Chapter 11
Site-Specific Sensing for Fungicide Spraying

Eiko Thiessen and Hermann J. Heege

Abstract Especially in humid moderate climates, high yields require the application of fungicides. Its site-specific application based on the biomass of crops is state of the art. Yet this technique does not take into account that fungal infections in most cases start and spread out from small, initial spots within a field. So a sensing technique to detect these initially small infected spots would be of great importance for saving fungicides, for reducing damage to crops as well as to the environment and for allowing higher driving speeds in uninfected areas. Reflectance indices of visible and near-infrared light as well as indices of fluorescent light are candidates for detecting these spots.

Detecting the fungi in early stages of infection (= latency stage) can be important for a successful treatment, because stopping the infection after this time gets more difficult. In this latency stage, the diseases might not yet be visible by human eyes. Fungal diseases often change the physiological state of plants either by means of the photosynthesis or by the formation of secondary metabolites like phenols. These changes can be detected in the smartest way by optical sensing. Hereby fluorescence is a sensitive method with the potential of detecting changes before infections are visible by human eyes.

Keywords Fluorescence • Fungal infection • Optical sensing • Site-specific

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11.1 The Situation for Site-Specific Fungicide Applications

It is a well known fact that fungal crop infections in most cases start with a few small and very discrete **patches**. At the beginning of a fungal epidemic, these few tiny infected loci within a field are hard to detect. Their visual patterns depend very much on the respective fungi, on the infection stage, on crop development as well as on varieties and on the weather. Therefore, traditionally crop epidemiologists have been **detectives of patterns** with microscopes as manual tools.

The early detection of an infection is crucial, because it allows timely counteractions, hence to eliminate further spreading of the disease and to prevent serious damage to the crop. It is also a prerequisite for site-specific application. Because at the far end of a fungal epidemic and much damage, a more uniform spatial distribution of the disease will exist.

If the challenge of an early and site-specific detection of infected patches is met, there probably are no other farming operations where treating the whole field uniformly can be so far off the actual needs. However, these challenges also mean that any successful site-specific application of fungicides must comply with extremely high spatial and temporal resolutions. So to come up to the prerequisites for site-specific spraying against fungi is not easy, yet the prospects might justify the efforts.

As a consequence of this situation, two different approaches for site-specific application of fungicides have evolved. The first approach is based on a **full-area preventive concept**. Since for practical applications the detection of the initial tiny infected loci is not yet solved, still the whole area is treated in a precautionary manner. The timeliness for these largely prophylactic applications is oriented at local epidemic forecasts of extension services. However, there might still be a site-specific control of the application rate. This control might be based on more general crop properties such as biomasses or leaf-area-indices.

The second approach aims at **discrete spot spraying** of the few just infected loci and if possible not beyond these. This approach – if well conceived and executed – would allow a radical reduction of fungicide use. However, whereas the full area preventive concept with site-specific application is state of the art, the discrete spot spraying of fungicides still is in an experimental stage. And especially the latter concept needs sprayers that allow for separate section- or even better separate nozzle control. Modern sprayers that use **direct injection** of the pesticides into the water close to the nozzles instead of premixed batches provide the technical prerequisites for such a resolution in the application (Vondricka and Schulze Lammers 2009).

11.2 The Full-Area Preventive Concept Based on Biomass

It is general experience that dense, lush crops are more susceptible to fungal infections than thin and less developed canopies. On a site-specific basis, the biomass densities or leaf-area-indices of crops vary as the soils do. With the usual

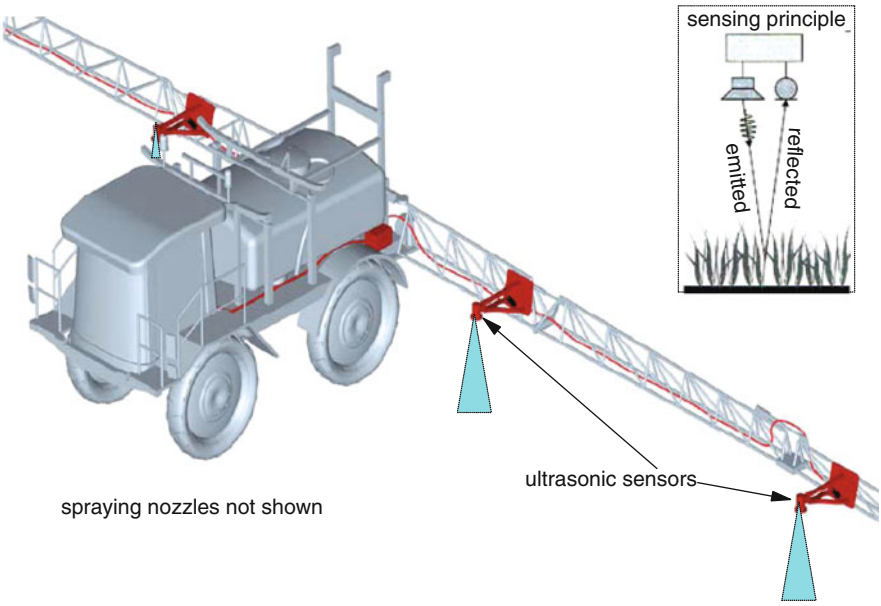

Fig. 11.1 Section control of a sprayer by ultrasonic sensing of biomass. The *insert* shows the sensing principle (From Reusch 2009 and Agri Con GmbH, Jahna, Germany, altered)

practice of applying a uniform fungicide rate across the whole field, any site-specific differences in crop densities are disregarded. Yet since in many cases the leaf-area is the object of a fungal attack, it seems logical to apply the same concentration of fungicides per unit of leaf surface area. This is the rationale for fungicide application according to site-specific crop densities or its surrogates, the biomasses or leaf-area-indices.

