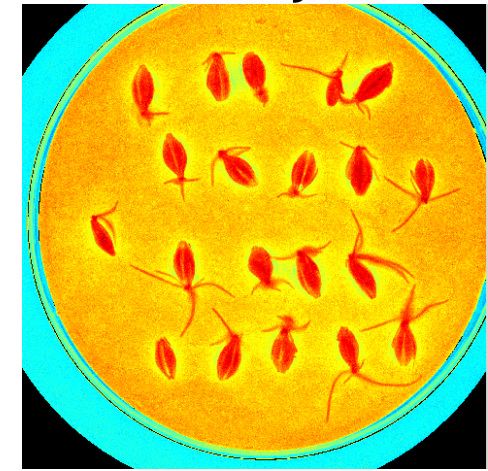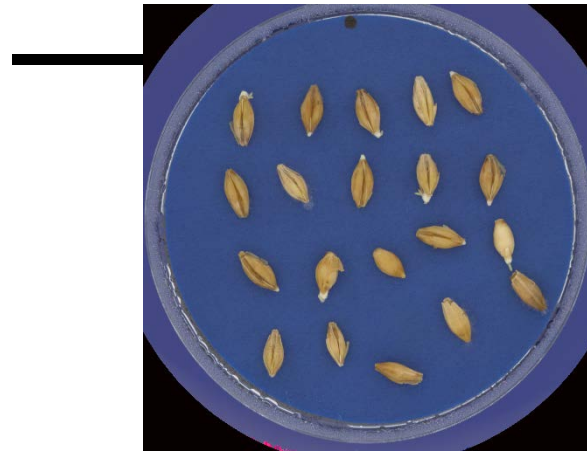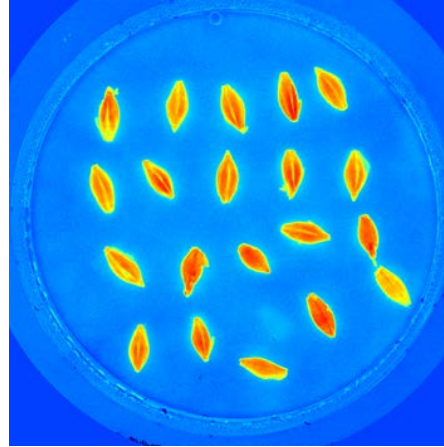
570 nm yellow

700 nm red

780 nm NIR

890 nm NIR

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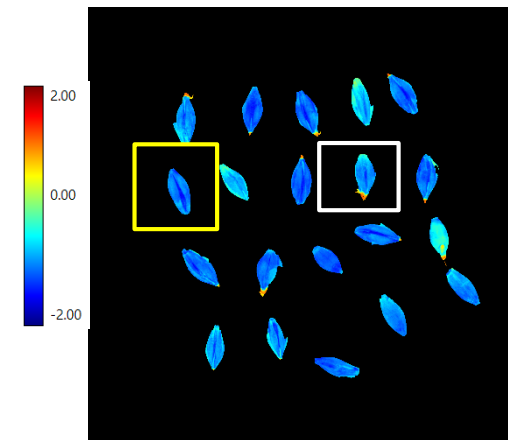ASSOCIATE PROFESSOR

VIDEOMETER SEGMENTATION AND CLASSIFICATION OF BARLEY SEEDS

Raw multispectral image

nCDA transformation
Foreground vs. Background

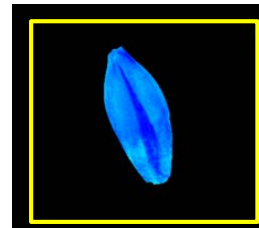Segmented multispectral image
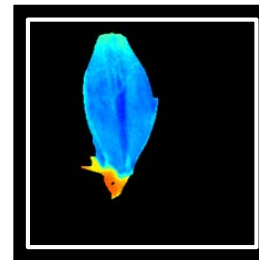
Germinated

