Plant disease detection by hyperspectral imaging: from the lab to the field

A-K. Mahlein[1], M.T. Kuska[1], S. Thomas[1], D. Bohnenkamp[1], E. Alisaac[1], J. Behmann[1], M. Wahabzada[1], K. Kersting[2]
[1]*INRES-Phytomedicine, University of Bonn, Germany*
[2]*Department of Informatics, TU Dortmund, Germany*
amahlein@uni-bonn.de

Abstract

The detection and identification of plant diseases is a fundamental task in sustainable crop production. An accurate estimate of disease incidence, disease severity and negative effects on yield quality and quantity is important for precision crop production, horticulture, plant breeding or fungicide screening as well as in basic and applied plant research. Particularly hyperspectral imaging of diseased plants offers insight into processes during pathogenesis. By hyperspectral imaging and subsequent data analysis routines, it was possible to realize an early detection, identification and quantification of different relevant plant diseases. Depending on the measuring scale, even subtle processes of defence and resistance mechanism of plants could be evaluated. Within this scope, recent results from studies in barley, wheat and sugar beet and their relevant foliar diseases will be presented.

Keywords: Plant diseases, crop protection, resistance, hyperspectral imaging, machine learning

Introduction

Precise evaluation of plant disease incidence, severity and negative effects on yield quality and quantity is relevant for precision crop production. For planning targeted plant protection activities in field or greenhouse production and to forecast temporal and spatial disease spread it is of importance to enable an accurate and timely assessment of plant disease occurrence and spread. Traditional estimation methods for the detection and identification of plant diseases are visual detection, microscopic, molecular, serological and microbiological methods (Bock et al. 2010). However, we are living in a technology characterized century and there is an increasing amount of optical sensors which perform non-invasively available. These sensors can support plant disease detection and identification in different fields of application. Especially precision agriculture and plant phenotyping for resistance breeding benefit from this movement. The application of hyperspectral imaging sensors on different scales, from investigating plant tissue on laboratory scale over the single plant scale in greenhouses or climate chambers to the canopy scale in field applications comes with numerous advantages compared to classical visual monitoring or analytical methods. Unlike e.g. molecular biologic analysis methods, the process of hyperspectral imaging is non-invasive, thereby enabling researchers and breeders to perform time series measurements of the sample plants. This leads to a reduction of required samples and makes long term experiments more efficient. Furthermore hyperspectral imaging is an objective method and can be set up as automated measurement system, significantly reducing the workload compared to manual rating (Mahlein 2016, Virlet et al. 2016, Walter et al. 2015). By this, economic and ecological costs in agricultural production can be reduced.

Diseases cause alterations in plant physiology, tissue colour and leaf shape, transpiration rate, crop canopy morphology and density and as a complex result of these manifold interactions variation in the optical properties of plants. Researcher applied hyperspectral imaging to the detection of plant diseases on different scales and in different pathosystems (reviewed in: Mahlein 2016, Sankaran et al. 2010, West et al. 2010). To name just some: Bravo et al. (2003) used in-field spectral images for an early detection of yellow rust infected wheat, Hillnhütter et al. (2011) successfully discriminated symptoms caused by the nematode *Heterodera schachtii* and the soil borne fungus *Rhizoctonia solani* in sugar beet fields. Delalieux et al. (2007) investigated *Venturia inaequalis* on apple plants by hyperspectral non-imaging techniques. Several researchers developed disease specific vegetation indices for efficient data analysis (Ashourloo et al. 2014, Mahlein et al. 2013, Oerke et al. 2016). Advanced data mining techniques enabled the definition of cardinal points during pathogenesis of foliar barley diseases in (Wahabzada et al. 2015a, 2016). Kuska et al. (2015) were able to characterize resistance reactions of barley to powdery mildew using a hyperspectral microscopic approach. With a similar technique, Leucker et al. (2016) established a link among QTLs and spectral signatures of sugar beet genotypes, with different levels of resistance to Cercospora beticola, the causal agent of Cercospora leaf spot. Apan et al. (2004) were able to detect sugarcane orange rust from EO-1 Hyperion hyperspectral imaging and Huang et al. (2007) found a good reliability and accuracy in detecting yellow rust in wheat by ground based spectral measurements and airborne hyperspectral imaging.
The present article is supposed to give examples and impression of applying hyperspectral imaging of plant disease detection and resistance screening on different scales.

