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Projektträger Bundesanstalt
für Landwirtschaft und Ernährung

aufgrund eines Beschlusses
des Deutschen Bundestages

Hyperspectral imaging in the UV-range – new steps in phenotyping?

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TECHNISCHE UNIVERSITÄT DARMSTADT

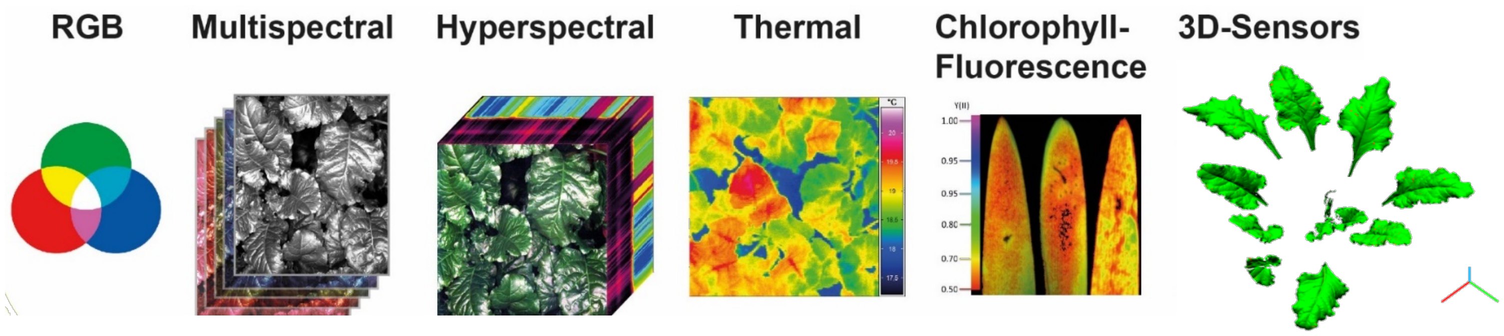
Optical sensors for the detection of diseases

- Host-pathogen interactions differ in their symptom severity, leading to specific spatial patterns and spectral signatures

Mahlein et al. 2012, Plant Meth

- Sensor technologies enable reproducible and objective detection of plant diseases

Rumpf et al. 2010, Comp El Agr



Optical sensors for the detection of diseases: RGB

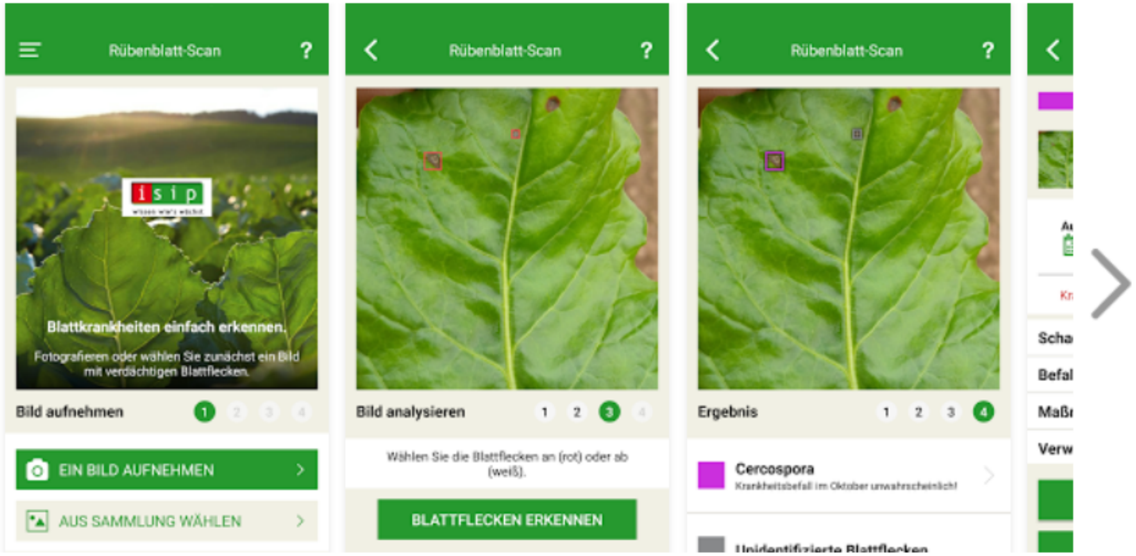
ISIP Rübenblatt-Scan

ISIP e.V. Tools ★★★★★ 3

USK ab 0 Jahren

Zur Wunschliste hinzufügen Installieren

“Smart” Diseases Detection



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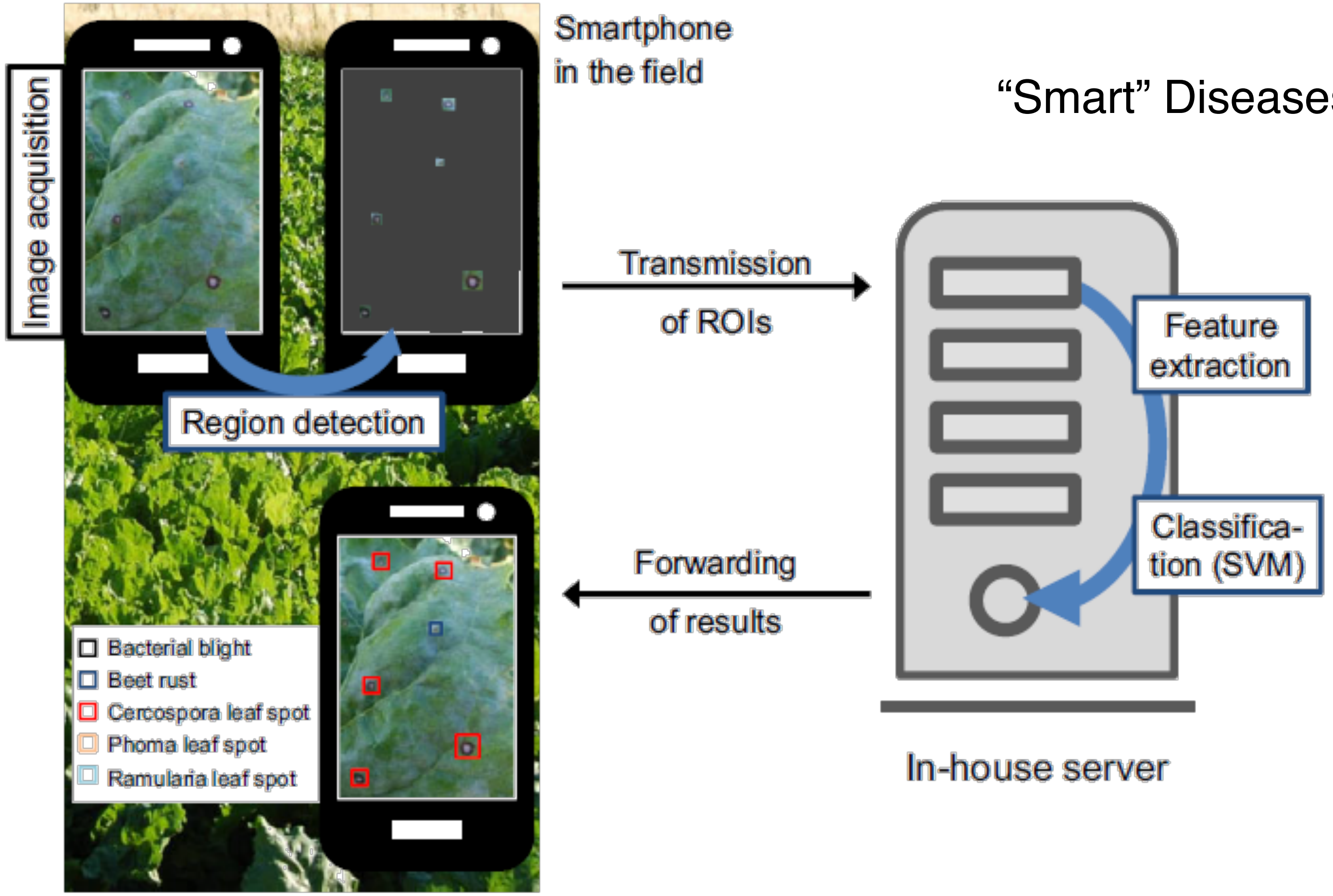
isip wissen wie's wächst

