Identification of powdery mildew (Erysiphe graminis sp. tritici) and take-all disease (Gaeumannomyces graminis sp. tritici) in wheat (Triticum aestivum L.) by means of leaf reflectance measurements

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Abstract: The ability to identify diseases in an early infection stage and to accurately quantify the severity of infection is crucial in plant disease assessment and management. A greenhouse study was conducted to assess changes in leaf spectral reflectance of wheat plants during infection by powdery mildew and take-all disease to evaluate leaf reflectance measurements as a tool to identify and quantify disease severity and to discriminate between different diseases. Wheat plants were inoculated under controlled conditions in different intensities either with powdery mildew or take-all. Leaf reflectance was measured with a digital imager (Leica S1 Pro, Leica, Germany) under controlled light conditions in various wavelength ranges covering the visible and the near-infrared spectra (380 -1300 nm). Leaf scans were evaluated by means of L*a*b*-color system. Visual estimates of disease severity were made for each of the epidemics daily from the onset of visible symptoms to maximum disease severity. Reflectance within the ranges of 490 - 780 nm ($r^2 = 0.69$), 510 - 780 nm ($r^2 = 0.74$), 516 - 1300 nm ($r^2 = 0.62$) and 540 - 1300 nm ($r^2 = 0.60$) exhibited the strongest relationship with infection levels of both powdery mildew and take-all disease. Among the evaluated spectra the range of 490-780 nm showed most sensitive response to damage caused by powdery mildew and take-all infestation. The results of this study indicated that disease detection and discrimination by means of reflectance measurements may be realized by the use of specific wavelength ranges. Further studies have to be carried out, to discriminate powdery mildew and take-all infection from other plant stress factors in order to develop suitable decision support systems for site-specific fungicide application.

Keywords: Plant disease, leaf reflectance, wavelength ranges, powdery mildew, take-all disease

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1 Introduction

The importance of spatial information in integrated pest management is well recognized [1, 2]. Plant diseases often occur in their initial phase of epidemiology in patches within the crop stand [3]. However, up to now the spatial relationships between disease development, crops and heterogeneous environmental parameters has received insufficient attention. Thus, the common practice of disease control in arable crops is to apply fungicides uniformly over the entire field. A variable rate application of fungicides according to disease incidence would help to optimize the use of production inputs and would help to reduce the input of biocides into the environment.

The potential of site-specific differentiated application of fungicides is still untouched due to the lack of sensors that are able to sense either crop parameters influencing growth of fungi, or fungi on or in the plants directly. Historically, early stress-induced changes in plants have mainly been detected after destructive sampling followed by biochemical and molecular determinations. As traditional field sampling of fungi infestation or plant parameters is time and cost consuming, there is an increased interest in finding simple and cost-effective optical means for remote sensing of diseases at the earliest possible stage. Imaging techniques that allow immediate detection of stress-situations before visual symptoms appear and adverse effects become established are emerging as a promising tool for crop disease and yield management. Sasaki et al. (1998) proposed a spectral reflectance technique for disease diagnosis on cucumber leaves [4]. Lorenzen and Jensen (1989) showed that reflectance of barley leaves infected with powdery mildew changed significantly 6 d after inoculation [5]. They suggested that the degradation of chlorophylls may lead to a change of reflectance in the visible spectra. Work has also been conducted using measurements of spectral reflectance from vehicle-mounted sensors as the basis for varying fungicide doses within a field with variable results. Results reported by Secher (1997) suggested that significant increases in yield of winter cereal crops could be achieved by distributing the dose of a fungicide in relation to crop canopy characteristics as measured using boom-mounted radiometers [6]. Nutter et al. (1993) reported that multispectral sensors could provide a non-destructive means of quantifying spot severity in bentgrass, as plant stresses modify the spectral characteristics of a canopy surface [7]. Multispectral sensors have also been used to assess the severity of early blight of tomato [8], rust and late leaf spot of peanut [9]. Nilsson (1991) reported significant correlations between spectral reflectance data and cereal plant growth, green biomass, yield, and symptoms of net blotch in barley, glume blotch in winter wheat, and take-all in spring wheat [10]. The existing techniques for disease detection can be applied on scales ranging from microscopic observation to airborne remote sensing and have recently been reviewed by Chaerle et al. (1999, 2001) [11, 12].

