Title: Spectral signatures in the UV-range can be combined with secondary plant metabolites by deep learning to characterise barley – powdery mildew interaction

Anna Brugger\textsuperscript{1*}, Patrick Schramowski\textsuperscript{2}, Stefan Paulus\textsuperscript{3}, Ulrike Steiner\textsuperscript{1}, Kristian Kersting\textsuperscript{4} and Anne-Katrin Mahlein\textsuperscript{3*}

\textsuperscript{1} University of Bonn, Institute for Crop Science and Resource Conservation (INRES)—Plant Diseases and Plant ProtectionPathology, Nussallee 9, 53115 Bonn, Germany

\textsuperscript{2} Technical University Darmstadt, Computer Science Department, Hochschulstrasse 1, 64289 Darmstadt, Germany

\textsuperscript{3} Institute of Sugar Beet Research, Holtenser Landstraße 77, 37079 Göttingen, Germany

\textsuperscript{4} Technical University Darmstadt, Computer Science Department and Centre for Cognitive Science, Hochschulstrasse 1, 64289 Darmstadt, Germany

*corresponding author: abrugger@uni-bonn.de mahlein@ifz-goettingen.de

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Abstract

In recent studies, the potential of hyperspectral sensors for the analysis of plant-pathogen interactions was expanded to the ultraviolet range (UV; 200-380 nm) to monitor stress processes in plants. A hyperspectral imaging set-up was established to highlight the influence of early plant-pathogen interactions on secondary plant metabolites. In this study, the plant-pathogen interactions of three different barley lines inoculated with *Blumeria graminis* f.sp. *hordei* (*Bgh*, powdery mildew) were investigated. One susceptible genotype (cv. Ingrid, wild type) and two resistant genotypes (Pallas 01, Mla1 and Mla12 based resistance and Pallas 22, mlo5 based resistance) were used. During the first five days after inoculation (dai) the plant reflectance patterns were recorded and in parallel plant metabolites relevant in host-pathogen interaction were studied. Hyperspectral measurements in the UV-range revealed that a differentiation between barley genotypes inoculated with *Bgh* is possible and distinct reflectance patterns were recorded for each genotype. The extracted and analyzed pigments and flavonoids correlated with the spectral data recorded. A classification of non-inoculated and inoculated samples with deep learning revealed that a high performance can be achieved with self-attention networks. The subsequent feature importance identified wavelengths, which were most important for the classification, and these wavelengths were linked to pigments and flavonoids. Hyperspectral imaging in the UV-range allows for a characterisation of different resistance reactions, can be linked to changes of secondary plant metabolites with the advantage of being a non-invasive method and therefore enables a greater understanding of the plants’ reaction to biotic stress as well as resistance reactions.

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Introduction

In recent plant phenotyping studies, the ultraviolet range (UV, 200-380 nm) has been used for the first time to record reflectance properties of plants by hyperspectral imaging (Brugger et al., 2019). An extension of spectral measurements to this range enables the consideration of secondary plant substances such as flavonoids with an absorption maximum in the UV-range (Taniguchi and Lindsey, 2018) (Tab.1). These secondary plant substances are produced in response to abiotic or biotic stress. Therefore, hyperspectral measurements in the UV-range can lead to more detailed knowledge about the plant’s response to stress factors.

The obligate biotrophic ascomycete Blumeria graminis f.sp. hordei (Bgh) causes powdery mildew on barley and influences the secondary plant metabolism. After infection with Bgh barley leaves show typical white pustules and the conidia of the pathogen are dispersed by wind. The primary and secondary germ tubes are developed within the first four hours and the penetration peg is developed 15 hours after infection. First fungal colonies are visible to the naked eye three to five days after infection (Both et al., 2005). An infection with Bgh leads to changes in plant metabolism and secondary plant metabolites like flavonoids, which are polyphenolic secondary plant metabolites. In the group of flavonoids, anthocyanins and flavonols have various tasks such as visual signals, auxin transport, and resistance against plant pathogens (Petrussa et al., 2013). High contents of flavonoids such as kaempferol and pelargonidin were found in plants exposed to high levels of UV-radiation (Monici et al., 1993). Anthocyanins protect chloroplasts from
UV-radiation but can also scavenge reactive oxygen species (Neill & Gould, 2003).

High levels were found in primary leaves of barley, where they are stored in the epidermis, because of their high absorptive properties of UV-light they can also be found in the mesophyll (Liu et al., 1995).