CROP.SENSe.net

https://www.isip.de/isip/servlet/isip-de/apps

Optical sensors for the detection of diseases: RGB



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Hallau et al., 2017 *Plant Pathology*

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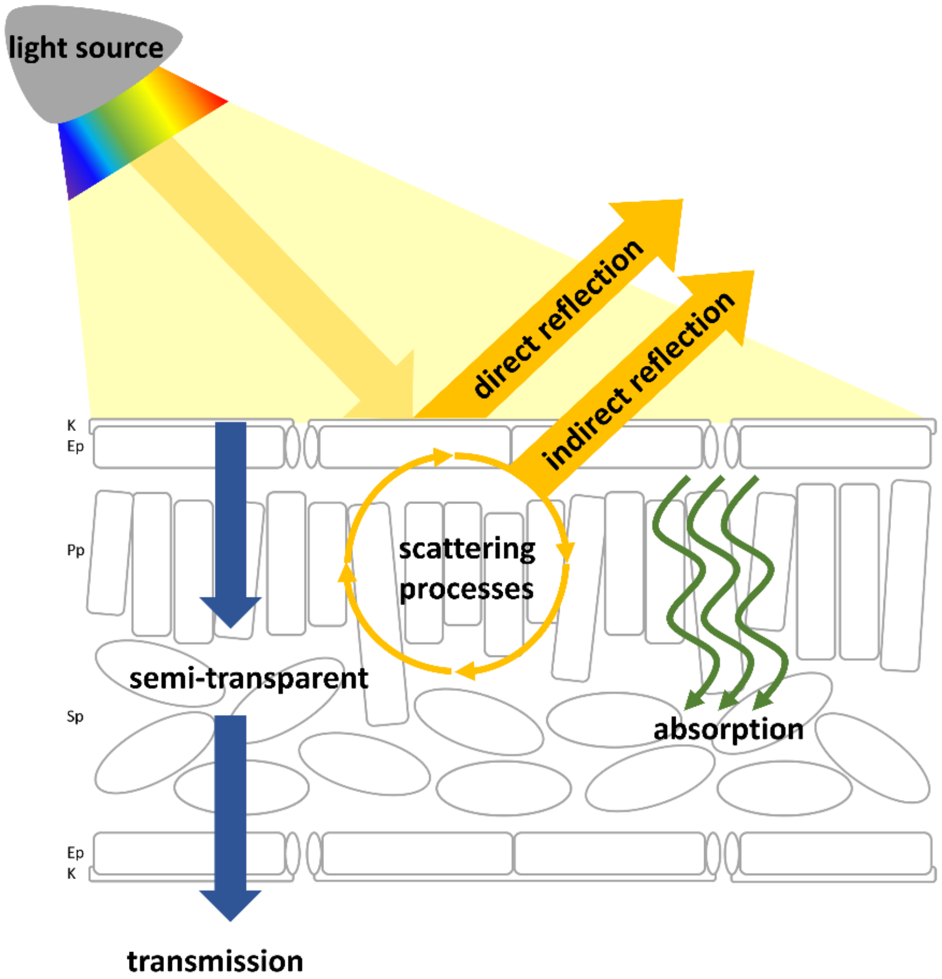
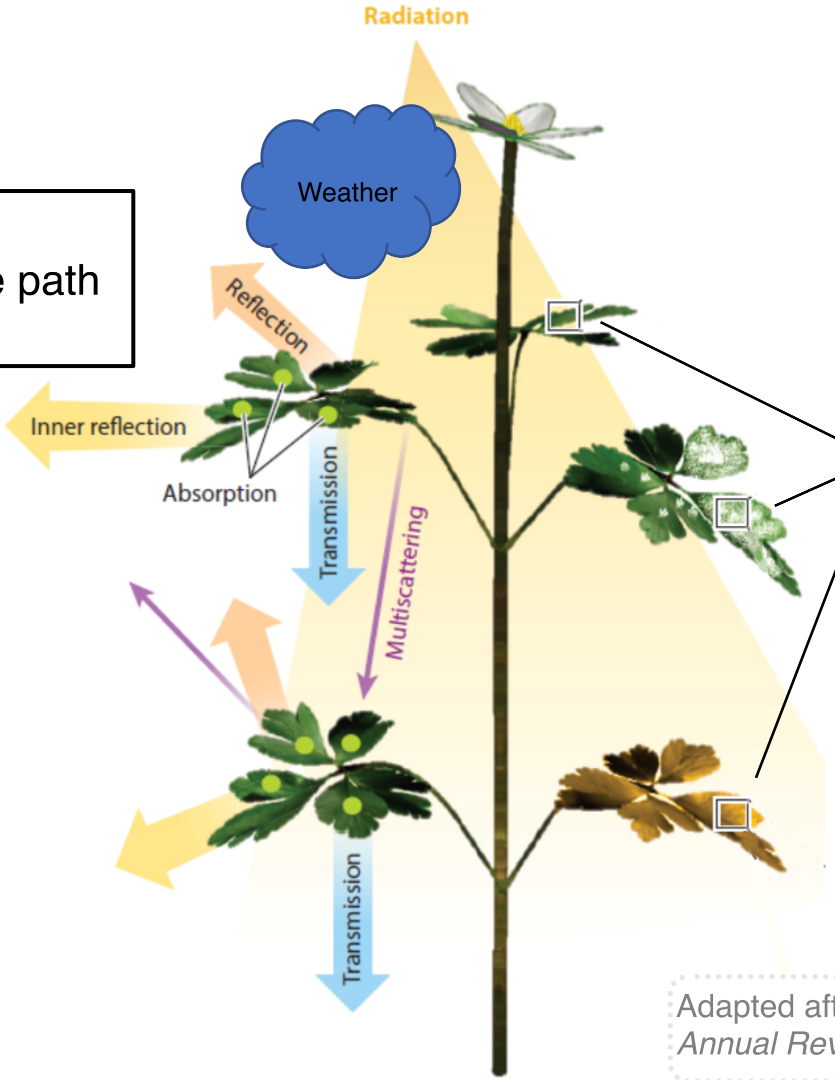
Hyperspectral imaging of plants