Several methods of detecting the site-specific biomasses or leaf-area-indices are available and have been dealt with:

- proximal or remote **reflectance** sensing (Fig. 6.7)
- mechanical sensing of the **bending resistance** (Fig. 9.33)
- sensing by **ultrasonics** (this section Fig. 11.1 and Sect. 9.4.6).

The growth stages at which fungal infections occur can be very different. For small grains, depending on the respective fungi, infections can develop at almost any growth stage. But the sensing methods listed above differ in their capabilities to detect well at various growth stages. Only the ultrasonic method can reliably sense biomass at any growth stage (Fig. 11.2).

With reflectance sensing, because of soil effects in less developed canopies, the **minimum growth stage** of small grains is about EC or BBCH 30. This is when tillering has ended. Reflectance indices with wavelengths from the red edge range should be preferred in order to avoid limitations at advanced growth stages for lush crops.

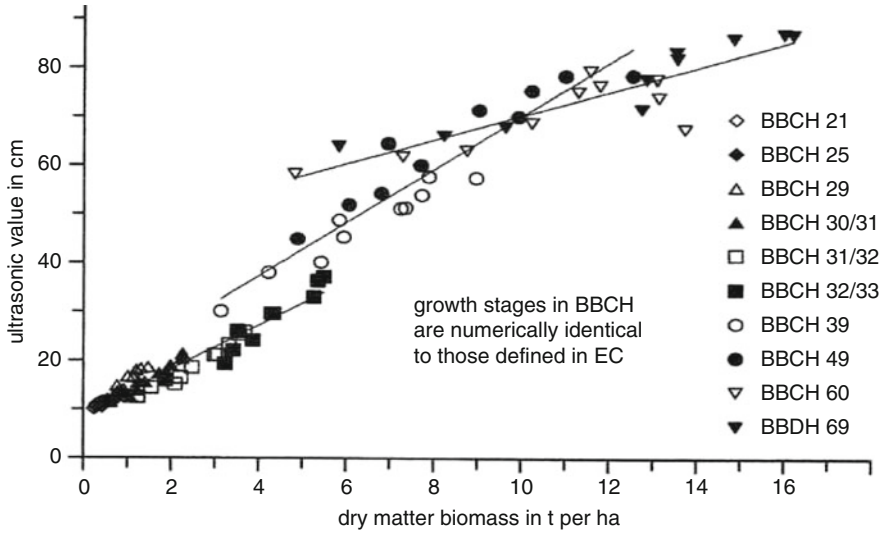

Fig. 11.2 Sensing the aboveground biomass of winter wheat at various growth stages by ultrasonics (From Reusch 2009, altered)

Finally, sensing leaf-area-indices or biomasses via the bending resistance of crops requires even a minimum growth stage of about EC or BBCH 35 (Ehlert et al. 2004). This is when the first flag leaf – still rolled – has appeared.

The **savings in fungicides** that can be realized with a site-specific control based on biomass via a full-area preventive concept will vary with the heterogeneity of a crop. When the sensing in ten fields with small cereals and growth stages between EC or BBCH 39 and 71 was based on the bending resistance, savings in pesticides between a minimum 7 % and a maximum of 48 % were realized (Ehlert and Dammer 2006). The average saving in fungicides was 23.1 %. The savings did not affect the grain yields.

11.3 The Discrete-Spot Sensing Concept Based on Reflectance

11.3.1 The Pinpointing Approach and the Field of View

Theoretically, the savings in fungicides could be much higher than indicated above if only at the start of a fungal epidemic the infected loci were treated. This **pinpointing approach** still is in an experimental stage.

Site-specific spraying against fungi is much more difficult than site-specific fertilizing of nitrogen, though the sensing might be based in both cases on the reflectance of the canopy. Because usually nitrogen deficiencies occur in much larger

patches or areas than fungal infections in their initial stages do. Hence for sensing fungal infections adequately, much smaller optical **fields of view** are needed. This will be difficult to obtain with sensing from satellites. But for proximal sensing from field based machines, principally it is possible to provide the small field of view by a narrow angle of vision and by a short distance to the canopy. In experiments with infections of yellow rust (*Puccinia striiformis*) and leaf blotch (*Septoria*) in winter-wheat, Moshou et al. (2011) used a field of view with 20 cm diameter on the canopy level for sensing from a sprayer-boom. However, small fields of view must be accompanied by many records in order to scan a field adequately. Already because of this, sensing for a discrete-spot effect will be more expensive than the conventional reflectance sensing for nitrogen fertilization.

Another point to consider is the fact that the **top leaf** of a plant in crops generally is not infected because it has only recently unfolded and incubation periods for the fungi hold (West et al. 2003). Hence in field operations, the viewing should be such that the top leaf is omitted as far as possible. Since with most crops – especially with small grains – the top leaf initially has a vertical position, oblique view directions towards the canopy should be avoided. With vertical viewing directions, primarily the horizontally oriented leaves get into the field of view, and the recently unfolded new vertical leaves affect the results less.

11.3.2 Spectra and Indices of Reflectance

There are many different types of fungal infections that can occur and these may affect the reflectance in different ways. Even within the same crop, different fungi can cause different **reflectance spectra**. This can be seen when the spectra of three different fungal infections of sugar beet leaves – cercospora leaf spot, powdery mildew and rust – are viewed (Fig. 11.3). The reflectances of the artificially infected leaves were recorded in a laboratory with increasing severities of the diseases (Mahlein et al. 2010).