In general, the detection of crop stress by remote sensing is based on the assumption that stress factors interfere with photosynthesis or the physical structure of the plant, affecting the absorption of light energy leading to an alteration in the reflectance spectrum of the plant [13–15]. Diseases may cause changes in pigment concentration and variation
in canopy gas exchange, leading to differences in color and temperature that can alter canopy reflectance characteristics [16]. In a healthy leaf, wavelengths in the 400 to 700 nm range are absorbed by photosynthetic pigments, particularly blue (425 to 492 nm) and red (645 to 700 nm) light [17]. Near infrared radiation (700 to 1300 nm) is reflected from plant cells deep within the leaf, and high internal scattering within leaf tissue is responsible for higher reflectance peaks compared to other wavelengths [18]. Plant stresses that cause a reduction in chlorophyll content may lead to an increase in light reflected in the visible range. Reflectance in the infrared region is also reduced as healthy leaf area is decreased by diseases and pests [19]. Remote sensing technologies would allow one to monitor disease development in their early stages, and help to avoid irreversible damage and substantial yield losses.

A further challenge of disease identification using remote sensing technologies lies in the fact that diseases may have various physiological impacts on their plants, such as stunting growth, causing leaf drop, interfering with nutrient uptake, or reducing seed viability [20]. The different physiological effects of the fungus depend on the nature of the interaction with the host. Foliar pathogens affect their host in a number of ways, including reducing photosynthesis in infected leaves, increasing water loss through transpiration [21], and diminishing water uptake by the roots [22]. However, root–born or systemic pathogens are often much harder to detect [20]. Therefore, suitable sensor technologies for disease identification under the scope of precision farming have to address and identify various disease categories.

Powdery mildew, caused by the obligate biotroph Erysiphe graminis sp. tritici, is the most dominant fungal disease encountered in German cereal production. Disease severity is dependent on many factors, including cultural practices, variation in weather conditions, level of cultivar susceptibility, and regional and in-field location. The disease may occur during all stages of growth. The fungus Gaeumannomyces graminis sp. tritici known as take-all disease affects the roots of wheat and is recognized as a serious limitation to wheat production [23, 24]. The fungus has received increasing attention in Europe over the past few years because of the increasing proportion of winter wheat crops, and cereal production in a monoculture favoring the conditions for take-all. The fungus can cause damage to the roots, which limits the uptake of nutrients and water and results in loss of yield.

The objective of this work was to assess the possibilities for non-destructive, site-specific sensor technologies for the identification of plant diseases. This study extends the work of Graeff et al. (2001, 2003) to determine if reflectance measurements in the visible and near-infrared (380-1300 nm) spectra can be effectively used to identify and discriminate different stress factors in plants [25, 26]. A greenhouse study was conducted with powdery mildew and take-all in wheat representing two different categories of fungi. As these diseases lead to different physiological effects at the plant level, it is assumed that reflectance patterns differ significantly between these diseases and enable the identification and discrimination. The overall aim of this study was: to assess the potential of leaf reflectance measurements to identify different categories of plant diseases, to evalu-
uate if diseases occurring at different loci of the plant limit the suitability of reflectance measurements for disease identification, and to investigate if reflectance measurements are to be of value for site-specific fungicide application and detection of diseases before the symptoms are readily visible. Due to a close relationship between leaf reflectance changes and occurring plant stresses, it is assumed that leaf reflectance measurements have a high potential for a site-specific on-line control of pathogens and thus for fungicide application rates.