Spectral sensors for the detection of diseases – the path of light

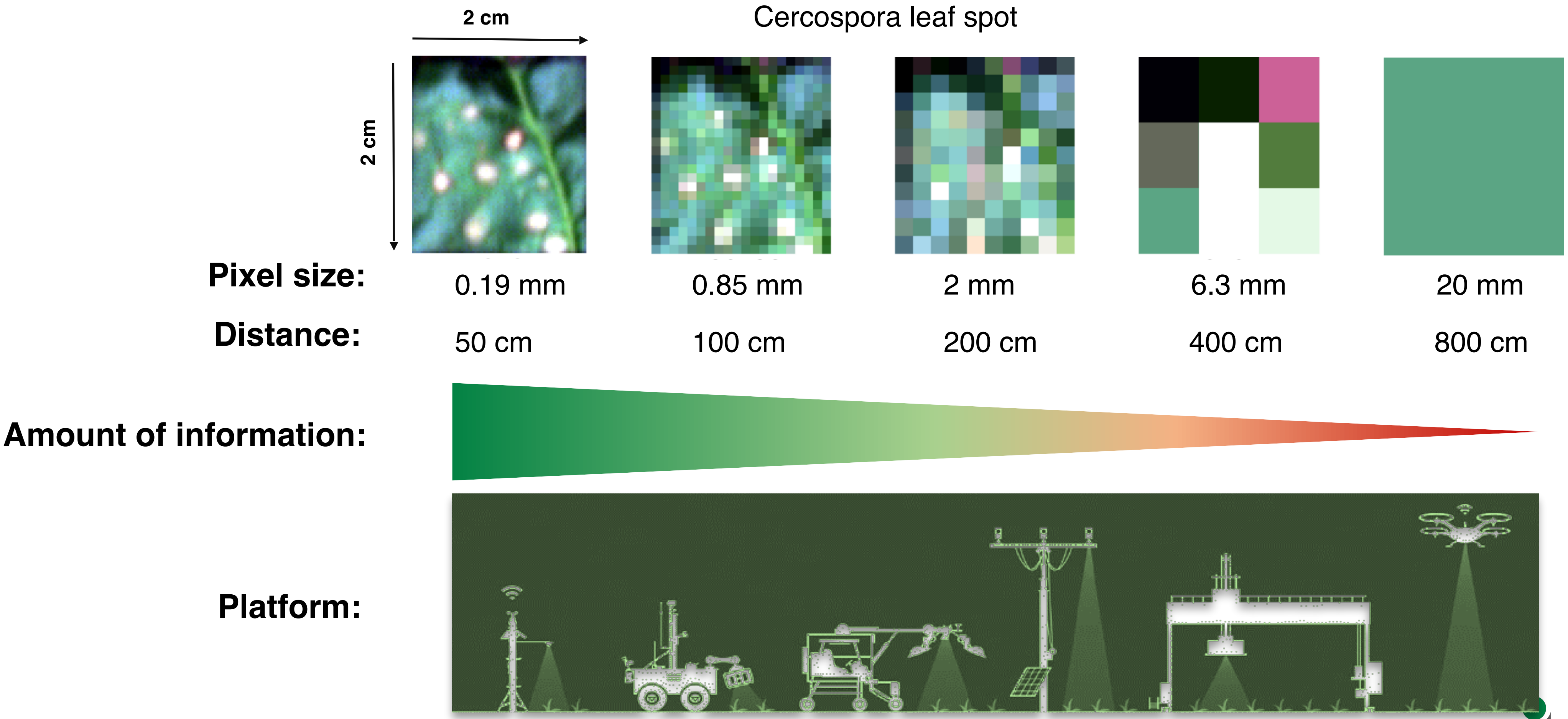
Various leaf physiological and meteorological effects influence the path of light



Abiotic and biotic factors influence the reflection of the leaf in a characteristic way

Adapted after Mahlein et al., 2018,
Annual Review of Phytopathology

Spectral sensors for disease detection – spatial resolution

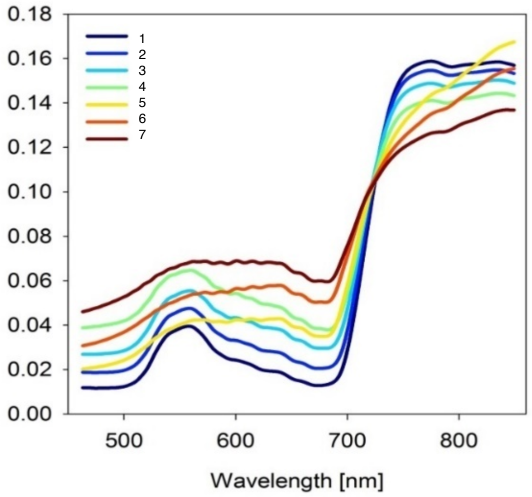


Adapted after Shakoor et al., 2017, *Current Opinion in Plant Biology*

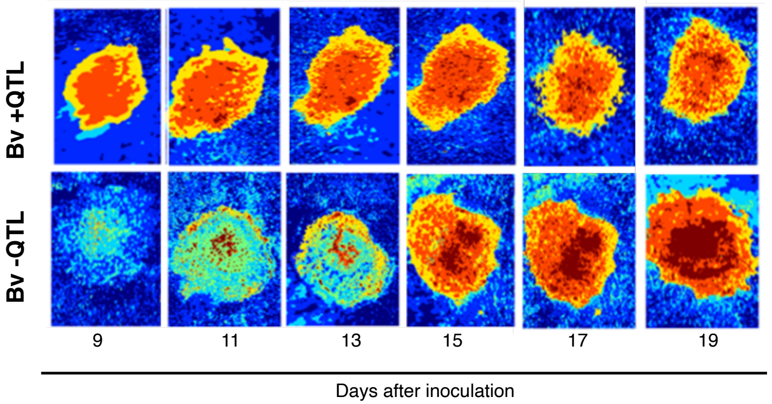
Hyperspectral measurements on different scales: symptom level

Clustering of Cercospora symptom-types

Representative spectral cluster

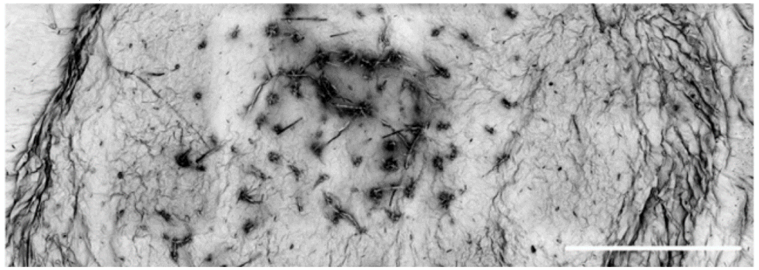


spectral symptom-phenotypes

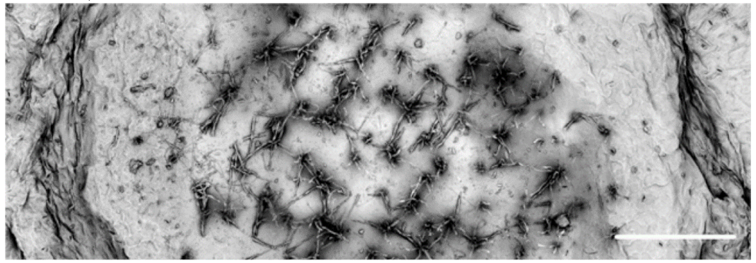


Scanning electron microscopy

Bv +QTL



Bv -QTL



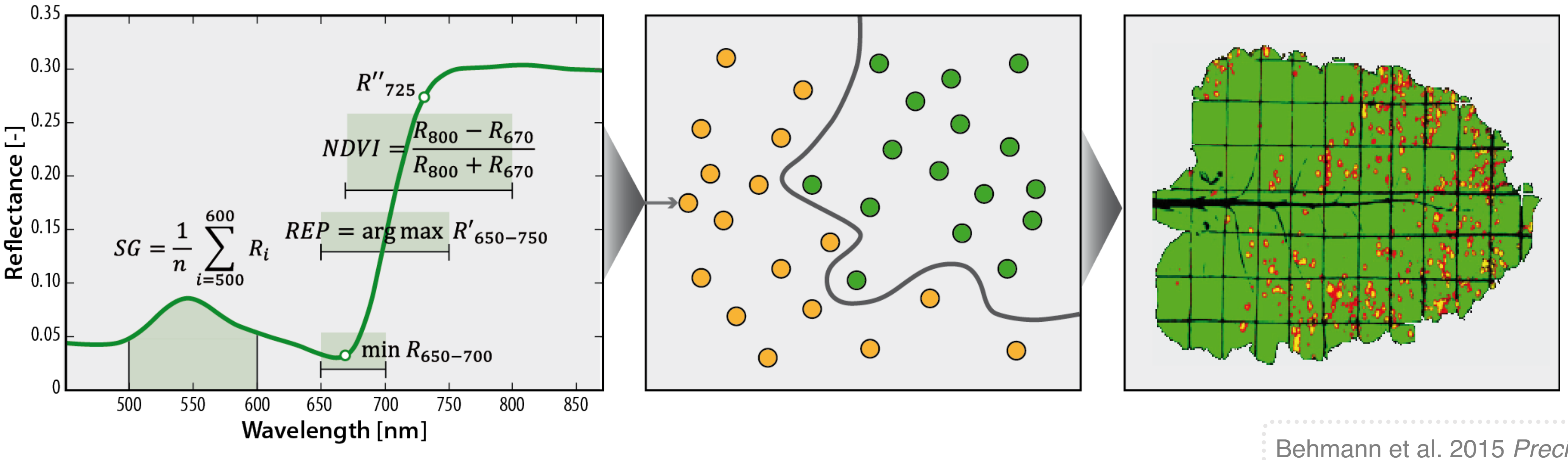
Connection of **spectral phenotypes** and **sporulation intensity**