The courses of the spectra for the non-infected leaves correspond to the known pattern within the visible and near-infrared wavelengths (Fig. 6.3). However, the deviations from these courses that result from the infections are different for each fungus. The powdery mildew infections cause rather uniform increases in reflectance in the visible as well as in the near-infrared range. The deviations for the infections of the cercospora and rust fungi do not comply with this in the range from about 720 to 900 nm wavelength. Within this range, the infections by these fungi result in decreases of the reflectance. And an unusually steep rise in the red reflectance evolves from cercospora fungi for highly infected leaves. But it has to be pointed out that these are results from one trial and its course of the reflectance could also be interpreted as the fact that *e.g.* 20 % of cercospora severity damages the crop the same as 100 % rust.

For the sensing of many crop properties, the use of **reflectance indices** has led to success. Generally indices allow to limit the sensing to just a few, narrow

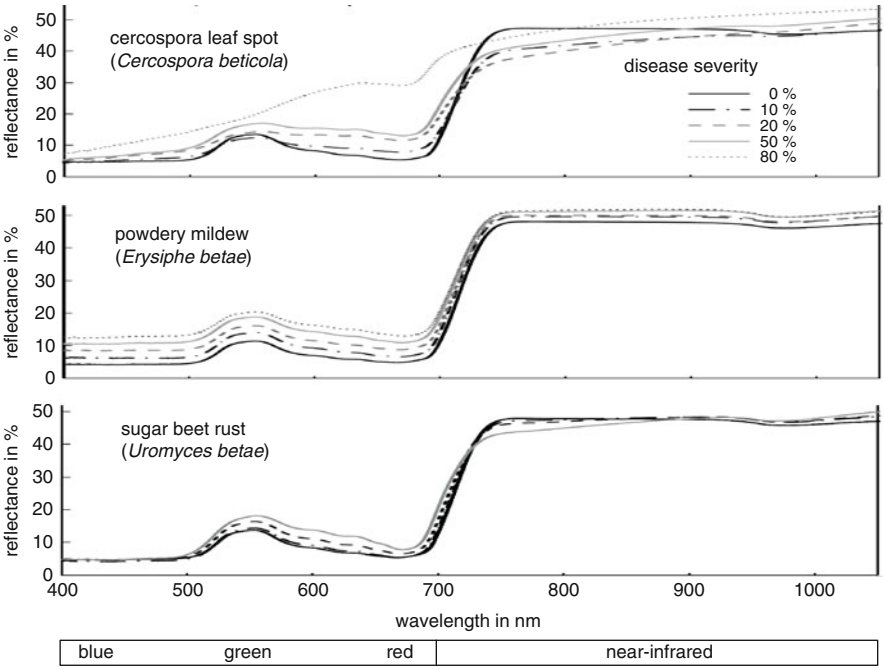

Fig. 11.3 Reflectance spectra of sugar beet leaves infected with various fungi (From Mahlein et al. 2010, altered)

wavelengths and thus to simplify the technology and – if properly selected – still can provide a high precision.

Attempts with **standard reflectance indices** have been made for sensing fungal infections in wheat. The leaves were artificially infected. Among many standard indices, the best **differentiations** between infected and healthy leaves for yellow rust (*Puccinia striiformis*) were provided by either

- the Photochemical Reflectance Index = $\frac{R531 - R570}{R531 + R570}$ (Huang et al. 2007) or
- the Anthocyanin Reflectance Index = $\frac{1}{R550} - \frac{1}{R700}$ (Devadas et al. 2009).

It should be noted that all standard reflectance indices originally were developed for defined purposes. The photochemical reflectance index was created for assessing the efficiency of radiation-use in photosynthesis (Penuelas et al. 1995). And the anthocyanin reflectance index was developed for estimating the anthocyanin accumulation in plants (Gitelson et al. 2001). The fact that these indices depend on the development of rust fungi does not imply that they are the best possible choice for any reflectance indices that are conceivable. Because none of the standard reflectance indices was developed with the aim of sensing fungi.

It might be helpful to remember that in-season nitrogen sensing also started with standard reflectance indices that too originally also were created for other purposes (Heege and Reusch 1996). It turned out that the prediction of these standard indices could be improved by special indices, which solely were determined for nitrogen sensing (Sect. 9.4.3.1, Table 9.5). These special nitrogen reflectance indices were developed by systematically checking all theoretically possible ratios of narrow reflectance bands from the visible range plus the adjacent near-infrared range (Reusch 2003, 2005; Müller et al. 2008).

Hence for sensing of fungi it probably is reasonable as well to systematically check mathematical combinations of discrete narrow wavelengths along a sensible full spectrum for their sensitivities in this respect. The efforts needed for this **systematic searching** will be immense, since many different fungi and various crops should be considered. A start in this direction has been made by Mahlein et al. (2013) for fungal diseases of sugar beets.

Instead of sensing by indices from narrow wavelengths, sometimes **image sensing** for the detection of fungi is proposed. These images rely on broad wavelength bands, *e.g.* the red, green and blue (RGB) bands of the spectrum. An obvious advantage of imaging is that the locations of the infected loci or discrete spots directly can be seen on the records. A disadvantage of images is that the broad wavelength bands tend to hide early effects of fungal infections. The detection is possible not until when symptoms appear as obvious lesions. Yet with suitable spectral indices from narrow wavelengths, the sensing can start already slightly before the first sporulation (Moshou et al. 2011). And a still earlier detection might be possible when fluorescence provides the signals. This will be dealt with in the next sections.