Material and Methods

Plant and pathogen systems
Investigations were performed in different plant-pathogen systems. Near-isogenic barley (*Hordeum vulgare*) lines cv. Ingrid wild type (WT) and Pallas with mlo 3 and Mla1 resistance were used for investigations on the microscopic scale. Barley plants were cultivated according to Kuska et al. (2015) and were investigated in a specific in vivo phyto-agar system. These plants were inoculated with the powdery mildew pathogen *Blumeria graminis* f.sp. *hordei* (Bgh), isolate K1.
Barley cultivars with differing susceptibility to powdery mildew infestation were cultivated in a mini-plot system. In this case, measurements were performed on the canopy scale. The cultivars used in this experiments were: Gesine, Grace, Tocada, Milford, Irina and Eileen. Inoculation took place with an unspecified field isolate of *Blumeria graminis* f.sp. *hordei.*
Sugar beet plants, diseased with the foliar diseases Cercospora leaf spot, sugar beet rust and powdery mildew were investigated on different scales. For cultivation please refer to Mahlein et al. (2012).

Sensor setup and hyperspectral image acquisition
A hyperspectral microscope system has been established to assess spectral changes on the leaf and cellular (Kuska et al, 2015, Leucker et al. 2016). This setup consist of a hyperspectral linescanner (spectral camera PFD V10E, Specim, Oulu, Finland) in the visible (400 – 700 nm) and near infrared (700 – 1000 nm) range, mounted on a foreoptic (Z6 APO, Leica, Wetzlar, Germany) with a magnification up to 7.3x. Symptom development and early resistance reactions of barley and sugar beet were measured in time series imaging.

Measurements on the plant scale were performed with a hyperspectral linescanner (spectral camera Imspector V10, Specim, Oulu, Finland) in the visible (400 – 700 nm) and near infrared (700 – 1000 nm) range, and with a SWIR-camera (Specim, Oulu, Finland) in the shortwave infrared. The spatial plane was covered, using a linear stage (Mahlein et al. 2012, Behmann et al. 2016). In addition to hyperspectral measurements, 3D-information from plants were assessed using a 3D laser scanner (Behmann et al. 2016, Paulus et al. 2015).
For measurements on the canopy scale, a high-throughput-scanner, installed in a greenhouse environment was established. In this system, a hyperspectral linescanner (spectral camera Imspector V10, Specim, Oulu, Finland), combined with a mirror-scanner was applied. Illumination was realized by six halogen-bulbs.

Data processing and analysis
Changes in spectral reflectance were extracted, analysed and classified manually and via data mining methods, respectively. Calculation of reflectance, relative to a white reference and the dark current was performed using the software ENVI 5.1 + IDL 8.3 (ITT Visual Information Solutions, Boulder, USA). After normalization the Savitzky-Golay filter (Savitzky & Golay, 1964) was applied to smooth hyperspectral images. These pre-processed images were used for further analysis using Python, Matlab or the ENVI 5.1 + IDL 8.3 software.

Automated data mining approach
Following the method of Wahabzada et al. (2015a), a data driven approach for an automated analysis of hyperspectral data was used. Simplex Volume Maximization (SiVM) is a recent, fast and interpretable factorization technique that represents the data (hyperspectral images and signatures) in terms of only a few extreme components (signatures). Furthermore, K-means clustering as an unsupervised and data driven approach was applied on the data (Wahabzada et al. 2015b, Leucker et al. 2016). Spectral classification of healthy and diseased pixels on the canopy scale were realized by the spectral angle mapper (SAM) algorithm. With the presented data analysis methods, all pixels of the hyperspectral images were considered.