Leucker et al. 2016 *Phytopathology*

Hyperspectral measurements on different scales: leaf level

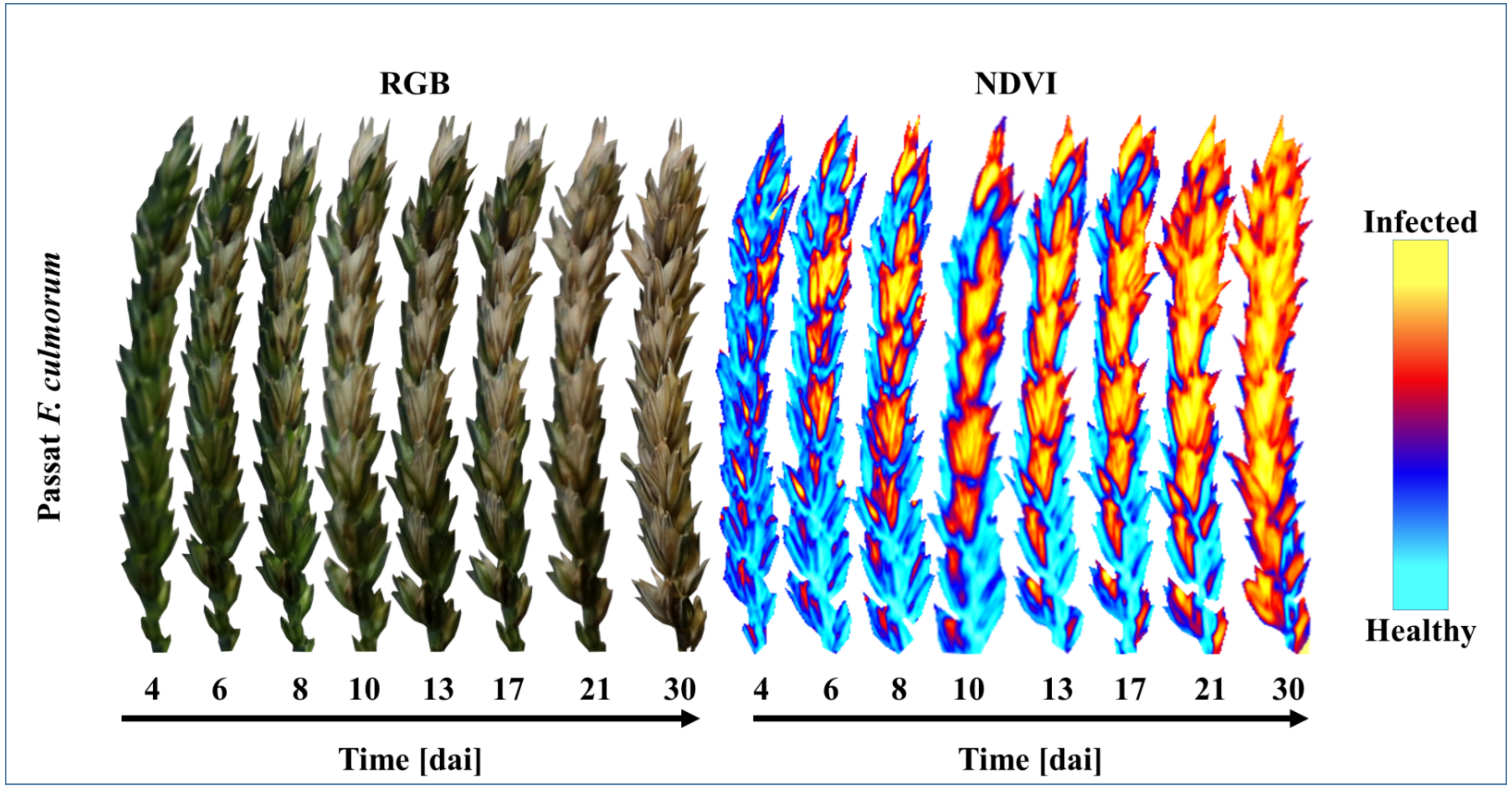
- Before symptoms are visible?
- Supervised classification using „Support Vector Machines (SVM)“

Calculation of spectral features | Automatic classification | Disease detection



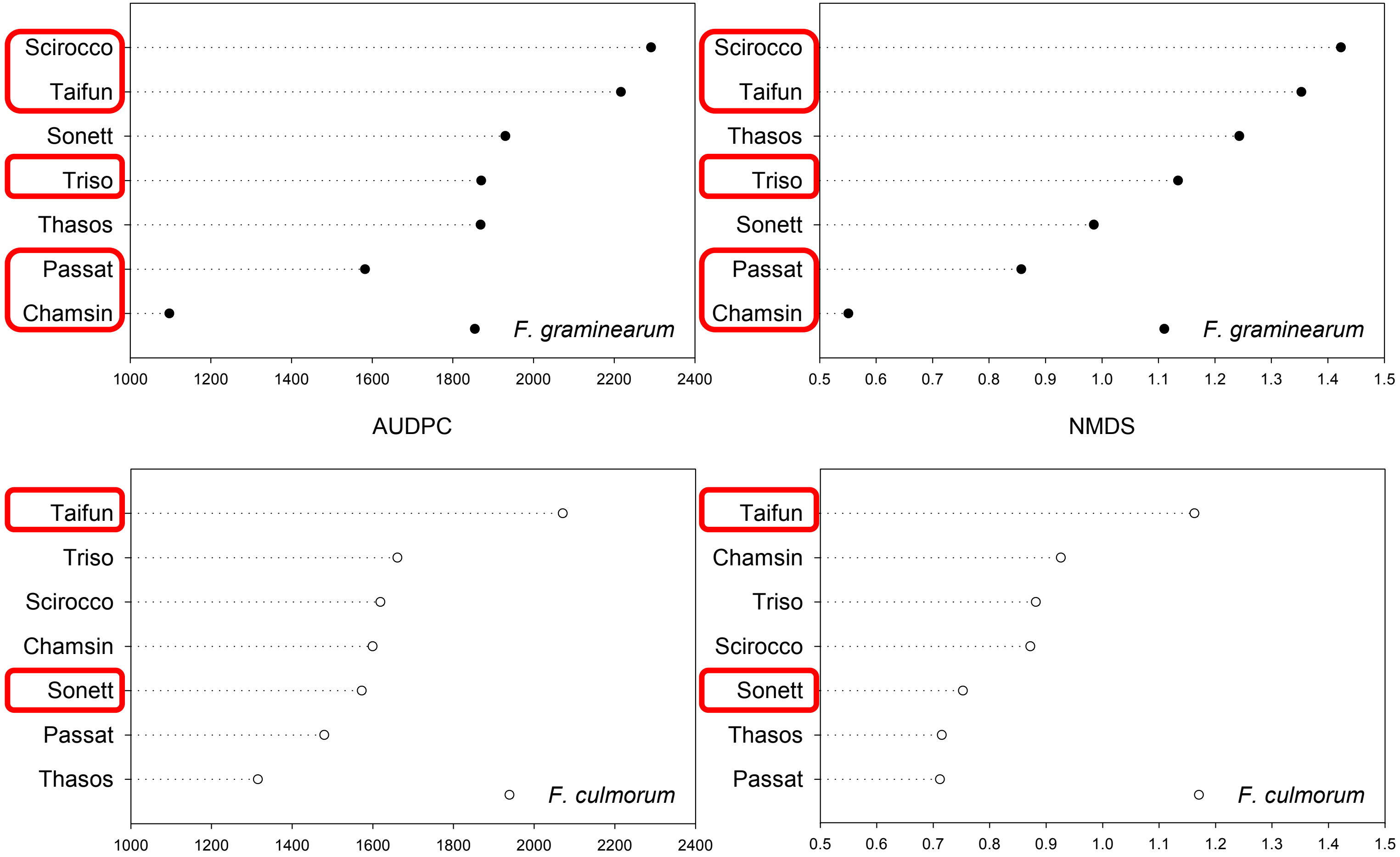
Behmann et al. 2015 *Precision Agriculture*