The flavone chrysin, present in different cereals (Liu et al., 2010) is often used to quantify flavonoids with spectrophotometric detection since their absorption maxima are at 240-290 nm as well as 310-370 nm (Mierziak et al., 2014). Flavonoids cannot only be synthesized by plants as a response to stress but also produced before the occurrence of stress and stored at important sites to play a direct role in the defence mechanisms (Treutter, 2006). Studies proposed that they can be stored in epidermal cells and are released into the infected tissue where they might be involved in hypersensitive responses (HR) (Beckman, 2000). In addition, degradation of plant pigments like carotenoids and chlorophyll a and b with an absorption maxima maximum at 400-500 nm (Taniguchi & Lindsey, 2018) can be linked to a changing photosynthetic activity due to compatible and incompatible interactions (Brugger et al., 2018). Not only flavonoids are affected by an infection of barley with \( Bgh \), but also other plant compounds. For example, genotypes susceptible to \( Bgh \) showed a reduced electron transport capacity due to a degraded photosystem II which results in a loss of chlorophyll during the infection development (Scholes et al., 1994).

Resistance breeding is a major protection strategy against \( Bgh \) infections in barley. The cultivar Ingrid (wild type (WT)) is susceptible to infections while the near-isogenic lines cv. Pallas has two isogenic lines 01 and 22, which are resistant and show no typical symptoms of a powdery mildew infection. Pallas 01 has a race specific resistance and possesses the resistant genes Mildew loci \( Mla1 \) and \( Mla12 \), which
cause a HR after recognizing specific $Bgh$ avirulence genes (Schulze-Lefert & Vogel, 2000). The near-isogenic line Pallas 22 contains a dysfunction in the mlo5 gene and has a broad spectrum broad-spectrum papilla based resistance against $Bgh$ (Kølster et al., 1986). A papilla or cell wall apposition (CWA) is quickly developed below the penetration point of the pathogen and prevents further infection development. CWAs contain phenols, belonging to the secondary plant metabolite group of flavonoids (Jørgensen 1992).

The susceptible and resistant barley-$Bgh$ interaction has previously been studied with hyperspectral imaging in the visible (400-700 nm) and near infrared range (700-1000 nm) with emphasis on reflectance and transmission data (Thomas et al., 2017; Kuska et al., 2015). Reflection measurements enabled an early detection of infection two days before colonies became visible by the naked eye (Thomas et al., 2017). In addition, the genotypes were differentiated according to their susceptibility to $Bgh$ (Kuska et al., 2015) and these data were combined with microscopic observations (Kuska et al., 2017) and results from invertase analysis (Kuska et al., 2018). At present, there is no research available that links the secondary metabolism of the plant with spectral changes of wavelengths. The genotypes used in this study serve as a model to prove the usability of the UV-range to describe phytopathogens and their effects on host plants.

Three hypotheses were investigated in this study: (i) an infection with $Bgh$ affects the secondary plant metabolites, (ii) the changes in secondary metabolism can be detected non-invasive with hyperspectral imaging and (iii) the relevant wavelengths can be narrowed down by combining the recorded hyperspectral data with deep learning algorithms (Fig. 1). The last point is particularly important in order to be able to limit future investigations to the relevant wavelengths of the UV-range in a
targeted and cost-saving manner.

Materials and methods

Plant cultivation and pathogen inoculation
The barley lines cv. Ingrid wild type (WT), Pallas 01 (Mla12), and Pallas 22 (mlo5) were grown in a greenhouse environment in plastic pots (5x5 cm) on commercial substrate (Topfsubstrat 1.5, Balster Erdenwerk GmbH, Sinntal-Altengronau, Germany) and watered, as necessary. After 10 days when reaching growth stage 11 (Witzenberger et al., 1989) the primary leaves were cut at 10 cm and placed on 10 g/l phyotagar (Duchefa Biochemie B.V, Haarlem, Netherlands) containing 0.34 mM benzimidazole (Kuska et al., 2015). For each genotype 10 leaves were kept untreated as a control group while eight technical replications with 5 leaves each were inoculated with fresh spores of Blumeria graminis f.sp. hordei isolate A6. The agar plates were sealed and incubated at 19°C in a controlled environment with 1100 cd x m⁻² illuminance and a photo-period of 16 h per day.

Extraction of secondary plant metabolites
For the extraction of secondary plant metabolites samples of inoculated and non-inoculated barley leaves of all three genotypes were collected 1 to 5 dai and kept in the freezer at -80°C until analysis. The extraction was carried out for 6 biological replications.

Chlorophyll and carotenoid extraction
Chlorophyll and carotenoid extraction was performed according to (Scholes et al., 1994). Frozen leaf samples with 0.5 M HClO₄ were grounded in liquid nitrogen to a
fine powder. Subsequently, 0.5 g per sample was transferred into a tube stored on ice and 1.5 ml of 80% acetone was added. The samples were kept for 3 h on ice in the dark and mixed every 20 min. Following the samples were centrifuged by 4°C at 13,000 rpm for 20 min and the absorption of the extract was measured at 470, 645, and 663 nm. The concentration of chlorophyll as well as carotenoid was calculated according to (Hiscox & Israelstam (1979).