11.4 The Discrete-Spot Sensing Concept Based on Fluorescence

11.4.1 Indirect Measuring with In-Situ Sensor System

Fluorescence is the emission of radiation that follows an absorption of light energy. The emitted radiation has longer wavelengths than the absorbed light. Fluorescence requires an adequate substance called **fluorophore**. Normally, the sensing of fungi by means of fluorescence is an indirect method. This means that the fungi are non fluorescent or only show a very weak fluorescence according to their low concentration in the leaf when compared with fluorescent plant pigments. Only some biotrophic fungi like powdery mildew and yellow rust show a blue fluorescence if excited with UV light (Zhang and Dickensen 2001).

The normally called “plant fluorescence” originates from substances insides the leaves that are natural fluorophores. This is different from the fluorescence detection tools used in serological or molecular methods where the samples must be prepared to show fluorescence.

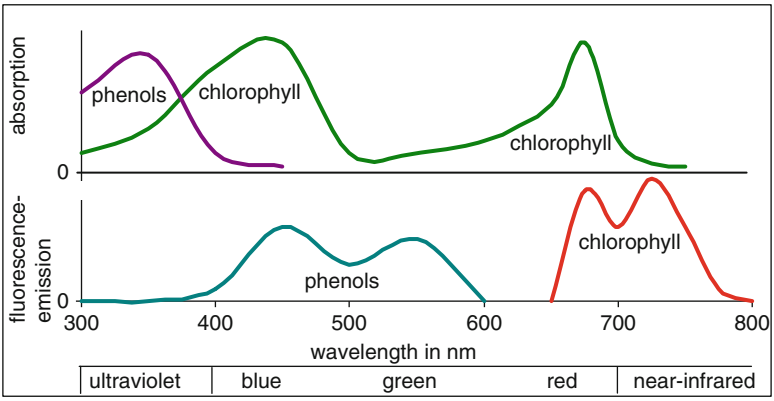

Fig. 11.4 Schematic diagram of plant fluorescence: The light absorption in the ultraviolet region by phenols (*violet line at top*) induces fluorescence in the blue-green (*blue line at bottom*), the light absorption in the blue and red region by chlorophyll (*green line at top*) induces fluorescence in the far-red region (*red line at bottom*)

In higher plants, the natural fluorophores are mainly chlorophyll and phenols. The latter are organic compounds that develop in plants during decomposition. The chlorophyll absorbes primarily in the blue as well as in the red and it emits fluorescence in the far red, whereas the phenols absorb dominantly in the ultraviolet and emit in the blue and green. The typical spectral characteristics of these optical properties are shown in Fig. 11.4.

A non-invasive measurement is only possible from the whole leaf or plant. This is of course not only a fluorescent dilution, but it is a very complex optical system with many other compounds in separated compartments and with a typical geometrical structure. For a first approach, one can summarise up to three types of fluorescence with their related measurement techniques:

- With excitation in the ultraviolet, the whole fluorescence **emission spectrum** is possible to measure. The excitation wavelength, which is also reflected, can be separated from the emission with wavelength-selective filters. But with blue or red excitation, only the chlorophyll fluorescence is measureable. Because these wavelengths are not short enough to induce phenolic fluorescence. So the fluorescence emission provides information about chlorophyll and/or phenols.
- Another method is measuring the **excitation spectra,** which induce the fluorescence**.** The excitation wavelength is changed consecutively to discrete bands or scanned continuously and the fluorescence is measured at a fixed emission wavelength. So a kind of absorption spectrum for substances inside the leaf is obtained. Normally the emission is detected in the far red (approximately 650–750 nm). So chlorophyll serves as sensor inside the leaf. Mainly two excitations are used: one within the ultraviolet and one within the visible region.
- As mentioned before in Sect. 6.4 “Fluorescence Sensing”, the fluorescence is also temporally variable. This so called “**Kautsky effect**” is typically measured

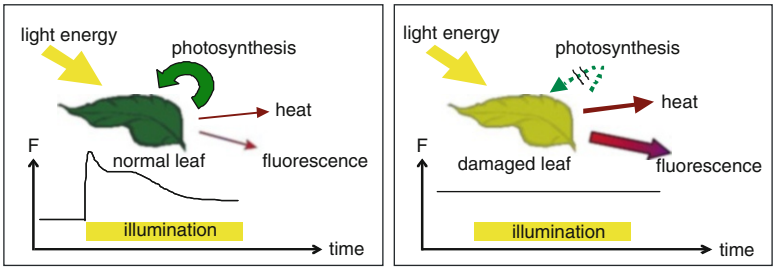

Fig. 11.5 Schematic of variable fluorescence (F) in a normal leaf and in a damaged leaf where photosynthesis is totally blocked. In the former, the fluorescence shows typical kinetics during illumination. In contrast to this, the damaged leaf has a higher but constant fluorescence

when a dark adapted plant is illuminated with intense light. The effect in normal plants is that the fluorescence rises quickly up to five times higher than an initial low level and after a few seconds it decreases to an intermediate stationary level. This is due to the processing of the light in photosynthesis, and it is obvious that a damaged leaf which is "dead" or "nearly dead" shows no or limited variable fluorescence (Fig. 11.5).

So in summary: there exist not only the spectral emission and excitation spectra of fluorescence, but also the temporal kinetic behaviour of the plant's fluorescence.

11.4.2 Fungi-Plant-Interaction and Physiology of Infected Plants

There exist many fungi (about 1.5 Mio) and plant species (250,000) and these have had much time during their evolution to develop strategies against each other. Fungi cause a **defence mechanism** in the infected plant. Elicitors (*e.g.* reactive oxygen species – ROS – that are produced by fungi) trigger a hypersensitive response of the plant. This could be the production of special substances – callose as a barrier for the fungi or phenols with antifungal properties – or the dying of hypersensitive cells.