2 Experimental procedures

2.1 Experimental design

Greenhouse studies were conducted at the Institute of Crop Production and Grassland Research, University of Hohenheim, Germany with summer wheat during the growing period April-August 2002. In one experiment wheat plants (*Triticum aestivum*, cv. Tha sos) were inoculated with four different spore concentrations (S0 = control, S1 = 2500; S2 = 5000, and S3 = 10000 spores per leaf) of powdery mildew (*Erysiphe graminis* sp. *tritici*) in the growth stage BBCH 14 [27] The spores were transferred onto leaf segments of the wheat plants and left to grow for 8 days before reflectance measurements started. In a second experiment, wheat plants (*Triticum aestivum*, cv. TX86A5606) were infected with take-all (*Gaemanniomyces graminis* sp. *tritici*) in five different inoculum concentrations. Take-all disease was induced by adding inoculated rye seeds to the soil at the sowing date. The inoculum was placed in a layer 5 cm below the wheat seeds in concentrations of 0, 5, 10, 15 and 20 g inoculum g$^{-1}$ soil (I0 = control, I1 = 5 g inoculum kg$^{-1}$ soil; I2 = 10 g inoculum kg$^{-1}$ soil, and I3 = 15 g inoculum kg$^{-1}$ soil, I4 = 20 g inoculum kg$^{-1}$ soil). The soil was sterilized at 120°C in a dry oven prior to seeding.

In each experiment wheat seeds were sown in Mitscherlich Pots containing 6 kg of soil. The soil type was a sandy loam with a pH of 7.4 and a total water holding capacity of 23 %. The soil was fertilized with 250 mg N [NH$_4$NO$_3$], 125 mg P [NaH$_2$PO$_4$], 125 mg K[K$_2$SO$_4$], 120 mg Mg [MgSO$_4$], 130 mg Ca [CaCl$_2$], 10 mg Mn [MnSO$_4$], 1.5 mg Zn [ZnSO$_4$], 1.0 mg Cu [CuSO$_4$], 0.3 mg B [H$_3$BO$_3$], and 0.02 mg Mo [Na$_2$MoO$_4$] prior to saving. Fifteen wheat seeds were sown in each pot and thinned after two weeks to 10 seedlings per pot. All treatments were replicated four times. Pots were randomly arranged in the greenhouse to minimize shading effects. Control plants were separated by transparent foils from infected plants especially in the powdery mildew experiment to avoid cross infections. The average temperature in the greenhouse was 18°C ± 3°C with a minimum of 13°C and a maximum of 24°C. The relative humidity was kept at 70 % ± 5 %, the average temperature was set to 20°C ± 5°C.
2.2 Image acquisition

Leaf scans were taken with a digital, light-sensitive (ISO 200–2400; spectral sensitivity of 250-1300 nm), high-spatial resolution (5140*5140 pixel) imager (S1 PRO, Leica, Germany). Settings of the imager were chosen according to Graeff (2000) [28]. The imager provided an entirely digital and fully automatic process to produce highly accurate digital image data. The imager gave output values in the form of trichromatic coordinates L*, a*, b* [29]. The imager was used in conjunction with a constant light source (Reporter 21D MicroSun, 21W, Sachtler, Germany). The chosen light source was equipped with a 21W daylight discharge bulb (Sachtler, Germany; color temperature 5500 – 6000 K), which produced more light than normal 50 W tungsten luminaries. It has a luminous flux of 71 lumens per watt, whereas the tungsten bulbs produce only 30 lumens per watt. No dichroic tungsten filter was used for the measurements. To ensure a constant light intensity over all measurements and sampling dates the light intensity was calibrated against known references as indicated by Graeff (2000) [28]. When the voltage dropped below 9 V, an automatic voltage controller switched off the luminary in order to ensure a constant power supply.