Results

Automated resistance screening on the microscopic scale
The automated assessment of spectral reflectance among the different genotypes during the experimental period from microscopic data were in accordance to the manually assessed spectral signatures and visual assessed processes during pathogenesis (data not shown). Based on the automatically assessed reflectance spectra a map system of the *Bgh* resistance was established (Fig. 1A). The resistance maps accentuated the location of *Bgh* diseased leaf tissue over time. This mapping process allows the visual observation of host-pathogen interaction and, if desired, a rapid visual identification of relevant pixels. Hypersensitive responses and papillae based resistance could be separated from healthy and diseased individuals in the following using matrix factorization (Fig. 1B). Embedding the spectral extremes emphasises differences among plant genotypes (Fig. 1C).

Assessment of plant diseases on the plant level
Spectral signatures of seven cluster means are given in Fig. 2 A. Each cluster was assigned to one colour and the clustering results were visualised by pseudo-colour images (Fig.2 B).

Typical spectral clusters of healthy tissue (green), leaf veins (blue) and Cercospora leaf spots (from yellow to red) could be assessed without human intervention automatically.

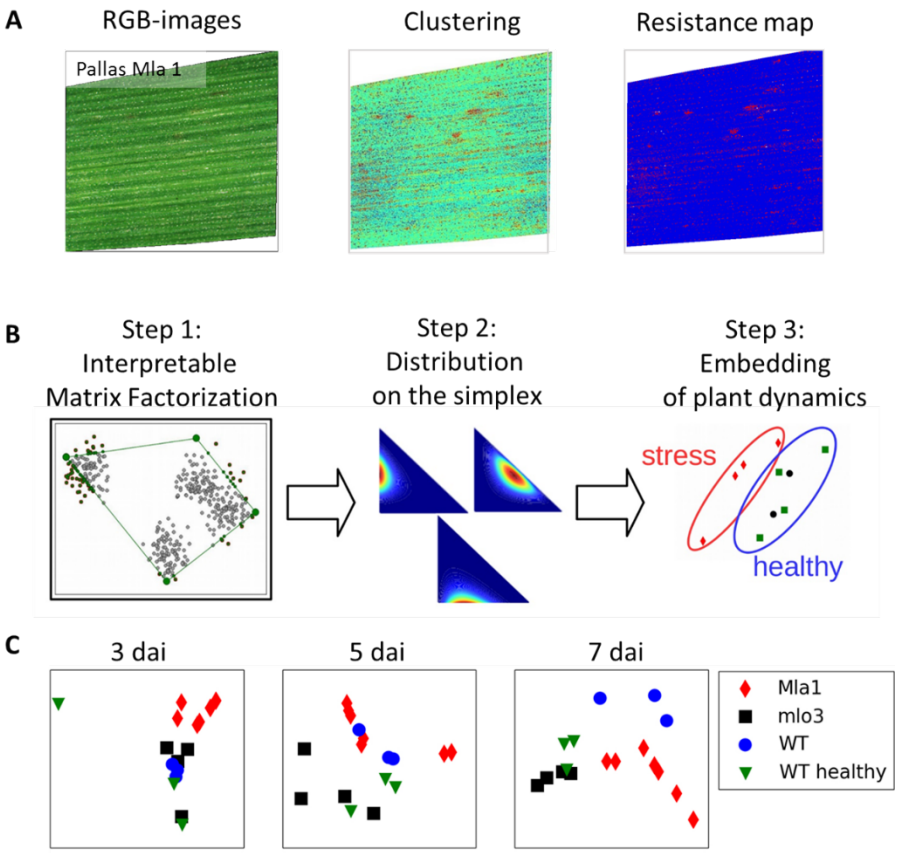

Figure 1 Hyperspectral phenotyping of disease resistance and monitoring of pathogenesis on the microscopic scale. (A) Example of hyperspectral tissue screening of barley, *cv*. Pallas *Mla 1* and assessment of resistance spectra by k-means clustering. (B) Based on this data setup, an embedding of spectral dynamics is presented. (C) This approach enables the monitoring and differentiation of different plant genotypes and plant-pathogen interaction types.