With common fungi in cereals, the defense reactions are either **necrotrophic** or **biotrophic**. For the first case: septoria leaf spot and fusarium head blight are typical examples in wheat. These fungi are destroying the plant cell walls with toxic substances and lead to necrosis of the plant leaf. Hereby compounds in the plant cells transform into phenols (Osbourn 1996).

The obligate biotrophic fungi need living plant material as they assimilate the plant's nutrients by haustoria (extraction by roots). Typical examples are powdery mildew (*Blumeria*) and rust (*Puccinia*) fungi. The effects on the plant parameters are normally reduced photosynthesis but increased respiration. The biotrophic fungi often form "green islands" on the leaf with a nutrient sink and higher nonphotochemical quenching (energy dissipation of the light excitation) than the surrounding area (Scholes and Rolfe 1996).

Table 11.1 Types of plant fluorescence and their applications

Fluorescence	Subtype and index	Properties	Trend when infection rises
Emission	Red: F680/F730	Chlorophyll	Long term falling
	Blue green : F450/F730	Phenols	Short term rising
Excitation	F_{UV}/F_{VIS}	Phenols	Short term rising
Kinetics	$(F_M - F_0)/F_M$	Photosynthesis	Decreased activity

Hypersensitive death of invaded cells is known as the most typical feature of rust resistance (Heath 1982), and this increases the concentration of phenols.

11.4.3 Fluorescence Indices Related to Infection

Indices are normally ratios of simultaneously measured radiation intensities. They are rather insensitive to changes in measurement geometry, to sensor drifting or to environmental conditions. Each index can be assigned to the corresponding measurement technique:

- The maxima in the fluorescence **emission spectra** correspond to chlorophyll and phenols (Fig. 11.4, bottom). The ratio of the intensities of the maximum chlorophyll fluorescence supplies a simple but powerful index. This relation of **F680/F730** is a measure for the chlorophyll content (Gitelson et al. 1999). As described before in Sect. 6.4.1, this ratio is negatively correlated to the chlorophyll content due to self-absorption of the radiation below 700 nm. The blue fluorescence around 450 nm related to the far red fluorescence 680 or 730 nm – *e.g.* **F450/F680** – is related to the content of phenols (Lichtenthaler and Schweiger 1998).
- The dual **excitation** of chlorophyll with light in the ultraviolet and visible region leads to the $\mathbf{F_{UV}/F_{VIS}}$ quotient. For this the fluorescence intensity in the far red excited with UV light is related to the one that is excited with VIS – normally blue or red light. This is a measure for the UV absorbing pigments in the leaf epidermis (Cartelat et al. 2005), namely the phenols.
- The **kinetics** of the Kautsky effect are very complex and can be influenced by the light scheme during measurement. The main parameter is the variable fluorescence, *i.e.* the difference between maximum (F_M) and minimum fluorescence (F_0) related to the maximum fluorescence, hence $\mathbf{(F_M - F_0)/ F_M}$. This variation occurs in the time of some seconds. The initial rise in the millisecond range can be described with a special method (Strasser et al. 2004).

In general the changes in plant properties – *e.g.* chlorophyll and phenol content or photosynthesis – are detectable with the corresponding fluorescence measurement techniques. This is summarised in Table 11.1. In detail there is a lot of literature dealing with stressed plants and fluorescence. In the following, only a few selected publications that deal with fungal infections as the stressor are listed:

- Lüdeker et al. (1996) analysed wheat and barley infected with rust (*Septoria*) and mildew (*Blumeria*) with ultraviolet excitation and found the blue **fluorescence**

emission ratio F440/F730 higher in infected leaf areas. They related the effect directly to the fungi, especially for the symptoms of mildew. But also for necrotrophic fungi like septoria leafspot it was found that this fluorescence ratio F440/F730 increases compared to uninfected wheat leaves even before symptoms are visible (Thiessen 2002). For the classification in healthy and infected leafs the whole fluorescence emission spectra could be used with multivariate analysis. Römer et al. (2011) demonstrated that there are mainly differences in the **blue-green region** of fluorescence spectra comparing healthy and rust (*Puccinia*) infected wheat leafs. This is due to the change in phenols in the early first days after infection. Differences in the far-red region due to chlorophyll degradation show up significantly later. This is in agreement with many other results in literature (*e.g.* Kuckenberg et al. 2009) where the fluorescence emission F680/F730 was used to quantify the disease. With this index, changes show up within a time period in which also visual symptoms and chlorosis appear. This could also be measured by reflectance.

- Scholes and Rolfe (1996) studied the **variable fluorescence** on oat leaves infected with crown rust (*Puccinia coronata*). They discriminated infected and healthy regions by differences in the fluorescence kinetics. The **fast kinetic method** shows differences between drought stress and infection with a fungal infection of wine – called esca disease – compared to the control (Christen et al. 2007).

11.4.4 Problems and Discussion

The **environmental light** also induces fluorescence that is reflected into the sensor. Normally this fluorescence is eliminated by modulating the active excitation light (*e.g.* by using **pulsed lasers**) and relating the detected fluorescence only to these light pulses. This works even in field conditions. But for the variable fluorescence**,** the uncontrolled **sun light** generates an additional effect to the photosynthetic state. And for this method, of course the measuring duration of some seconds is a problem when sensing in the field from a driving vehicle.