Reflectance measurements started in the powdery mildew experiment eight days after inoculation and were carried out every five days until most infested wheat plants were dead. In the take-all experiment reflectance measurements started in BBCH 30 and were carried out once a week until flowering. Reflectance measurements were carried out on the infested leaves in the powdery mildew experiment and on the youngest unfolded leaf as well as the flag leaf in the take-all experiment. One plant per pot was used as a sub-sample of the whole pot for the reflectance measurements. Reflectance spectra were taken without removing the leaf from the plant. A leaf area of 4.5 cm² was scanned at a point about one-quarter of the way from the base to the tip. The leaf to be measured was laid on a black aluminum plate mounted 15-20 cm away from the optics (1.28/60 mm, LEICA, Germany) of the imager. To exclude the effects of solar light as well as of stray background light the imager, light source and sample were surrounded by a black aluminum box. Individual leaf samples were placed horizontally on a black aluminum plate that was illuminated by the constant light source, which was positioned 13 mm from the black aluminum plate surface and oriented 45°from vertical. The imager was oriented vertically, 15-20 cm from the aluminum plate surface and the reflectance was scanned through the visible (380–780 nm) and NIR (750–1300 nm) wavelength ranges at given long pass filter intervals at a rate of 1 scan per 24 milliseconds. Selected long-pass filters (Maier Photonics, Manchester, VT, USA), were active at wavelengths longer than 380 nm (400-2000 nm transm. avg. 90%), 490 nm (500-2000 nm transm. avg. 90%), 510 nm (520-2000 nm transm. avg. 90%), 516 nm (525-2000 nm transm. avg. 90%), 540 nm (550-2000 nm transm. avg. 90%), and 600 nm (610-2000 nm transm. avg. 90%), respectively. The long-pass filters had the following general specifications: 3 mm thickness, hard-oxide coating surface, quality 80/50 per MIL-O-13830A, coating quality 60/40 per MIL-O-13830A, and temperature limits - 50 to 100 °C. For each plant, scans
were performed with the aforementioned long-pass filters in conjunction with a LEICA Daylight Filter IRa E55 to cut all scans at 780 nm (wavelength ranges indicated with X780 nm). A second set of scans was taken without this daylight filter in order to scan in the near-infrared ranges, indicated with X1300 nm.

Spectral data were studied to select feature wavelengths, that is, the wavelengths at which contrasts in spectral responses between major object categories became distinct. Scans were analyzed with ADOBE® Photoshop 5.0 (file type *.psd, 8-bit) in the L*a*b*-color system [29] by splitting the scans into a* and b* parameters in the different wavelength ranges. In the L*a*b*-color system the values L*, a* and b* are plotted using a Cartesian coordinate system. Equal distances in the space approximately represent equal color differences. Value L* represents lightness; value a* represents the red/green axis; and value b* represents the yellow/blue axis. The spectra of the 11 filter classes revealed that, at certain wavelengths, the contrasts between major object categories were maximized. The technique used in this study transforms the CIELAB chromaticity parameters into mathematical equations not only for the visible spectrum, but also for the near-infrared spectrum. Characteristics of chromaticity parameters are related mathematically to the response of plants due to stress factors. The evaluation of individual stress factors is based on the mathematical expression of the difference between CIELAB chromaticity values, and not on the expressed color itself. The final spectral features used to quantify plant stress factors should not be confused with human perceived colors. Giving an equation of colorimetric alteration directly connected to the healthy conditions of a plant, could help the final user in the interpretation of the raw data, regardless of the color.

2.3 Harvest and analyses

After scanning, the leaves and shoots of the measured plants were harvested and the fresh weight was determined at once. The visible degree of infection was monitored according to the guidelines of BSA (1988) [30] on a percentage scale. Plant samples were then dried at 60 °C and total dry matter was determined. The remaining leaf tissue was ground, dry-ashed and analyzed for nutrient concentration via atomic absorption spectrometry (AAS) [31]. Total N was determined according to Dumas (1962) [32] by means of Heraeus macro-N-analyzer (Hanau, Germany).

2.4 Statistical analysis

Analysis of variance (ANOVA) was carried out on all crop and reflectance data using the general procedures of the Tukey minimum significant difference (MSD) test at the 5 % significance level of the Statistical Analysis System (SAS) version 6.12. Correlation and regression analyses were performed to determine the association among the monitored physiological parameters, infection levels and the leaf spectral reflectance.
3 Results

No visible signs of infection were recognized in inoculated leaves between the beginning of the experiment and the first measurements. During the following 20 days the amount of mildew hyphae and conidia increased on the leaves and led finally to the senescence of the plants. However, even when the leaves were populated with mildew colonies in the late stage of infection, green islands of apparently healthy leaf areas could be observed among the colonies.

Figure 1 shows the reflectance changes in the wavelength range 516\textsubscript{780} nm over time for the control and the 2500 spore treatment of powdery mildew infection (S1). The reflectance parameter b* of infected leaves changed significantly in the visible wavelength ranges 17 d after the inoculation in the S1 treatment. A similar result was obtained for S2 and S3 treatments 9 d after the inoculation (results not shown). The b*-parameter increased in all treatments S1-S3 over time and accompanied increasing spore concentration. However, due to the dynamics of the mildew infection the different infestation levels could not be discriminated spectrally, especially in later stages of infection. In general mildew infection was low (0-15%) at the beginning of the experiment and increased to 100 % at the end of the experiment. A similar response was obtained in the near-infrared wavelength ranges (results not shown). Over the duration of the experiment the wavelength ranges 510\textsubscript{780} nm and 516\textsubscript{1300} nm were selected for the identification of powdery mildew infection, because leaf reflectance was significantly affected in an early stage of disease.