Classification
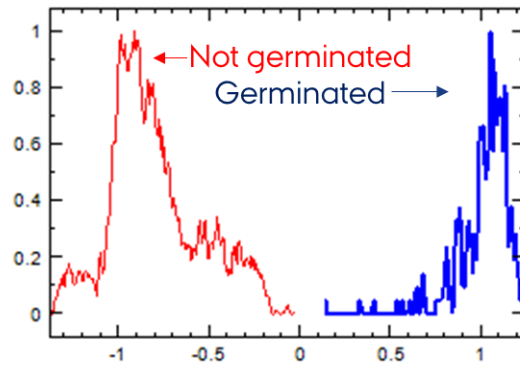
Not Germinated

Germinated if
RegionMSI Threshold -> 0.5

nCDA Transformed Image

nCDA: Normalized Canonical
Discriminant Analysis

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GERMINATION SCORING- nCDA Model (Barley)

Blob database- Collection of each seed

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Detection on germination of aged barley seed by Videometerlab (cv. Pepino and Quench), %

Legend:
- Pepino 0
- Pepino 30
- Pepino 60
- Pepino 90
- Pepino 120
- Quench 0
- Quench 30
- Quench 60

X-axis: Hours (0, 20, 40, 60, 80)
Y-axis: 0, 20, 40, 60, 80, 100
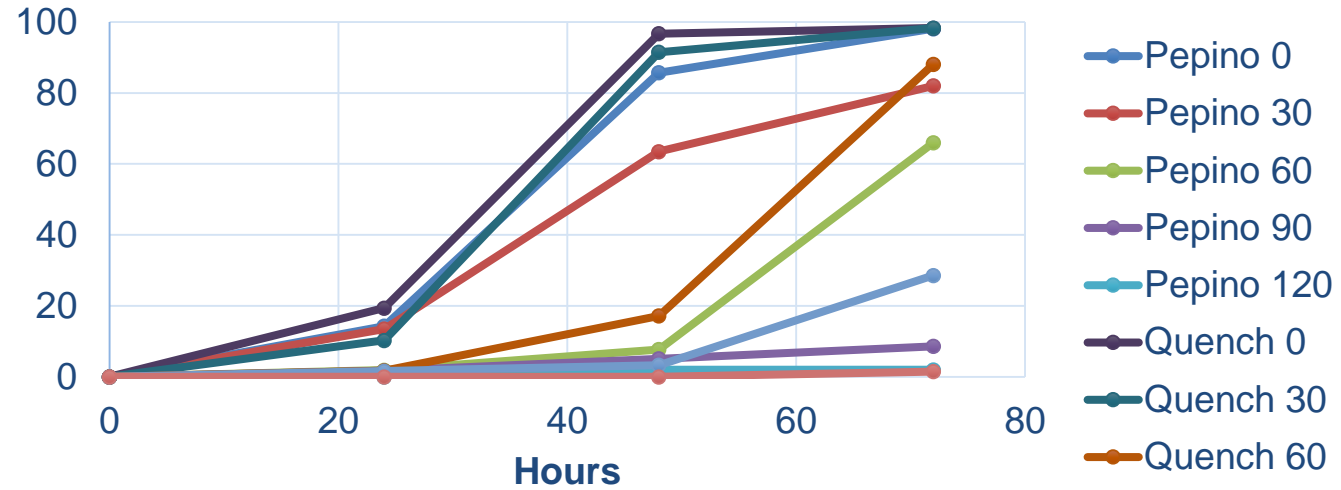
Raw multispectral image

Germinated vs ungerminated seed
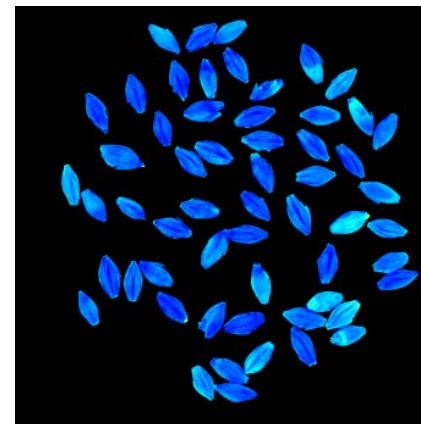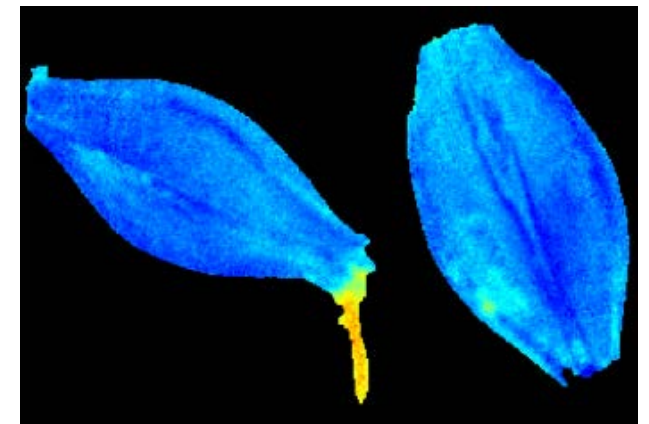
nCDA Transformed Image