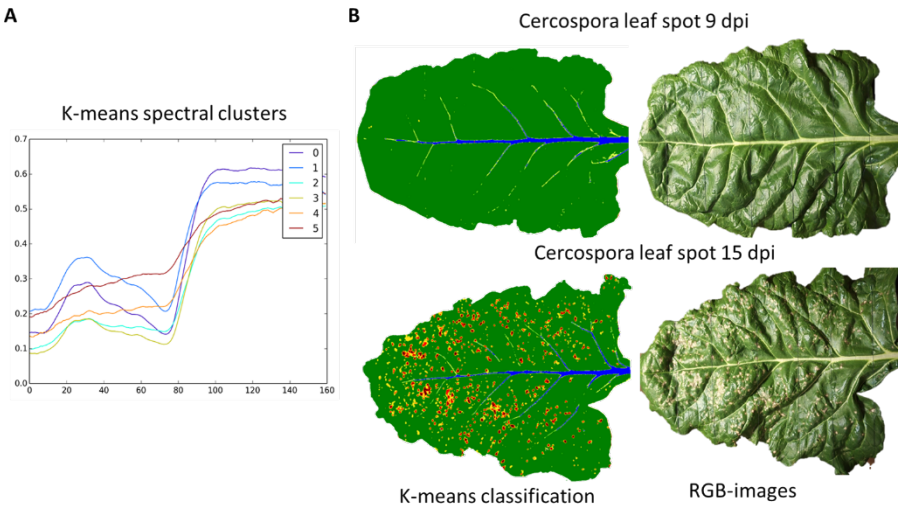

Figure 2 Clustering of hyperspectral signatures of sugar beet leaves, diseased with Cercospora leaf spot on the leaf scale. (A) Seven characteristic clusters for healthy and diseased parts and for leaf veins were evaluated and (B) plotted on hyperspectral images.

Hyperspectral 3D models – case study

Fusion of hyperspectral imaging and 3D laser scanning data provided hyperspectral 3D models according to Behmann et al. (2016). The application of hyperspectral 3D models has been investigated in a case study of sugar beet plants and canopy, inoculated with Cercospora leaf spot. With this approach we could demonstrate, that spectral signals are affected by both, the geometric sensor configuration and the plant geometry (Fig. 3). Based on the presented hyperspectral 3D models, normalisation and calibrations routines can be applied to increase the data quality and resulting classification results.

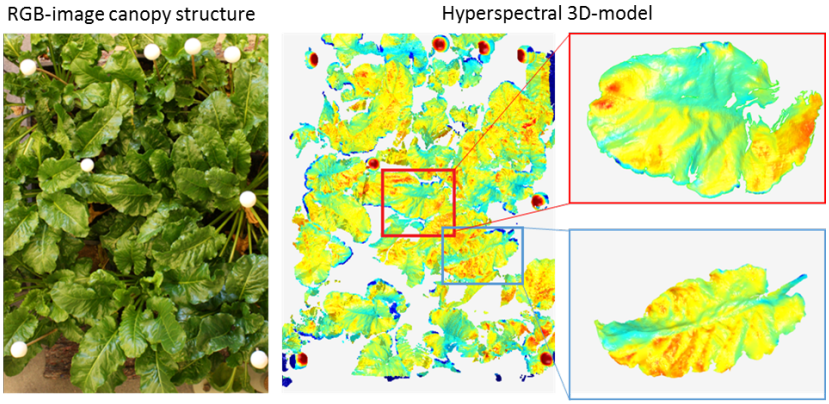

Figure 3 Hyperspectral 3D-plant canopy model of sugar beet plants, considering the distance among the plant canopy and plant parameters, such as leaf inclination.

Automated disease detection in plant canopies by hyperspectral imaging

Investigations of barley cultivars on the canopy scale resulted in a multi-dimensional and multi-temporal data set. The erectophile architecture of barley plants result in multiple sensor to object distances, shadowing and angular effects on the hyperspectral data (Figure 4). To contribute to different illumination situations in the canopy scenes of barley, the spectral angle mapper algorithm was chosen for data analysis. SAM calculates the spectral similarity of spectra and reference spectra using the spectral angle between the two spectra in an n-dimensional space dependent on the number of spectral bands.