**Total flavonoid extraction**

Total flavonoid extraction was performed according to Mihai et al., (2010). Frozen leaf samples were grounded in liquid nitrogen to a fine powder and were extracted with 96% ethanol. For this 1 g of each sample was mixed with 30 ml ethanol and kept overnight with constant stirring. Following the samples were filtered on qualitative filter paper for three times before the volume was added up to 100 ml for an initial extract concentration of 1%. Of this extract 3 ml were mixed with 1 ml 2.5 % ZrOCl₂ and 21 ml methanol were added. After 30 min the absorption was measured at 288 nm against a blanc solution consisting of methanol. A calibration curve was established using chrysin. For this, a stock solution with 0.1 mg/ml was prepared and aliquots of 0.25, 0.5, 1, 1.5, and 2 ml were used. The measured absorbance was plotted against the concentration to establish the calibration curve.

**Hyperspectral image acquisition and data preprocessing**

Spectral reflectance was recorded with a hyperspectral imagine line scanner in the UV-range according to (Brugger et al., 2019). with an exposure time of 800 ms, a frame rate of 0.4 frames per second and a linear axis speed of 0.3 mm/s. The reflectance was daily measured 1 to 5 days after inoculation (dai). The relative

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reflectance was calculated using the software Headwall Hyperspec III (Headwall Photonics, Bolton, MA, USA) and for this purpose, a white reference image of 95 % barium sulfatesulphate and a dark current image were recorded. Data was then analysed with the software ENVI 5.5 (Exelis Visual Information Solutions, Boulder, CO, USA). An amount of manually selected 7500 pixels of each sample was used to calculate the average reflectance. The selected pixels covered the entire barley leaf. A Savitzky-Golay filter with a window size of 7 and a 3rd degree polynomial was used to pre-process the data. The Savitzky-Golay filter and the corresponding parameters were selected to reduce the signal noise while preserving the properties of the signal distribution (Madden, 1978). Figures were generated with SigmaPlot 14 (Systat Software GmbH, Erkrath, Germany). RGB visualization was performed 1 to 5 dai.

**Experimental Setup of classification of non-inoculated and inoculated samples**

The superpixels of the data were used as input for the (deep) learning algorithms to distinguish the measurements into non-inoculated and inoculated samples. A superpixel is defined as the average of $P \times P$ neighboring pixels. A spatial area with $P=7$ was selected so it is likely to contain symptoms. The computed areas are non-overlapping. The data were split into two different sets for training and testing. The test set contained 20% of the data and the results were cross-validated so that five different models were trained and evaluated for each classification task.

**Determination of relevant features with self-attention classification networks**

To determine the meaning of the characteristics of the hyperspectral data, the neural network architecture Self-Attention Networks (SAN, Skrlj et al., 2020) is used. SANs are motivated by recent advances in natural language processing, e.g., through the
language model BERT (Devlin et al., 2018) with the Transformer Network Architecture (Vaswani et al., 2017). A key feature of these architectures is the so-called self-attention mechanism. Skrlj et al., (2020) have shown that self-attention can also be used to identify the relevant features per data point and that doing so can result in better classification accuracy than previous methods. The network architecture can be described as follows

\begin{equation}
K
\end{equation}

\begin{equation}
\Omega
\end{equation}

where $K$ is the number of parallel self-attention blocks $\Omega$, $X$ is the input, and $W$ as well as $b$ are learnable parameters of the network. The functions $a$ and $\sigma$ are activation functions. In this case $a$ is a SELU (Klambauer et al., 2017) and $\sigma$ softmax function. The symbol $\square$ corresponds to the Hadamard product while the symbol $\oplus$ refers to the Hadamard sum formation over individual blocks. The first neural network layer is used especially for maintaining the connection with the input features $F$. The input vectors are first used as input for the softmax-activated layer, whose neurons match with the number of features $F$. The softmax function is defined as following:

\begin{equation}
F
\end{equation}

The self-attention mechanism effectively creates a sparsely populated input area and only emphasizes relevant features for solving the task at hand (e.g. language comprehension). In this way, the self-attention mechanism is often used to, e.g., better learn the relationships between words (Skrlj et al., 2020). In the present paper, SAN is used for the classification of individual signatures (pixels) or combined signatures (superpixels) of hyperspectral data in the UV-range, classified between healthy and inoculated samples of different genotypes. The networks’ self-attention mechanism weights the input features and uses the weighted output for the neural
classification network. Two self-awareness heads and a neural classification network with 64 latent neurons were selected for the Self-Attention Networks (SAN). The final feature importance is calculated by averaging the feature importance of the two heads

\[ \text{(4)} \]

where \( X \) is the evaluated set of inputs and in this case \( k=2 \). The training setup of by Skrlj et al., 2020 was followed and the network was trained with the commonly used Adam optimization algorithm (Kingma & Ba, 2014). However, the described batch size seemed to be very low. To achieve a more stable training it was increased to 128 samples. A learning rate of 0.001 was used and it was gradually reduced during training. In a pre-processing step, the first wavelengths were removed, since they were characterized by increased noise, resulting in an input wavelength range from 260.232 nm to 501.219 nm.