Hyperspectral measurements on different scales: kernel level



Alisaac et al., 2018, *European Journal of Plant Pathology*

Two Ways Variety Ranking



Area under Disease Progress Curve

Non-Metric Multi-Dimensional Scaling

Alisaac et al., 2018, *European Journal of Plant Pathology*

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Fusarium infected kernels cv. (‘Sonett’)

Control | *F. culmorum* | *F. graminearum* | *F. poae*

10^4 Spore/ml

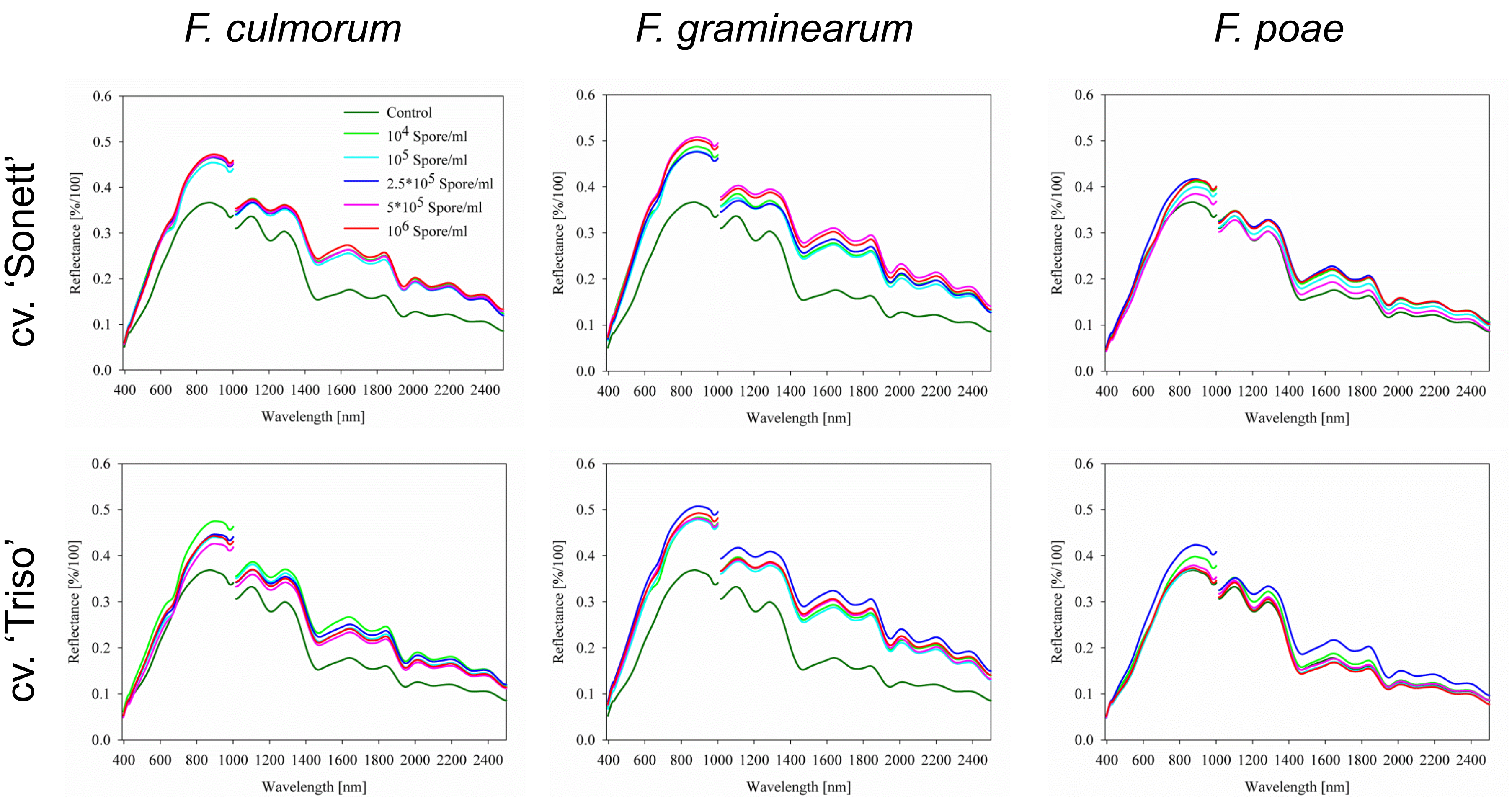
$2.5x10^5$ Spore/ml

10^6 Spore/ml

Alisaac et al., 2019, *Toxins*

Summer wheat kernels cv. (‘Sonett’) inoculated with *Fusarium* species (*F. culmorum, F. graminearum* and *F. poae*) at different spore densities