Fluorescence indices are also influenced by other factors like abiotic stress, environmental condition or even age of the leaves (Gorbe and Calatayud 2012). So it is difficult to state that *e.g.* "F440/F730>0.8" means "infected" and "F440/F730<0.8" means "healthy". As with other indirect measurements of plant parameters like reflectance or mechanical resistance, the fluorescence is a **relative measure** and needs to be compared with "reference" plants. So a calibration for the special situation is essential. One possible solution to differentiate between stressors is a multivariate calibration**.** For this, more then one index is measured, *i.e.* some spectral region or even a complete spectrum is obtained and analysed according to its whole profile.

Another fact is that an infection shows a spatial pattern on the leaf. If symptoms appear pronounced in small spots (like sporulation or insertion points of the fungi), the use of sensors with a high spatial resolution – in the range of some mm – might be needed. Consequently, the variation of the detected property along the leaf is an

Fig. 11.6 Experimental robot in maize (Photo: Ruckelshausen)

identifier for an infection. This can be detected with a spot sensor by successive scanning or with images from cameras (Kuckenberg et al. 2009; Moshou et al. 2005). The image analysis of fluorescence pictures is a very promising technique and could be automated with increasing computational capacity.

For an effective sensing in a field, the **sample size** is an important aspect: Fungicides are applied typically in an area within the working width of a sprayer. However, optical sensors for fluorescence detect an area in the range of only some mm^2 up to a few cm^2 when acting with artificial light. Small plots of infections might start with a diameter of maybe some meters. Hence if the sensor is operating in an on-the-go manner, there might be problems:

- The measured area is only a very small spot sample of the whole field (*e.g.* a line of some mm of the 24 m working width).
- The concerned leaf area (which is affected by fluorescence changes due to infection) is too small and the sensed surface of some cm^2 shows up in an average signal, which is not significantly different from healthy plants.
- The infected crop area is not measured, because the driving direction of the sensor is not in line with the infected plot.

The solution could be smaller sensed sample areas that are obtained with **scanning laser techniques** *or a* **series of small sensors** perpendicular to the driving direction. A large size of the sensed area would be a good choice if the infection and its fluorescence signals extend over whole leaves or plants. Though this probably is not the rule, it still might be necessary to have a signal which describes the average plant property of an area where the application should be done. Depending on the spraying technique, this could be within the range of the working width, within sections of the boom length or even only within the range of individually controlled nozzles (Vondricka and Schulze Lammers 2009).

The best way out of this dimensional problem between current spraying techniques and the infected loci probably is to leave the detection and perhaps also the treatment to small **scouting robots** (Fig. 11.6). In the future, these small robots might loiter through the fields and carefully inspect as well as treat individual plants.

11.4.5 Sensors for Practice and Research

For an on-line use, the **MiniVegN** (Fritzmeier, Germany) or **Laser-N-Detector** (Planto, Germany) are commercially available. These systems are able to measure the fluorescence emission ration F680/F730 of a small spot which is scanned beyond the tramline. This principle is sensible to the chlorophyll content and therefore mainly used for nitrogen application.

When using handheld commercial sensors, it is possible to measure almost everything. Spectralfluorimeters (*e.g.* RF5001PC, Shimadzu, Japan) with ultraviolet excitation can be applied in a laboratory or a field to monitor the fluorescence emission. For full **excitation spectra of plant fluorescence,** the **Dualex** and **Multiplex** (Force-A, France) or the **UV-PAM** (Walz, Germany) are available. The **kinetics** can be measured *e.g.* with the **PAM** (Walz, Germany) or the fast kinetics with the **PEA** (Hansatech, England).

11.5 Differentiation Between N Deficiency and Effects of Fungi

For any site-specific application of farm chemicals there is the problem that the control signals of sensors may be ambiguous. Nitrogen deficiencies in crops cause increases of reflectances in the visible range. The same effect can result from fungal infections (Fig. 11.3). And there can be further examples of abiotic stress factors – *e.g.* lack of water – that affect the signals of a sensor in a similar way as biotic stress factors do. Because all signals of optical sensors are substitutes for the respective site-specific crop properties. These substitutes can be influenced by other factors than the one that the control should rely on.

The interaction which thus might arise between the effects of **nitrogen and fungi** is especially disturbing. Because it is general experience that high nitrogen rates promote the development of fungi. Hence if the control of site-specific nitrogen application results in increasing rates because of high reflectances in the visible range that are caused by fungal infections, the result on the development of the crop might be disastrous.

To some extent, the present reflectance indices for nitrogen sensing do prevent this misinterpreting of the situation. Because these indices do not only include details of the visible spectrum, but of the near-infrared radiation as well. This even holds if the indices are extracted from the red edge range. In the near-infrared and red edge range, the effects of fungi can deviate from the result of nitrogen (see Figs. 9.19 and 11.3).

Reusch (1997) designed a field experiment with winter barley and strips that were either sprayed against fungi or not. In addition, different nitrogen application rates were included on all strips. The strips where no fungicides had been applied and where higher nitrogen rates had been given were more severely