**Gradient Boosting as classification method**

The framework XGBoost (Chen & Guestrin, 2016) was used to implement the baseline Gradient Boosting (GB) classifier. The machine learning technique GB can be used for classification as well as regression problems and generates a predictive model in the form of an ensemble of weak predictive models, usually decision trees.

As in other boosting methods the model is build up stage-wise. The hyperparameters to optimize the GB model were chosen empirically. The reported results were achieved using a maximum tree depth of 6 and a learning rate of 0.3. Further, a L2 regularization was used with the weight \( \lambda = 1 \).
Results

**Impact of compatible and incompatible barley Bgh interactions on pigment and flavonoid concentration**

Chlorophyll, carotenoid, and total flavonoid content presented changes depending on the resistance of the host plant. The total chlorophyll content of all genotypes decreased from 1 to 5 dai. In WT leaves there was a strong decrease from 5.2 µg/ml 1 to 3.1 µg/ml 5 dai, whereas in mlo5 leaves only a slight decrease from 5.7 µg/ml 1 dai to 5.1 µg/ml 5 dai was observed (Fig. 2). The carotenoid extraction revealed a similar pattern for WT leaves. The highest value was measured 1 dai and decreased by 76% until 5 dai. Mla1 leaves demonstrated a strong decrease from 2.2 µg/ml 1 dai to 1.4 µg/ml 3 dai but exhibited no further decrease 5 dai. mlo5 leaves were represented by a constant carotenoid concentration. The flavonoid content of WT leaves decreased from 49% 1 dai to 40% 5 dai. Mla1 leaves displayed a decrease of 12% from 1 to 3 dai and stayed constant 5 dai. mlo5 leaves demonstrated an increase from 47% 1 dai to 59% 3 dai and remained constant 5 dai.

**Phenotypic development of Bgh on barley leaves**

The phenotypic development of non-inoculated as well as with Bgh inoculated barley leaves of Ingrid and the near-isogenic lines Pallas 01 and Pallas 22 were visualized with RGB images (Fig.3 and Fig.4). Non-inoculated leaves of all three genotypes were represented by constant vitality and showed no visible disease symptoms (Fig.4). Inoculated leaves of the susceptible WT exhibited senescence starting 3 dai and as well as typical white pustules 4 dai. These pustules were unevenly distributed on the leaves and covered approximately 60% of the leaves 5 dai. Chlorotic tissue was seen in the area around powdery mildew pustules. Resistant Mla1 and mlo5
leaves showed no typical symptoms of infection with *Bgh*. Starting 4 dai *Mla1* leaves displayed HR, visible under the microscope as necrotic brown spots heterogeneously distributed over the leaves.

**Spectral signatures of non-inoculated and with *Bgh* inoculated barley lines**

Spectral signatures enabled a differentiation between the different host-pathogen interactions. Distinct peaks between 250 and 312 nm e.g. 258 nm, 266 nm, or 271 nm characterized the reflectance of each sample. Between 410 and 440 nm, additional peaks are visible at e.g. 421 nm, 423 nm, or 439 nm (Fig. 5). The highest reflectance of each measurement was recorded at 254 nm at a value of 0.4%. Non-inoculated barley leaves demonstrated constant spectral signatures with a decrease in reflectance from 0.4% at 255 nm to 0.15% at 450 nm. The reflectance remained constant during the entire time-series measurements from 1 to 5 dai. Inoculated susceptible WT leaves presented a decreasing reflectance at 254 nm from 0.42% at 1 dai to 0.38% at 3 dai before reaching the highest value of 0.47% at 4 dai. This change in reflectance was continuous from 250 to 400 nm before the reflectance of all measurements was similar between 418 to 430 nm at 0.14%. The smallest reflectance at 500 nm was measured at 1 dai with 0.12% and increased until 5 dai to 0.145 %. The race-specific resistant genotype *Mla1* exhibited the smallest reflectance at 254 nm 1 dai, increased each day, and reached 0.47% at 5 dai. The highest value was recorded 4 dai with 0.48%. Similar to the susceptible WT the reflectance of all measurements was homogeneous from 418 nm onward. The highest reflectance at 500 nm was measured at 5 dai at 0.145%. The broadband resistant genotype *mlo5* showed the highest reflectance at 254 nm 1 dai with 0.44% and decreased until 0.37% 5 dai. At 418 nm all measurements displayed a similar
reflectance except 5 dai, which was characterized by a smaller reflectance. From 460 nm onward, the reflectance of all measurements was similar and reached 0.1% at 500 nm.