Germinated vs ungerminated seed

GERMINATOR

- The GERMINATOR package contains three modules:
- (i) design of experimental setup with various options to replicate and randomize samples;
- (ii) automatic scoring of germination based on the color contrast between the protruding radicle and seed coat on a single image; and
- (iii) curve fitting of cumulative germination data and the extraction, recap and visualization of the various germination parameters.
- Samples were photographed with a RGB.
- 20 cereal seeds were incubated on moistened blue filter paper in transparent rectangular incubation trays and at intervals of 12 hours the trays were photographed.
- Using visual scripting, germinated and non- germinated seeds could be distinguished.
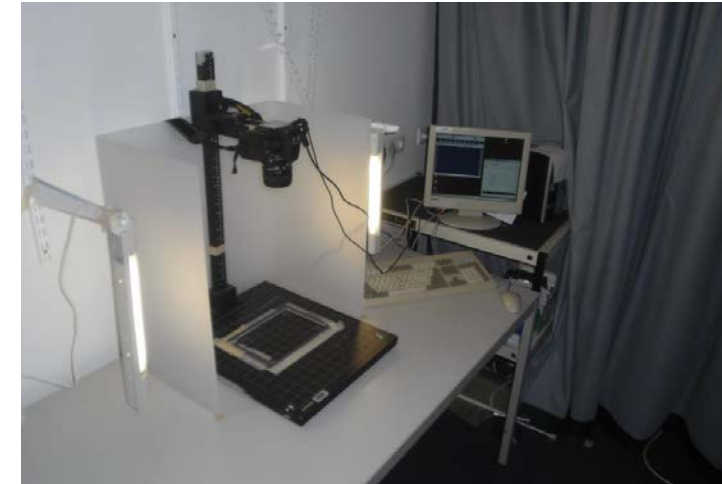
RESULTS GERMINATOR

Original image
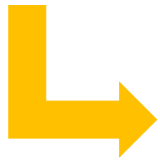
Processed image – seed coats, radicles and acrospires selected