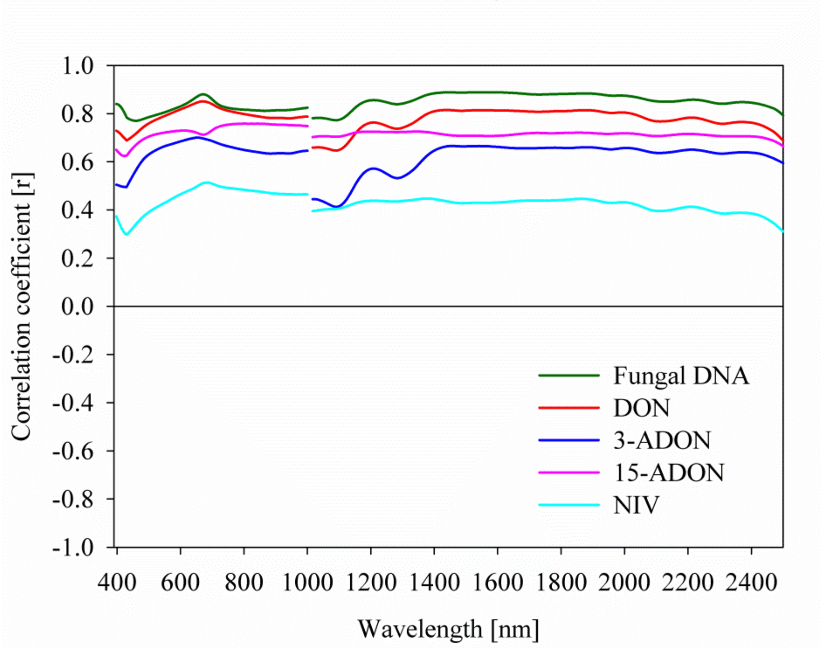
Spectral signature of infected kernels



Alisaac et al., 2019, *Toxins*

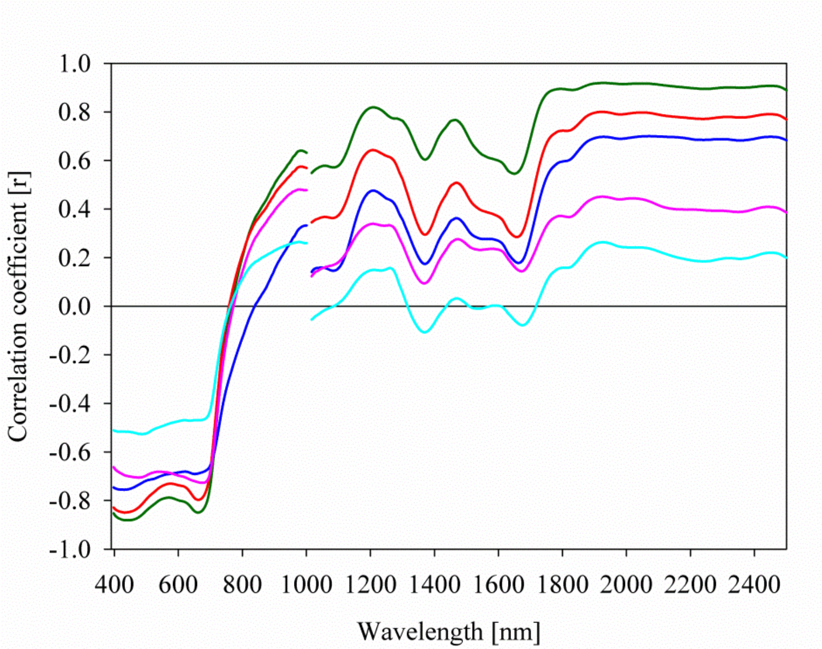
Correlations between spectral signature in relation with fungal DNA and mycotoxin contents

Kernels



F. graminearum

Flour



F. graminearum

Alisaac et al., 2019, *Toxins*

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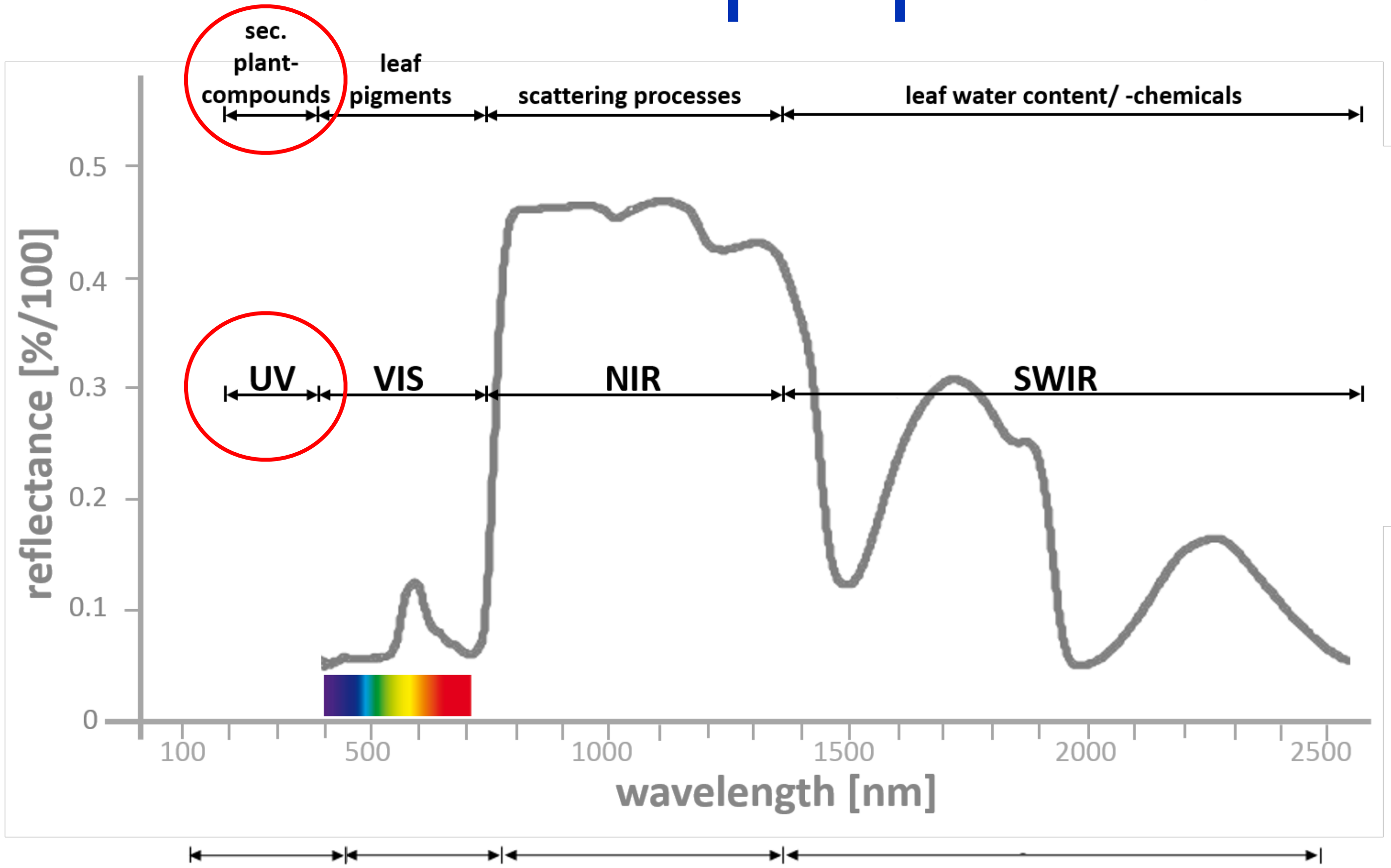
Spectral reflection properties of plants