All non-inoculated leaves demonstrated constant spectral signatures while inoculated WT leaves presented an overall decrease in reflectance until 3 dai before increasing and reaching the highest values 4 dai. Resistant Mla1 leaves displayed an increase from 250 to 418 nm which daily increased until the highest values were reached 5 dai. Resistant mlo5 leaves daily decreased in the range from 250 to 418 nm and were represented by low values 5 dai.

**Classification of non-inoculated and inoculated samples with deep learning**

The superpixels of the data were used as input for the (deep) learning algorithms to distinguish the measurements into non-inoculated and inoculated samples.

A superpixel is defined as the average of $P \times P$ neighboring pixels. A spatial area with $P=7$ was selected so it is likely to contain symptoms. The computed areas are non-overlapping. As classification method, a neural network architecture with attention mechanism so called self-attention networks (SAN) (Skrlj et al., 2020) was chosen. Attention-based neural networks are a rather novel deep learning architecture which have achieved recent advances in various fields. However, they have not been considered as feature importance extractors for biological or hyperspectral data. The attention mechanism in the first layers of the network was used to classify particularly important elements of the feature space and filter them out of the rest. Afterward, the classification layer used the as important identified parts to classify the input. The data were split into two different sets for training and testing. The test set contained 20% of the data and the results were cross-validated.
so that five different models were trained and evaluated for each classification task. Table 2 shows the achieved accuracy (average and standard deviation) of the SAN compared to the well-established Gradient Tree Boosting (GB) method (Chen & Guestrin, 2016). With this, SAN consistently achieved higher performances and both methods have the highest accuracy at 1 and 5 dai. WT genotype displayed values of 89% 5 dai while Mla1 and mlo5 achieved 91% for the SAN method.

**Feature importance identifies relevant wavelengths**
The self-attention block of the trained network was used to determine the feature importance. For this, the softmax-activated output of the self-attention block was extracted. Fig. 6 visualizes the average over the cross-validated models. Only features whose weighting is above 5% are highlighted for easy presentation. In all three genotypes, 262 or 264 nm were identified as important wavelengths, for WT leaves 263 nm was identified as well. In addition, 280 and 478 nm were also identified for the susceptible genotype. For both resistant genotypes, 501 nm was additionally identified.

**Changes of secondary plant metabolites can be linked to relevant wavelengths**
The wavelengths identified by feature importance are in the range from 262 to 291 nm and 442 to 500 nm. In both ranges, WT and Mla1 leaves were represented by an increase of reflectance from 1 to 5 dai and also showed a decrease of chlorophyll, carotenoid, and flavonoids. mlo5 leaves displayed a decrease of reflectance in the range from 262 to 291 nm and an increase of flavonoids but similar values of reflectance were recorded in the range from 442 to 500 nm from 1 to 5 dai and extraction experiments presented a small decrease of chlorophyll and constant...
value of carotenoid content.

Discussion

In this study, hyperspectral imaging in the UV-range was used to investigate changes of secondary plant metabolites as well as pigments in susceptible and resistant barley powdery mildew interactions. It is well described that hyperspectral imaging can provide non-invasive information on host-pathogen interactions by assessing specific changes in plant reflectance patterns, which can be associated with physiological processes (Thomas et al., 2018, Mahlein et al. 2018). Near-isogenic barley lines with different susceptibility towards Bgh have been previously studied with hyperspectral imaging and revealed different dynamics over time (Kuska et al., 2015; Kuska et al., 2017). First studies connected multispectral imaging in the visible and near infrared range with the activity of invertase isoenzymes and analyzed the activity of cell wall, cytosolic, and vacuole invertase. Susceptible and resistant genotypes demonstrated specific dynamics and resistant genotypes showed an increased cell wall invertase (Kuska et al., 2018). Although this shows that multispectral imaging and invertase analysis complement each other, the correlation between hyperspectral imaging data and plant metabolites has not been previously studied. Therefore, this study of resistant and susceptible plant-pathogen response demonstrates the ability of UV hyperspectral imaging in combination with deep learning to identify changes of secondary plant metabolites and follows the workflow of Figure 1..

The development of Bgh on WT leaves was as described (Both et al., 2005) and typical white pustules were visible 4 dai. Inoculated resistant leaves containing a mlo mutation showed no symptoms as CWAs were formed at the penetration site, thus

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stopping further development (Jørgensen et al., 1994). Inoculated plants containing
the resistant Mla1 gene also showed no symptoms but from 4 dai on brown necrotic
spots were visible on the leaves. Once the penetration peg ruptures the cell wall and
enters the epidermal cell, a race-specific resistance gene recognizes the Bgh
avirulence factors and H₂O₂ is produced (Caldo et al., 2004). H₂O₂ triggers a HR and
causes cell death of penetrated epidermal cell, which is visible in brown, necrotic
spots (Schulze-Lefert & Vogel, 2000).