![Figure 1](image)

**Fig. 1** Change of reflectance parameter b* in the wavelength range 516\textsubscript{780} nm due to powdery mildew infection (mean, SE, n = 4). Significant changes at the $\alpha = 0.05$ probability level are indicated with *.

Figure 2 shows the reflectance changes for the different inoculum levels of take-all infection 33 d and 54 d after the infection. As shown in the figure, the reflectance param-
eter a* of infected leaves changed significantly in wavelength range 540–1300 nm. The a* parameter decreased in all treatments over time and accompanied decreasing inoculum concentration. Take-all disease affected reflectance, both in the visible and near-infrared spectra. To relate leaf reflectance response to the intensity of take-all infection, reflectance changes were correlated with the inoculum concentration in the soil (Figure 3). Based on these data, there is a linear correlation between inoculum density and reflectance difference (between infested and control plants). This relationship enabled the quantification of take-all infection ($r^2 = 0.78$).

![Fig. 2](image_url) Change of reflectance parameter a* in the wavelength range 540–1300 nm due to take-all infection (mean, SE, n = 4). Significant changes are indicated at the $\alpha = 0.05$ probability level.

Table 1 presents an overview of the correlation coefficients of the relationship between the induced inoculum level and the change of the b* parameter at different wavelength ranges. The best correlations were found in the wavelength ranges of 490–780 nm ($r^2 = 0.69$), 510–780 nm ($r^2 = 0.74$), 516–1300 nm ($r^2 = 0.62$) and 540–1300 nm ($r^2 = 0.60$). Using these correlations take-all disease could be detected in various wavelength ranges. According to Table 1 most visible bands were more sensitive to take-all infestation than the near-infrared ranges. The higher sensitivity of visible wavelengths may be due to leaf chlorosis that is typically caused by take-all disease. The reflectance in these ranges was then subjected to the sensitivity analysis and correlation analysis mentioned in the methods section.

There were some consistent results in the two experiments. The 490–780 nm wavelength range was most sensitive to damage caused by powdery mildew and take-all infestation, because there were larger differences in reflectance between infested and control plants.
Fig. 3 Correlation of leaf reflectance response in the wavelength range 540–1300 nm and take-all inoculum densities.

Table 1 Coefficients of determination for the relationships between the change of the reflectance parameter b* due to take-all disease and the induced inoculum level 54 days after the inoculation in the visible and near-infrared wavelength ranges.

<table>
<thead>
<tr>
<th>Wavelength range</th>
<th>$R^2$</th>
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<tbody>
<tr>
<td>380–780 nm</td>
<td>$r^2 = 0.56$</td>
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<tr>
<td>490–780 nm</td>
<td>$r^2 = 0.69$</td>
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<tr>
<td>510–780 nm</td>
<td>$r^2 = 0.74$</td>
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<tr>
<td>516–780 nm</td>
<td>$r^2 = 0.58$</td>
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<tr>
<td>540–780 nm</td>
<td>$r^2 = 0.52$</td>
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<tr>
<td>600–780 nm</td>
<td>$r^2 = 0.54$</td>
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<tr>
<td>490–1300 nm</td>
<td>$r^2 = 0.05$</td>
</tr>
<tr>
<td>510–1300 nm</td>
<td>$r^2 = 0.38$</td>
</tr>
<tr>
<td>516–1300 nm</td>
<td>$r^2 = 0.62$</td>
</tr>
<tr>
<td>540–1300 nm</td>
<td>$r^2 = 0.61$</td>
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<tr>
<td>600–1300 nm</td>
<td>$r^2 = 0.22$</td>
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for both diseases. The change of reflectance in some wavelength ranges seemed to be similar for both diseases. However in contrast to take-all disease, the b* parameter increased with powdery mildew infection in the visible spectra, while it decreased for take-all disease (Figure 4). Thus, the distinguished spectral response of wheat plants to different pathogens enabled the discrimination of the two investigated pathogens.
Fig. 4 Change of reflectance parameter $b^*$ for take-all (A) and powdery mildew infection (B) in the visible spectra at 490–780 nm. The $b^*$ parameter decreased (a) with take-all disease, while it increased with powdery mildew infection (b). Significant changes are indicated at the $\alpha = 0.05$ probability level.