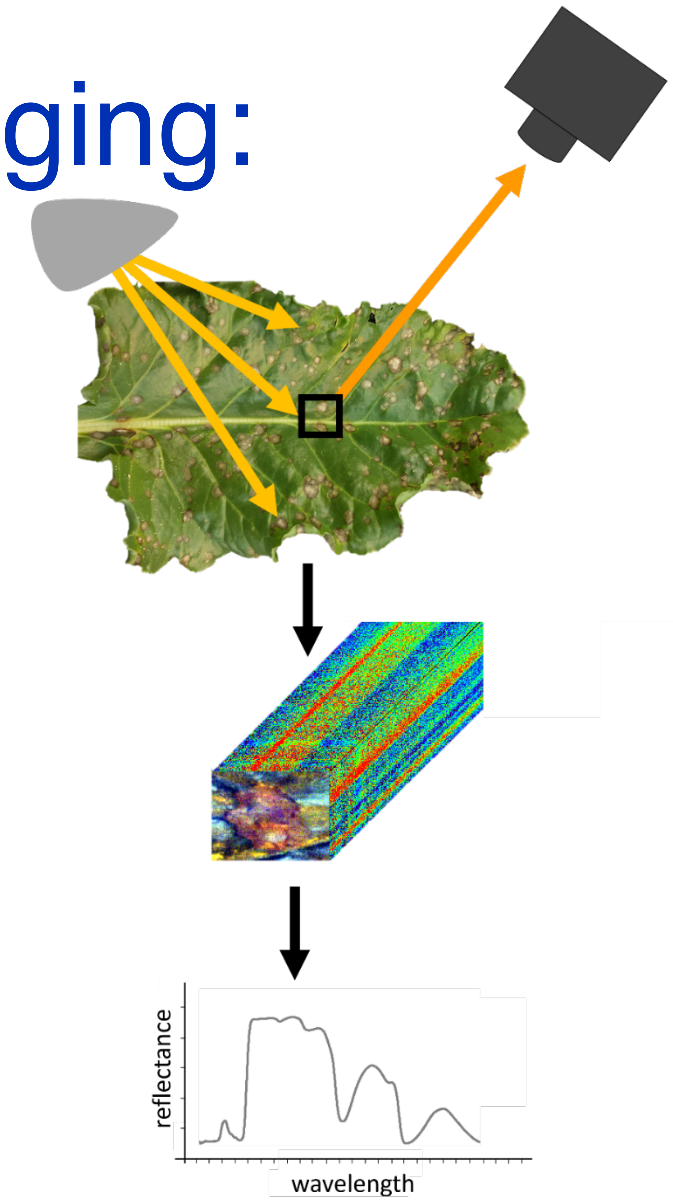


250-380 nm 380-1030 nm tomato - *Botrytis cinerea* (Kong et al. 2014)
? 400-1000 nm sugar beet – *Cercospora beticola* (Mahlein et al. 2012)
400-1000 nm barley - *Erysiphales* (Kuska et al. 2015)
400-2500 nm rape seed - *Alternaria* (Baranowski et al. 2015)

Motivation for UV-imaging:

Early host-pathogen interactions show an influence on plant substances like secondary metabolites (flavones, phenol etc.)

Hypothesis: Changes in the hyperspectral range can be visualized in the UV-range



Requirements to be developed:

- Homogeneous illumination in the spectral range 250-400 nm
  - → Tissue damage to plant material must be avoided
  - → Safety aspects during measurements must be taken into account
- Hyperspectral camera with UV-sensor
  - → Establishment of measurement protocols
- Development of a processing and evaluation routine
- Biological interpretation of hyperspectral UV signatures

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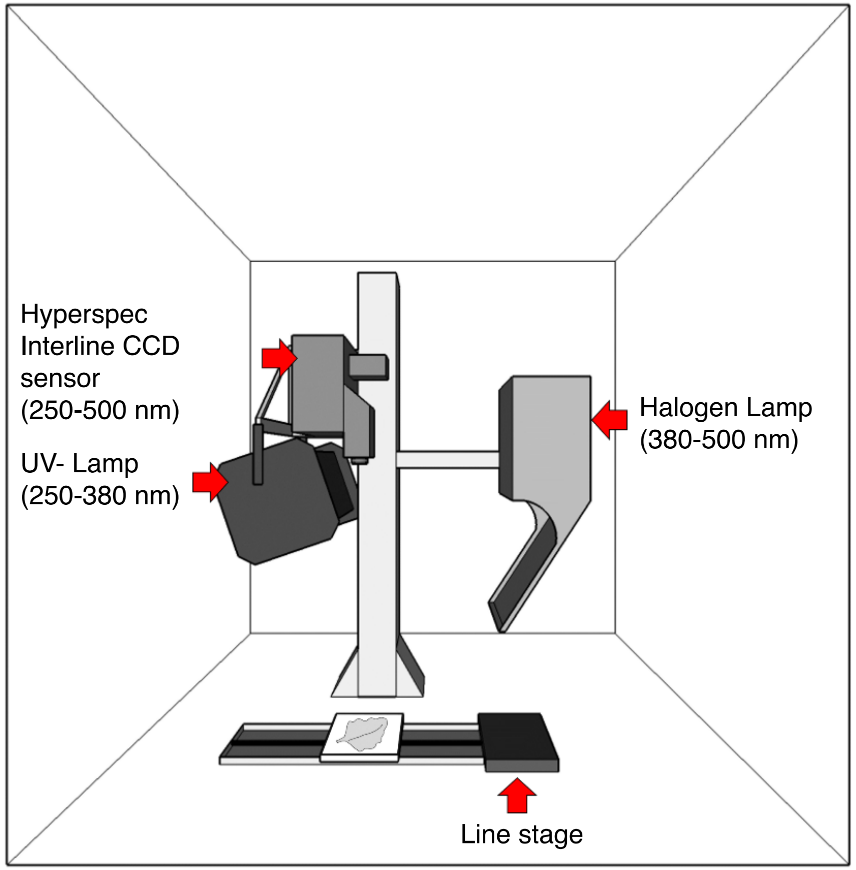
Processing and evaluation of the data

- **Hyperspectral Measurement:**
  - UV-VIS-Interline CCD Sensor (250-500 nm)
  - Spectral resolution 14 nm and focal length 28,3 mm
- **Measurement cabinet:**
  - opaque cabinet
  - 92 cm x 61 cm x 92 cm
- **Illumination:**
  - UV-lamp *Guardian* von UniLux
  - Halogen lamp *21 DC* von TechniQuip
- **Evaluation of data:**
  - ENVI 5.4

Spectral changes of host-pathogen interactions



Brugger et al., 2019, *Remote Sensing*

Phototoxicity of UV-irradiation

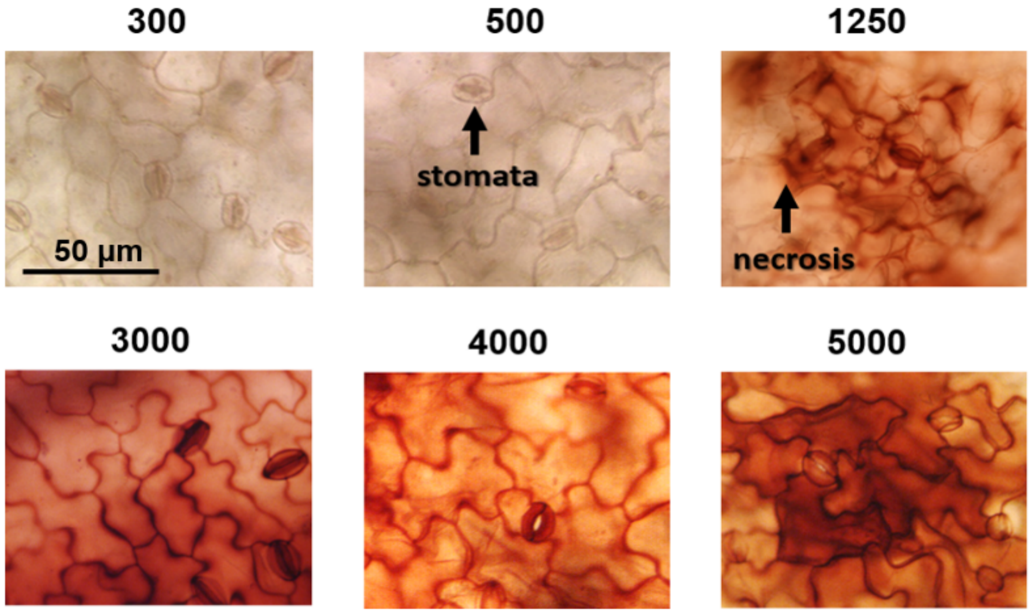
- First measurements of sugar beet leaves revealed tissue damage

- Determination of non-phtotoxic UV-intensities
  - Detection of cell death by staining H_2O_2 (DAB-staining after Thordal-Christensen *et al.* 1997)

- **Sugar beet** leaves display no phototoxic effect after illumination with intensities from **300-1250 lux**
- **Barley leaves** display no phototoxic effect after illumination with intensities from **300-2500 lux**

Brugger et al., 2019, *Remote Sensing*

Tissue damage of sugar beet leaf after illumination with 3500 lux



Microscopic observation of sugar beet leaves after illumination with different intensities

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Phototoxicity of UV-irradiation during time-series measurements

measuring
every 2 days

8 dai

16 dai

- Time series measurements of sugar beet leaves showed tissue damage after 5 measurements and illumination with 1250 lux

Time-series measurements can not be conducted with the same sample for sugar beet leaves
Assessment of symptom stages with one sample per measurement