The interaction of the sensor and illumination caused significant peaks from 250 to
321 nm as well as 410 to 440 nm which could not be removed by normalization.
Therefore, a correlation of these wavelengths to specific secondary plant metabolites
cannot be verified. Untreated barley leaves showed no changes in spectral
signatures and no senescence during the observation period, which corresponds to
the phenotypic development in of barley leaves (Fig. 3). The increase in reflectance
of inoculated WT leaves from 450 nm onward can be linked to a decrease in plant
pigments like chlorophyll and carotenoids (Brugger et al., 2018.; Scholes & Rolfe,
1994) and is also reflected in the pigment analysis. Previous studies showed that
already 1 dai, susceptible WT leaves show a reduced photosynthetic performance
due to reduced pigment content in the leaves (Brugger et al., 2018). Similar toLike
WT, Mla1 leaves showed an increase in reflectance from 450 to 500 nm starting 2
dai and a corresponding decrease in chlorophyll and carotenoid content. It is
described that Mla resistance leads to a deterioration of photosynthesis and pigment
metabolism, as HR causes necrosis (Matile et al., 1999). Previous studies with
hyperspectral imaging showed that a reduction of plant pigments can be already
detected 2 dai on Mla1 leaves (Kuska et al., 2017) confirming the results in the
UV-range. Infected mlo5 leaves showed no changes in reflectance at 450-500 nm

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and demonstrated an almost constant pigment content. Due to the formation of CWA mlo, resistant leaves are known for only small decreases in photosynthesis activity (Scholes & Rolfe, 2009) resulting in a high pigment content. Compared to mlo5, WT and Mla1 leaves exhibited a faster senescence visible as an increase in reflectance while mlo leaves are known to display a delay in senescence and greening effect after inoculation (Kuska et al., 2015).

During the pathogenesis 1 to 5 dai, reflectance increased in the WT leaves from 250 to 370 nm, which can be associated with a decrease of flavonoids, since their absorption maxima are in the range of 240-290 nm and 320-370 nm (Mierziak et al. 2014). The flavonoid content of WT leaves slightly decreased justified by the fact that demonstrated high values 1 dai and decreased by 8% until 5 dai. Flavonoids act as catalysts in photosynthesis, which is downregulated in Bgh infections, resulting in a reduced need for flavonoids and subsequent downregulation of the flavonoid metabolism. In addition, flavonoids are categorized into preformed and induced compounds, whereby preformed flavonoids are produced during normal plant development and stored at important sites, e.g., epidermal cells. There they can play a direct role in the defense of pathogens or act as signaling compounds (Treutter, 2005). For this reason, a decrease of flavonoids in susceptible barley leaves after Bgh infection is plausible and is evident both in the recorded spectral reflectance and in the analysis. An influence of the UV-radiation on the leaves can be excluded, as this would have led to higher levels of flavonoids due to their protective function against UV-light (Monici et al., 1993). Mla1 leaves displayed a decrease of flavonoids from 1 to 3 dai before exhibiting constant values until 5 dai. Phenolic compounds to which the group of flavonoids belongs, are stored in epidermal cells and especially in the case of HR and the associated cell death, are
released rapidly in the infested tissue (Beckman, 2000). As this occurs in a
Mla1-based resistance and results in a decrease in the flavonoid amount, the
decrease of flavonoids in inoculated Mla1 leaves can thus be explained. In addition,
a previous study suggested that the release of flavonoids occurs particularly in early
stages of infection, which explainings the strong decrease of flavonoids from 1 dai to
3 dai in the present study. In contrast to inoculated WT and Mla1 leaves, mlo5 leaves
featured a decrease of reflectance from 250-370 nm during time-series
measurements corresponding to the strong increase of flavonoids 3 dai. Flavonoids
are involved in auxin metabolism, which causes a tightening of the plant tissue
(Beckman, 2000). This leads to the formation of callose and cell wall-phenolics
(von Roepenack et al., 1998), explaining an increase of flavonoids 3 dai. Additionally,
studies with barley and Fusarium head blight revealed an accumulation of flavonoid
glycosides as a resistance response against the fungi to strengthen cell walls and
limit the infection (Karre et al., 2019). Similar patterns between WT and Mla1
leaves can be explained by the previous mentioned early senescence after an
inoculation while mlo5 leaves displayed a characteristic delay in senescence. The
analysis of flavonoids and pigments reflected the spectral signatures of each
genotype so that a connection between both can be established.