4 Discussion

Crop protection decisions are currently determined at the field scale. As demonstrated by Secher (1997) [6], disease control might be optimized if fungicides are applied on a site-specific scale, resulting in more sustainable agricultural cropping systems and reduced environmental impact. In order to apply fungicides on a site-specific scale, non-intrusive sensor technologies for disease identification and tools to support fungicide spray decisions must be developed. Remote sensing and reflectance measurements have gone through rapid development over the past two decades and there is a trend towards the use of images in the application of stress identification for precision farming [33]. Remote sensing techniques have been extensively discussed for the purpose of site-specific
disease identification, because they potentially allow for the early detection of disease outbreaks. However, little is known in detail about the effects of diseases on plant spectral properties raising the need for accurate and reliable spectral information under various environmental conditions. Hence, the potential of leaf reflectance measurements as a tool for identifying, quantifying and discriminating powdery mildew and take-all disease has been assessed in this study.

The results of this study indicated that in principle, leaf reflectance measurements and thus remote sensing techniques may be applied to detect crop stress or damage inflicted by pathogens. Studies by Lorenzen and Jensen (1989) [5] and Malthus and Madeira (1993) [34] have shown that disease detection and assessment by means of reflectance spectra may be possible when changes in pigments or cell constitution create a detectable qualitative or quantitative difference in the spectral reflectance between healthy and diseased plants. Polischuk et al. (1997) used spectral reflectance measurements to make an early diagnosis of symptoms in Nicotiana debneyi plants at different stages of tomato mosaic virus infection [35]. Reduction in chlorophyll content in the leaves could be detected by reflectance measurements within 10 days after inoculation even though significant visible differences between the control and the infected plants were not noted until after 3 weeks. Furthermore, several studies have been conducted to assess the effects of disease-induced cellular changes in plants on reflectance at individual wavelengths [7, 9, 10, 36–39]. Most of these studies have shown that canopy reflectance within the red (600-710 nm) and near infra-red (750-900 nm) wavelengths were correlated with disease severity estimates. In the visible range reflectance is considered to be influenced by leaf pigments such as chlorophyll and in the near-infrared range reflectance is affected by changes in the anatomical structure of leaves [40, 41].

The infection of wheat plants with take-all and powdery mildew in this study lead to changes in reflectance in the visible and near-infrared. These differences may be caused by changes in pigments or nutrient status of plants. Further investigations are necessary to evaluate the physiological parameters responsible for the reflectance changes.

The findings of this study indicate the potential of using leaf reflectance measurements to detect and discriminate powdery mildew and take-all disease infestation on wheat. If one could detect pathogen infection using reflectance of sensitive bands before pathogen densities reach their maximum, one could initiate directed field scouting and take necessary control measures. These methods would protect non-infested and lightly infested crops before the infestation spreads across the whole field or to adjacent fields. Therefore, total yield loss caused by pathogen infestation could be decreased.

To determine the potential of the non-destructive method to differentiate between diseases in crops and other stress factors, the spectral properties of leaves with various stress factors will have to be investigated in the field. Practical implementation of this procedure requires that an appropriate sensor, independent of controlled light conditions, be made available to farmers. This could enable continuous monitoring of the plant vitality status from a tractor and the direct transfer of the collected information into management algorithms. Future studies also have to define the reflectance profile of
plants under different growth stages and infestation levels. Furthermore, the pathogen infestation often appears when the wheat crop is suffering from other stress factors. Therefore, a clear discrimination of crop stress factors has to be ensured for appropriate site-specific fungicide application strategies.

5 Conclusion

The results of this study indicate that reflectance measurements may be used for disease detection and discrimination in an early stage of infection by use of specific wavelength ranges. For site-specific fungicide application, further studies should focus on the discrimination of powdery mildew and take-all infection from other plant stress factors by means of reflectance measurements.

References