Summary

- Optical sensors can detect, quantify and identify plant diseases
- Smartphone based solutions spread information about plant disease detection
- Application possible on different scales : from cell to field
- Spectral changes also visible from infected kernels

- Hyperspectral measuring protocols in the UV-range established
- Phototoxicity of UV-illumination was evaluated, time-series measurements can not be conducted for sugar beet leaves
  - Symptom stage assessment with one sample per measurement for sugar beet leaves
- Sugar beet leaves inoculated with *C. beticola* and *U. betae* causes changes in the reflectance properties in the UV-range
- Susceptible and resistant barley genotypes lead to different spectral signatures

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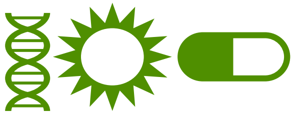
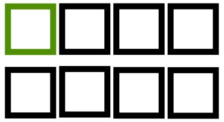
Plant phenotyping

Aim: accelerate resistance breeding and selection of relevant genotypes

Influenced by:
- genotype
- environmental conditions
- management



Size: small plots



Measurement: various traits of genotype, host-pathogen interaction, susceptibility, yield, quality

Technique: RGB imaging, thermal imaging, hyperspectral imaging

Data processing: Delayed data analysis, related to genotyping

Result: Development of new cultivars

Challenges:

- advancement of phenotyping techniques for improving the selection efficiency
- automated monitoring
- cost efficiency

Plant phenotyping examples

Greenhouse Scanalyzer, an automatic plant-to-sensor system in which hyperspectral cameras can be mounted into measurement cabins.

Field Scanalyzer, an automatic sensor-to-plant system in which a camera can be mounted on the probe head.

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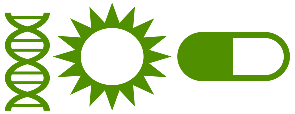
Precision agriculture

Aim: detect spatial heterogeneity within crop stand

Influenced by:
- genotype
- environmental conditions
- management



Size: fields

Measurement: real-time mapping systems of crop, soil and environment variables

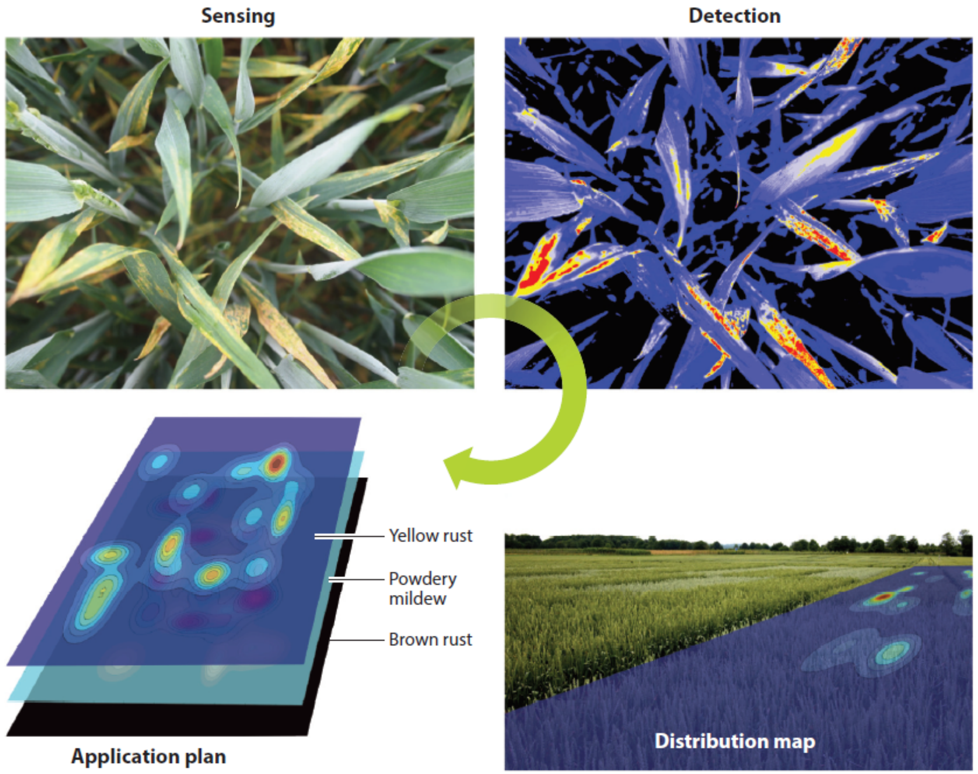
Technique: Optical sensors, GPS, imaging platforms, robots, UAVs, satellite images

Data processing: Real time data analysis, online, automated data analysis

Result: Decision support for management practice

Challenges:
- quality of the acquired data and data analysis
- automated monitoring
- cost efficiency

Precision agriculture



Mahlein et al., 2018, Annual Review of Phytopathology

Outlook

→ Research findings have to be transferred into practice

→ Implement sensor into plant phenotyping to accelerate resistance breeding and selection of relevant genotypes

→ Implement sensor into precision agriculture with the aim to exam spatial heterogeneity

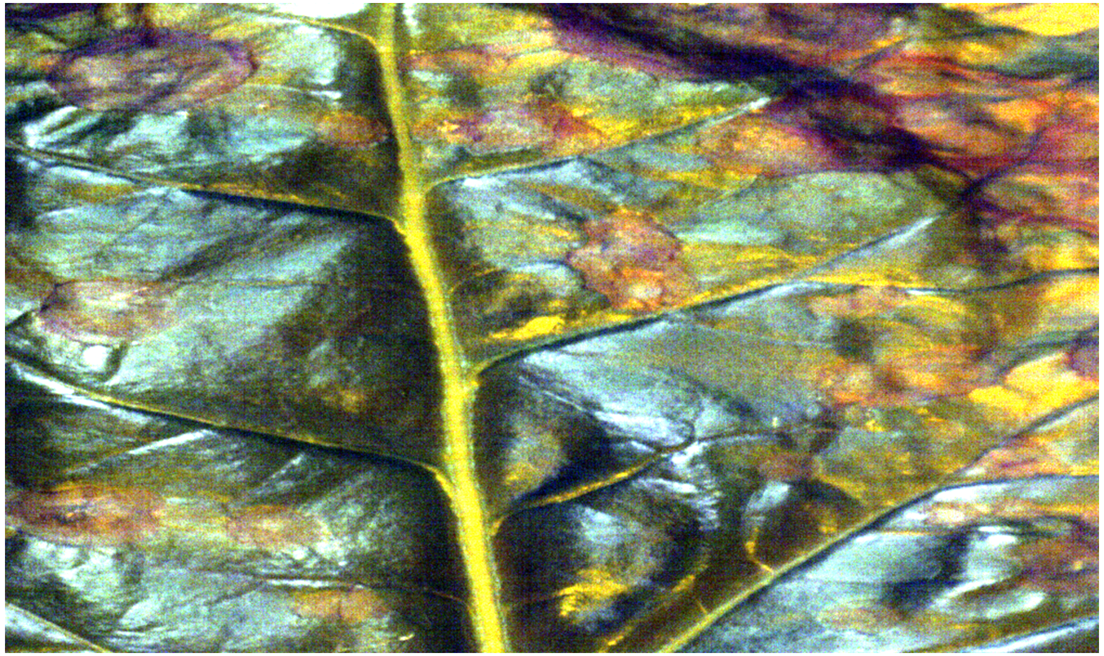
  → Imaging platforms, robots and UAVs

→ Data has to be evaluated with machine learning techniques and data mining methods

→ Hyperspectral changes will be linked to microscopically and molecular biologically recorded interaction parameter

→ Cooperation with project partners for evaluation with data-driven/ “non-supervised” high-throughput methods

Thank you for your attention



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