In this work neural networks with self-attention mechanisms were used for the
classification of healthy and diseased plants, identifying the most relevant parts of the
input for this task. Due to the decreasing costs of cameras, hyperspectral imaging
becomes more and more popular, researchers and developers have better access to
this technology (Mahlein et al., 2019). Since hyperspectral imaging leads to large
amounts of data, deep learning methods have been used to identify plant diseases
such as the use of deep convolutional neural networks to identify charcoal rot

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disease in soybean stems (Nagasubramanian et al., 2019) and other applications (Tetila et al., 2019; Polder et al., 2019, Schramovski et al. 2020).

Data mining methods were also used to localize HR from hyperspectral imaging data before they were visible on RGB images (Kuska et al., 2017). A study reviewed the trends and future perspectives of using deep learning techniques for plant stress phenotyping and state the great promise for improving the speed, accuracy, reliability, and scalability of disease phenotyping (Singh et al., 2018). The classification of non-inoculated and inoculated samples with deep learning revealed that SAN consistently achieved higher performance compared to GB. This aligns with previous findings that applying SAN results in an improved performance on data sets with a large number of many features (Skrlj et al., 2020). The classification performance was cross-validated as well as the feature importance identification utilizing SANs and the results were verified by a biological investigation. However, different (deep learning) approaches, for instance attribution methods such as LIME (Riberio et al. 2016) and saliency maps (Karen et al. 2014), could result in a deviating importance of elements in the input space, which depends among other things on the general performance of the underlying model. But one of the advantages of SAN is that the feature importance is computed during inference and one has not to rely on an external linear approximate of the deep learning model such as used in LIME (Riberio et al. 2016). Nevertheless, future work should address different kinds of (deep) feature importance extractors and analyze their differences.

It is striking that both SAN and GB have the highest accuracy at 1 and 5 dai. The high accuracy of 1 dai can be explained by the inoculation methodology since the samples were inoculated by using a brush to apply the Бgh spores on the leaf immediately before the first measurement. While non-inoculated leaves were treated
with a clean brush, fresh spores covered the surface of inoculated leaves and therefore changed the optical properties of the plant, which eased the classification. High classification accuracy 5 dai are linked to visible symptoms on WT and Mla1 leaves and noticeable higher reflectance of mlo5 leaves. The feature importance identified 264 nm as the most important wavelength for the classification of all three genotypes, and since flavonoids feature an absorption maximum at 240-290 nm (Mierziak et al., 2014) this wavelength can be linked to flavonoids. Other wavelengths with a slighter feature importance value were identified at 442 as well as 478 nm and since chlorophyll and carotenoids feature absorption maxima from 400-500 nm (Lichtenthaler 1987) these wavelengths were linked to a change in pigments.

Secondary plant metabolites which can be linked to the by feature importance identified wavelengths revealed in extraction experiments changes in content that were also consistent with the spectra recorded in the UV-range. This can be used as information to characterize different host-pathogen interactions as they lead to differences in secondary plant metabolites, which was also reflected in changes in reflectance. Thus, it could be shown that hyperspectral imaging in the UV-range leads to information about changes in secondary plant metabolites such as chlorophyll, carotenoid, and flavonoids depending on susceptibility or resistance reaction.

This study showed that spectral information in the UV-range of different host-pathogen interactions corresponds to changes of secondary plant metabolites. Specific resistance responses in incompatible barley-Bgh interactions can also be differentiated by spectral reflectance. In addition, deep learning revealed that these secondary plant metabolites can be linked to wavelengths which are of importance.
for the classification of healthy and diseased plants. In summary, hyperspectral imaging in the UV-range and deep learning allows for a better understanding of susceptible and resistant responses of plants and can be used as a non-destructive tool to achieve a deeper knowledge about plant-pathogen interactions.

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Competing interest
The authors declare that they have no competing interests.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Table 1:** List of important secondary metabolite which feature an absorption maximum in the UV-range.

**Table 2:** Cross-validated classification results (mean ± standard deviation) of all three genotypes 1, 3 and 5 days after inoculation. The accuracy of self-attention

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networks (SAN) is shown in comparison to the established Gradient Tree Boosting (GB) method and standard deviation is mentioned for each classification result.

**Figure legends**

**Figure 1:** Work-flow of the investigation if spectral images in the UV-range can be linked with changes of secondary plant metabolites.

**Figure 2:** Pigment and flavonoid analysis of susceptible and resistant barley-*Bgh* interactions. Total chlorophyll, carotenoid and flavonoid extraction of non-inoculated barley leaves as well as barley leaves inoculated with *Bgh* of Ingrid WT, Mla1 and mlo5. Analysis was performed 1, 3 and 5 days after inoculation and standard deviation is indicated. Small letters stand for significant difference between dai per genotype for which data was tested with Kolmogorov-Smirnov-Test for normal distribution with $p \leq 0.05$ and aTukey’s range test was applied with $\alpha = 0.05$.