Processed image – only seed coats selected

Sample 18: Low germination capacity, sample 21: High germination capacity
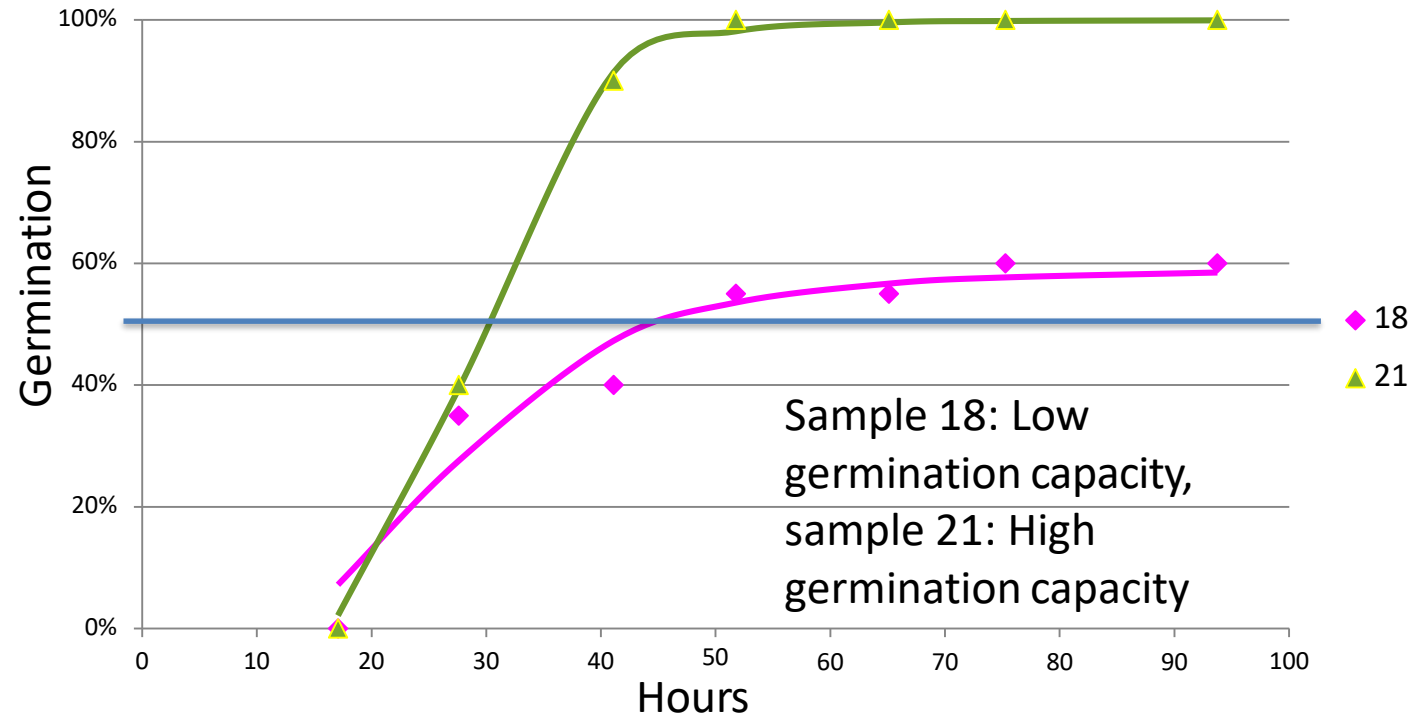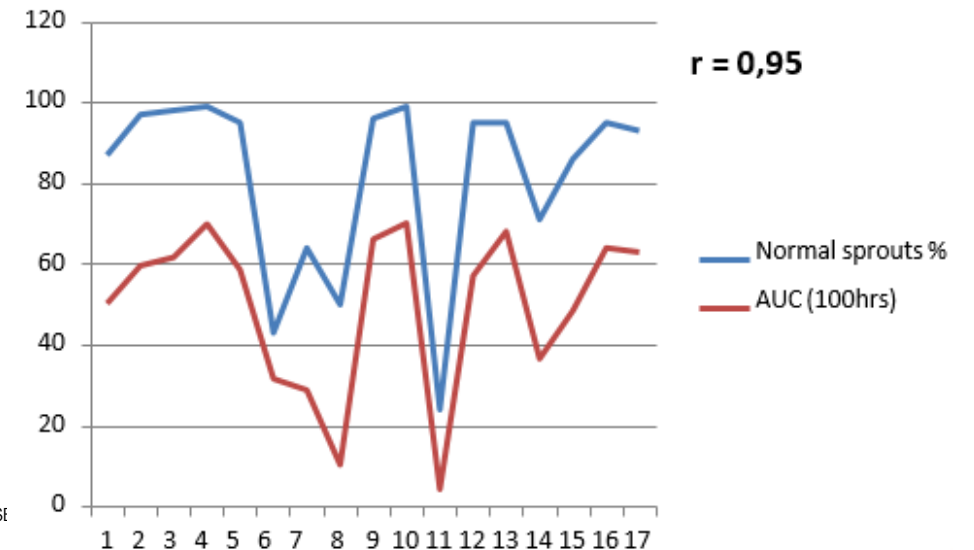
Different statistics can be calculated from the curves such as:
- time for 50% germination (**t50**),
- maximum germination (**gMAX**)
- mean germination time (**MGT**) and,
- Area Under Curve (**AUC**).

ISOTHERMAL CALORIMETRY

Isothermal calorimetry is a technique in which heat generation in a sample can be very precisely monitored.

Heat production rate (thermal power) is measured at constant temperature.