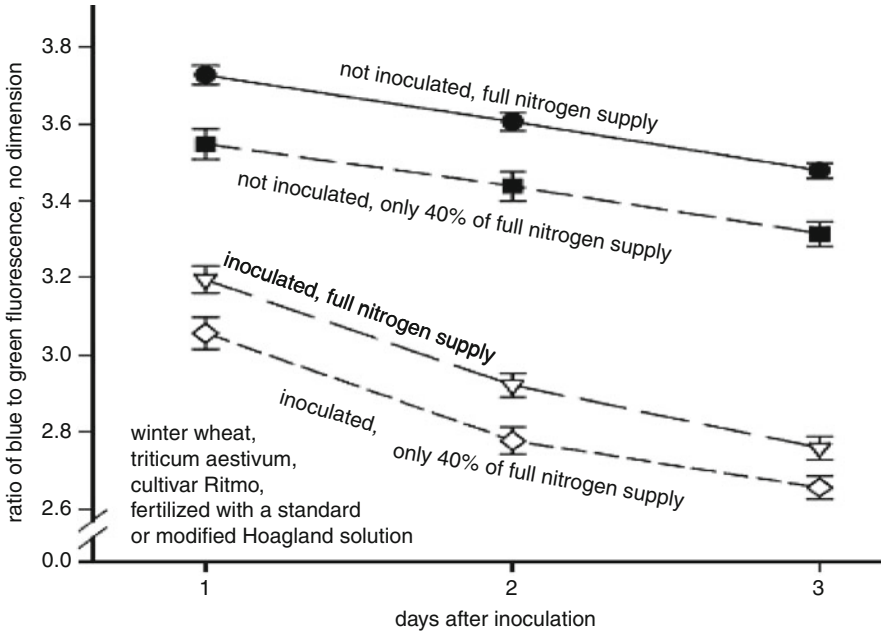

Fig. 11.7 Effects of nitrogen supply and inoculations by powdery mildew – *Blumeria graminis* – on the ratio of blue to green fluorescence (From Bürling et al. 2011, altered)

infected by leaf blotch (*Rhynchosporium secalis*), leaf rust (*Puccinia hordei*) and powdery mildew (*Erysiphe graminis*). However, the effects on the **red edge inflection point indices** were much higher for the nitrogen rates than for the mode of fungicide application. The red edge inflection point indices thus represented mixed effects from nitrogen as major contributor and fungi as minor participants.

Mixed effects of nitrogen and fungi may also be hidden in fluorescence signals. However, the weighting of the contributors nitrogen and fungi for the sensed index might be reversed (Bürling et al. 2011). In Fig. 11.7, the sensing index is the ratio of the blue to green fluorescence emission amplitudes (Fig. 11.4 bottom, blue curve). The results were obtained in a laboratory and refer to wheat leaves from plants that were artificially infected or not by powdery mildew and either fully or only partially supplied with nitrogen. There is an effect of the nitrogen supply on the fluorescence index, yet it is small. The influence of the fungal infections on the index is much more pronounced.

So regarding reflectance sensing by a red edge inflection index, the signals represent predominantly the nitrogen supply, whereas instead with the blue to green fluorescence index mainly the fungal infections matter. In the experiments by Bürling et al. (2011) this held not only for powdery mildew (*Blumeria graminis*) but for leaf rust (*Puccinia triticina*) as well. The discrimination among the infected and

not infected groups was possible as early as 1 or 2 days after inoculation for powdery mildew and leaf rust respectively.

In short, the problems with mixed effects from nitrogen supply and from fungal infections might be dealt with by **fusing** reflectance and fluorescence sensors. The latter, however, are not yet capable to sense fungi in an on-the-go mode in fields. And the multitude of different fungi and various crops still calls for much research.

11.6 Summary and Prospects

A pinpointed, site-specific application of fungicides is one of the most ambitious and challenging aims in precision farming. Because extraordinary high spatial- as well as temporal resolutions are needed. If these resolutions can be realized in an on-the-go mode, very substantial savings in fungicides and consequently environmental reliefs might become feasible.

Yet as long as such extraordinary high spatial resolutions cannot be obtained in practice, site-specific application based on the varying biomass or leaf-area-indices of crops must be regarded as the sole present alternative. This method fits well to a precautionary, prophylactic approach of spraying against fungi. Though prophylactic methods hardly provide options for drastic reductions in the use of fungicides, they might even be needed in combination with pinpointed, site-specific applications. The timeliness of both methods might be greatly improved by local epidemic forecasts of extension services.

The pinpointed approach might rely either on reflectance or on fluorescence. Both methods still are not state of the art, and its prospects for applications in practice are difficult to predict. Solely reflectance sensing for biomass and for nitrogen is well established. This technique depends largely on leaf-area-indices and chlorophyll. Fluorescence can be based on either emission spectra that sense chlorophyll, on excitation spectra that are influenced by phenols or on its kinetics that depends on the photosynthesis.

The earliest detections of fungal infections theoretically can be provided when the indications occur via phenols or via the photosynthetic process. Hence in this respect, sensing by the blue-green fluorescence or by the kinetics of the variable fluorescence is an advantage. Whenever the indications rely on biomasses, leaf-area-indices and chlorophyll, the earliest detection is postponed by a few days until visual symptoms like chlorophyll degradation or other canopy alterations occur. This holds regardless of the sensing method that is used for these indicators.

Whether in practice any earliest possible detection as outlined above will be feasible and reasonable, depends not only also on the ability of sensing but also on control techniques that deal with the tiny initially infected loci. Since managing the spreading of fungicides with a temporal precision of 1 or 2 days might be difficult in practice anyway, the use of reflectance sensing methods should not be excluded. For any pinpointing approach, sophisticated scanning, processing and spraying techniques will be essential.