**Figure 3:** Spectral signatures of non-inoculated and inoculated barley leaves and RGB visualization. Spectral signatures of non-inoculated barley leaves (a,c,e) as well as barley leaves inoculated with *Bgh* (b,d,f). Ingrid WT, Mla1 and mlo5 were used and incubated on phytoagar. RGB images of inoculated leaves 1 to 5 days after inoculation are displayed on the right.

**Figure 4:** RGB images of non-inoculated and inoculated WT, *Mla1* and *mlo5* 1 to 5 days after inoculation. Non-inoculated leaves of all genotypes displayed a constant vitality while inoculated leaves showed a typical resistant or susceptible reaction.
**Figure 5:** Visualization of relevant peaks due to the interaction of the sensor and illumination which were not considered in this investigation. Peaks are identified between 250 and 312 nm (258 nm, 266 nm, 271 nm) as well as between 410 and 440 nm (421 nm, 423 nm, 439 nm).

**Figure 6:** Resulting feature importance of the classification network to differentiate between inoculated and non-inoculated data of the genotypes WT, Mla1 and mlo5. For each genotype, wavelengths with a weighting of more than 5 % are highlighted and standard deviation is indicated. Wavelengths from 262 to 291 nm were connected with flavonoids while wavelengths from 442 to 500 nm with chlorophyll as well as carotenoids.
| Secondary plant metabolite | Absorption maxima | Source |
|----------------------------|-------------------|--------|
| Acriflavine                 | 396 nm            | Reiber, A., Leyshon, L., Saunders, D., Mijovic, M., Bright, A., Bogie, J.: Fluorescence of aromatic benzoxazole derivatives. Journal of the American Chemical Society94(7), 2411–2421 (1972) |
| Anthocyanin                | 270-290 nm        | Woodall, O., Stewart, C.: Do anthocyanins play a role in UV protection of the red juvenile leaves of actyioum? Journal of Experimental Botany46(325), 1447–1450 (1995) |
| Carotenoids                | 400-500 nm        | Lichtenhaker, H.K., Buschmann, C.: Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical chemistry1(1), 4–3 (2001) |
| Chlorophyll a              | 428 nm            | Seely, O., Jensen, R.: Effect of solvent on the spectrum of chlorophyll. Spectrochimica Acta21(16), 1835–1845 (1965) |
| Chlorophyll b              | 455 nm            | Seely, O., Jensen, R.: Effect of solvent on the spectrum of chlorophyll. Spectrochimica Acta21(16), 1835–1845 (1965) |
| Coumarin                   | 311 nm            | Abu-Eittah, R.H., El-Tawil, B.A.H.: The electronic absorption spectra of some coumarins. A molecular orbital treatment. Canadian Journal of Chemistry82(6), 1173–1179 (1995) |
| Flavonoids                 | 240-290 nm, 310-370 nm | Mierzlak, J., Kostyn, K., Kulma, A.: Flavonoids as important molecules of plant interactions with the environment. Molecules19(10), 16240–16265 (2014) |
| Hydroquinone               | 294 nm            | Stalin, T., Devl, R.A., Rajendran, N.: Spectral characteristics of ortho, meta and para dihydroxy benzenes indifferent solvents, pH and β-cyclodextrin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy61(11-12), 2495–2504 (2005) |
| Phenol                     | 271 nm            | Stalin, T., Devl, R.A., Rajendran, N.: Spectral characteristics of ortho, meta and para dihydroxy benzenes indifferent solvents, pH and β-cyclodextrin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy61(11-12), 2495–2504 (2005) |
| Quinoline                  | 313 nm            | Snyder, R., Tesia, A.: Influence of electron-donor-acceptor complexation on electronic relaxation of quinoline. The Journal of Physical Chemistry68(24), 5948–5950 (1984) |
### Table 2

| Genotype | dai | GB (in %) | SAN (in %) |
|----------|-----|-----------|------------|
| **WT**   | 1   | 84.73±1.71| 99.35±0.75 |
|          | 3   | 76.00±1.39| 79.59±1.87 |
|          | 5   | 84.31±1.94| 89.26±0.82 |
| **Mfa1** | 1   | 85.96±1.51| 96.10±1.77 |
|          | 3   | 75.99±0.66| 78.65±1.54 |
|          | 5   | 81.70±0.81| 91.09±1.63 |
| **mlo5** | 1   | 83.62±1.15| 99.19±0.51 |
|          | 3   | 74.99±2.28| 82.12±2.48 |
|          | 5   | 84.34±2.22| 91.06±2.35 |