A commercial calorimeter with 125 mL vials (Biocal 2000, Calmetrix Inc.) with aeration was used.

Each sample consisted of 100 grains placed on two levels on pleated germination paper.

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TYPICAL RESULTS OF ISOTHERMAL CALORIMETRY

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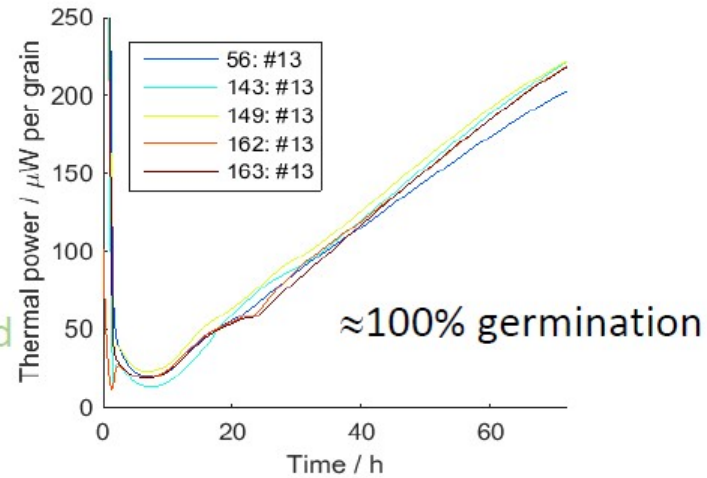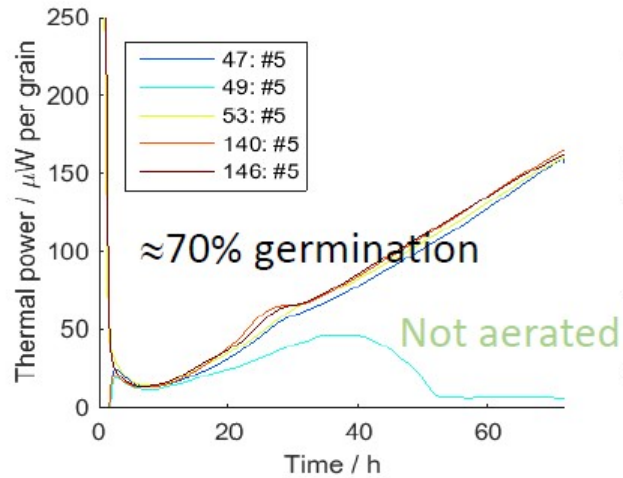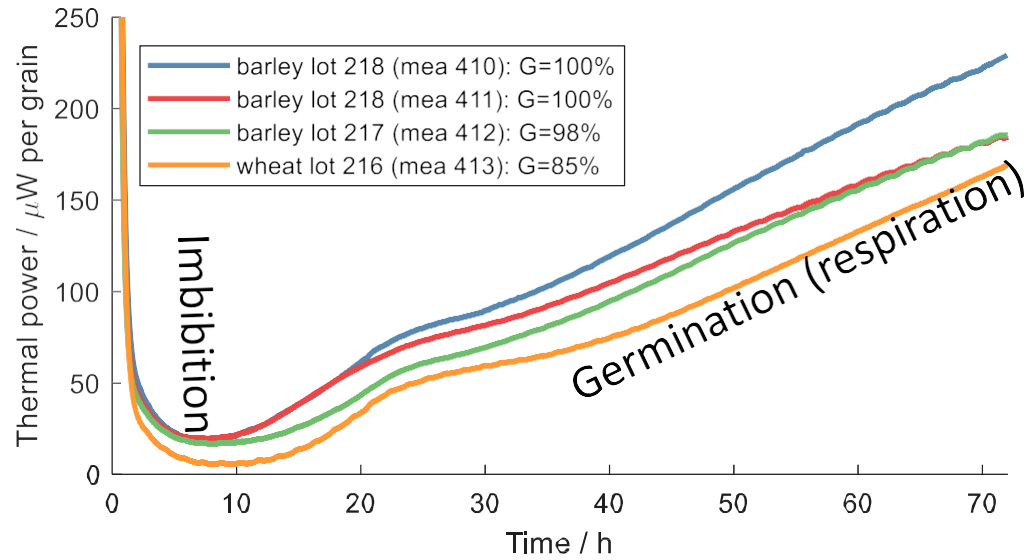
PRELIMINARY RESULTS: GOOD CORRELATION BETWEEN GERMINATOR, MEAN GERMINATION TIME (MGT) AND ISOTHERMAL CALORIMETRY (TIME TO MINIMUM)

Potential to determine germination capacity (normally determined after 72 h) already after 10 h.