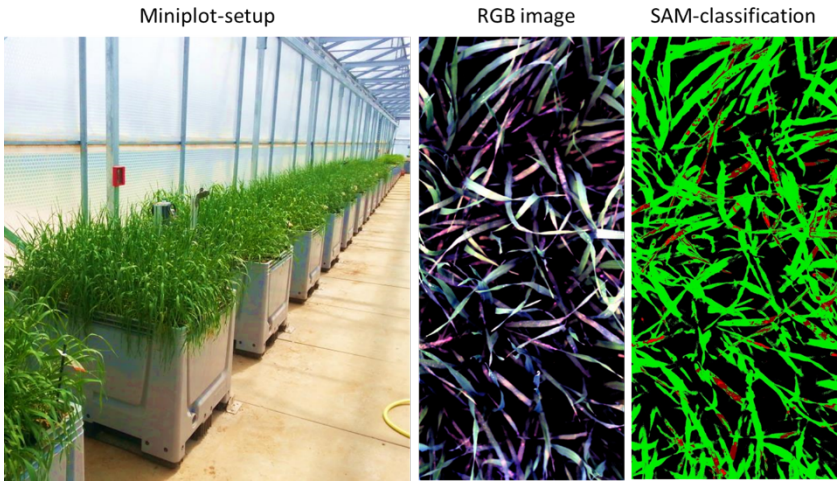

Figure 4 Measuring setup for the detection of plant diseases on the canopy scale and classification of powdery mildew diseased pixels of barley by spectral angle mapper classification (SAM).

Because the analysed spectra are transferred as vectors, variable illuminations due to the surface structure of the barley canopy were attenuated (darker pixel will plot along the same vector, but closer to the origin). With this approach a detection of disease symptoms (red pixels, Fig. 4) and an evaluation of the susceptibility of different cultivars was realized.

Discussion

It has been shown that hyperspectral imaging can be performed on different scales, from the tissue level to the canopy level. This offers several new applications for plant phenotyping or precision agriculture. Nevertheless, the scalability between hyperspectral close range-, greenhouse-, field- and remote imaging has to be investigated in mode detail in future research. From the technical side, a calibration of camera systems and different kind of measuring setups is essential (Behmann et al. 2015). Furthermore, the 3D structure of the object of interest influences the information in hyperspectral data. Thus, optical sensing benefits from combined hyperspectral and 3D measuring approaches (Behmann et al. 2016, Roscher et al. 2016, Wahabzada et al. 2015b). Another important factor, influencing the information content of hyperspectral images, is the spatial resolution and the amount of mixed pixels. This parameter strongly depends on the distance between the sensor and the object, thus airborne or space-borne, far-range systems have lower spatial resolution compared to near-range or microscopic systems. Sensors with a spatial resolution of about 1m are hardly suitable for the detection of single symptoms or diseased leaves and plants; here proximal sensor platforms are preferable (Oerke et al. 2014). The spatial resolution has a strong influence on the detection of plant diseases or plant-pathogen interactions (Mahlein et al. 2012). Increasing the distance between sensor and plant pathogen are challenging for the specificity and sensitivity. Nevertheless, identified characteristic hyperspectral signatures seems to be transferable on different scales. This enables a new approach for basic research in the lab and in the greenhouse to analyse the plant phenotype and genotype under different conditions and transferring this knowledge to remote sensing methods. Future research also has to consider improved sensor platforms and vehicles for field and high-throughput applications more intensively (Polder et al. 2015, Sankaran et al. 2010, Virlet et al. 2016,

Walter et al. 2015). It is expected, that new developments in the field of robotics will speed up this process.

Conclusion

There are multiple opportunities and technical solutions for an automated, objective and reproducible assessment of plant diseases by hyperspectral. As presented in this work, many of these technologies are still in a developmental phase. The time frame for a standardized invention into agricultural practice in precision agriculture or plant phenotyping can only be roughly estimated. Especially for field applications the impact of environmental factors, the plant architecture and the measuring setup has to be considered carefully.

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