COMPARISONS

Correlations with reference methods and examples of measures registered with the different quick methods.
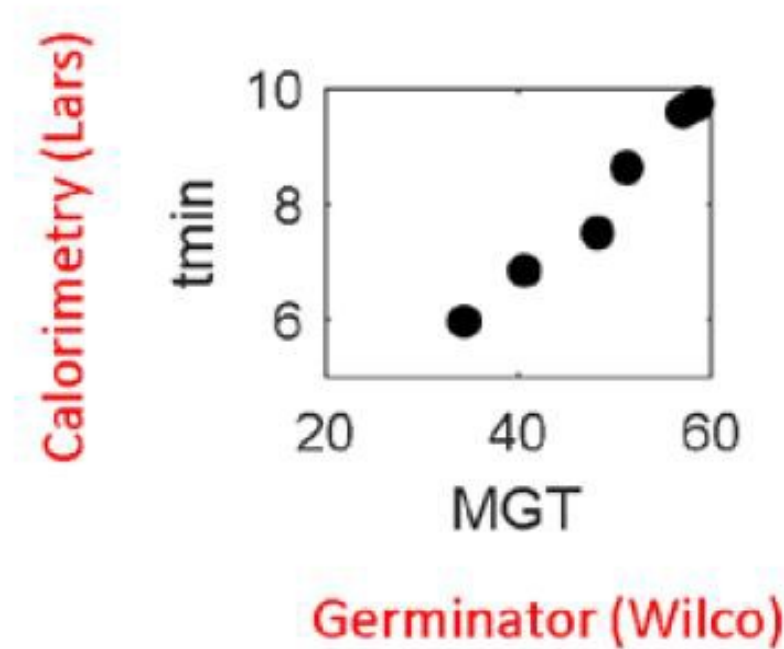N = 20 barley samples from 2015 without microbial contamination.

Normal Sprouts (ISTA)

Videometer			Calorimeter	GERMINATOR*	
24h	48h	72h	72h	Gmax	AUC
0.69	0.91	0.88	?	0.87	0.89

Germination energy 3 days (EBC)

Videometer			Calorimeter	GERMINATOR*	
24h	48h	72h	72h	Gmax	AUC
0.68	0.90	0.90	?	0.90	0.91

Germination energy 5 days (EBC)

Videometer			Calorimeter	GERMINATOR	
24h	48h	72h	72h	Gmax	AUC
0.61	0.80	0.83	?	0.82	0.82

* Based on one randomly selected sample from 4 replicate analyses.

CONCLUSIONS - ADVANTAGES OF THE DIFFERENT TECHNOLOGIES

- **VideometerLab3**
  - Good agreement with reference methods after 48 h.
  - Images of individual kernels can be saved.
  - Easy to modify settings
  - Fungal infections and early onset of germination can be monitored using the right set of wavelengths.

- **The isothermal calorimetry**
  - Allows larger sample sizes than the other methods.
  - Possible to get early results using the slopes of the heat generation curves.

- **The GERMINATOR**
  - Non-complicated and inexpensive technology.
  - Good agreement with reference methods after 48 h.
  - Can be scaled up using larger trays.

PROJECT PARTNERS / ACKNOWLEDGEMENT

- Thomas Börjesson, Agroväst, coordination, scale-up GERMINATOR
- Johannes Ravn Jørgensen, Aarhus University, VideometerLab, Artificial aging
- Wilco Ligterink, Wageningen University, GERMINATOR
- Lars Wadsö, Lund University, Isothermal calorimetry
- Elisabeth Janver, Viking Malt, reference malting barley analyses
- Pernilla Andersson, Swedish Board of Agriculture, reference seed analyses

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