References

Bürling K, Hunsche M, Noga G (2011) Use of blue-green and chlorophyll fluorescence measurements for differentiation between nitrogen deficiency and pathogen infection on winter wheat. J Plant Physiol 168(14):1641–1648

Cartelat A, Cerovic ZG, Goulas Y, Meyer S, Lelarge C, Prioul JL, Barbottin A, Jeuffroy MH, Gate P, Agati G, Moya I (2005) Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum L.*). Field Crops Res 91(1):35–49

Christen D, Schönmann S, Jermini M, Strasser RJ, Défago G (2007) Characterization and early detection of grapevine (*Vitis vinifera*) stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. Environ Exp Bot 60(3):504–514

Devadas R, Lamb DW, Simpfendorfer S, Backhouse D (2009) Evaluating ten spectral vegetation indices for identifying rust infection in individual wheat leaves. Precis Agric 10:459–470

Ehlert D, Dammer KH (2006) Widescale testing of the crop-meter for site-specific farming. Precis Agric 7:101–115

Ehlert D, Dammer KH, Völker U (2004) Application according to plant mass (in German). Landtechnik 59(2):76–77

Gitelson A, Buschmann C, Lichtenthaler HK (1999) The chlorophyll fluorescence ratio F735/F700 as an accurate measure of the chlorophyll content in plants. Remote Sens Environ 69(3):296–302

Gitelson A, Merzlyak MN, Chivkunova OB (2001) Optical properties and nondestructive estimation of anthocyanin content in plant leaves. Photochem Photobiol 74(1):38–45

Gorbe E, Calatayud A (2012) Applications of chlorophyll fluorescence imaging technique in horticultural research: review article. Sci Hortic 138:24–35

Heath MC (1982) Host defense mechanisms against infection by rust fungi. In: Scott KJ, Chakravorty AK (eds) The rust fungi. Academic, London, pp 223–245

Heege HJ, Reusch S (1996) Sensor for on-the-go control of site-specific nitrogen top dressing. International Meeting in Phoenix, AZ. American Society of Agriculture Engineering, St. Joseph, MI, Paper No. 961018

Huang W, Lamb DW, Niu Z, Zhang Y, Liu L, Wang J (2007) Identification of yellow rust in wheat using in-situ spectral reflectance measurements and airborne hyperspectral imaging. Precis Agric 8:187–197

Kuckenberg J, Tartachnyk I, Noga G (2009) Detection and differentiation of nitrogen-deficiency, powdery mildew and leaf rust at wheat leaf and canopy level by laser-induced chlorophyll fluorescence. Biosyst Eng 103(2):121–128

Lichtenthaler HK, Schweiger J (1998) Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants. J Plant Physiol 152:272–282

Lüdeker W, Dahn HG, Günther KP (1996) Detection of fungal infection of plants by laser-induced fluorescence: an attempt to use remote sensing. J Plant Physiol 148(5):579–585

Mahlein A-K, Steiner U, Dehne H-W, Oerke E-C (2010) Spectral signatures of sugar beet leaves for the detection and differentiation of diseases. Precis Agric 11:413–431

Mahlein A-K, Rumpf T, Welke P, Dehne H-W, Plümer L, Steiner U, Oerke E-C (2013) Development of spectral indices for detecting and identifying plant diseases. Remote Sens Environ 128:21–30

Moshou D, Bravo C, Oberti R, West J, Bodria L, McCartney A, Ramon H (2005) Plant disease detection based on data fusion of hyper-spectral and multi-spectral fluorescence imaging using Kohonen maps. Real-Time Imag 11(2):75–83

Moshou D, Bravo C, Oberti R, West J, Ramon H, Vougioukas S, Bochtis D (2011) Intelligent multi-sensor system for the detection and treatment of fungal diseases in arable crops. Biosyst Eng 108:311–321

Müller K, Böttcher U, Meyer-Schatz F, Kage H (2008) Analysis of vegetation indices derived from hyperspectral reflection measurements for estimating crop canopy parameters of oilseed rape (Brassica napus L.). Biosyst Eng 101:172–182

Osbourn AE (1996) Preformed antimicrobial compounds and plant defense against fungal attack. The Plant Cell 8:1821–1831

Penuelas J, Filella I, Gamon JA (1995) Assessment of photosynthetic radiation-use efficiency with spectral reflectance. New Phytol 131:291–296

Reusch S (1997) Development of an optical reflectance sensor for recording the nitrogen supply of agricultural crops (in German). Doctoral dissertation, University of Kiel, Forschungsbericht Agrartechnik der Max-Eyth-Gesellschaft Agrartechnik im VDI 303

Reusch S (2003) Optimization of oblique-view remote measurement of crop N-uptake under changing irradiance conditions. In: Stafford J, Werner A (eds) Precision agriculture. Papers from the 4th European Conference on Precision Agriculture, 16–18 June 2003, Berlin. Wageningen Academic Publishers, Wageningen

Reusch S (2005) Optimum waveband selection for determining the nitrogen uptake in winter wheat by active remote sensing. In: Stafford J (ed) Precision agriculture '05. Wageningen Academic Publishers, Wageningen, extended paper of Precision Agriculture '05 conference, pp 261–266

Reusch S (2009) Use of ultrasonic transducers for online biomass estimation in winter-wheat. In: van Henten EJ, Goense D, Lockhorst C (eds) Precision agriculture '09. Wageningen Academic Publishers, Wageningen, pp 169–175

Römer C, Bürling K, Hunsche M, Rumpf T, Noga G, Plümer L (2011) Robust fitting of fluorescence spectra for pre-symptomatic wheat leaf rust detection with support vector machines. Comput Electron Agric 79(2):180–188

Scholes JD, Rolfe SA (1996) Photosynthesis in localized regions of oat leaves infected with crown rust (*Puccina coronata*): quantitative imaging of chlorophyll fluorescence. Planta 199:573–582

Strasser RJ, Srivastava A, Tsimilli-Michael M (2004) Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou G, Govindjee C (eds) Chlorophyll a fluorescence: a signature of photosynthesis, vol 19, Advances in photosynthesis and respiration. Springer, Dordrecht, pp 321–362

Thiessen E (2002) Optical sensing techniques for site-specific application of farm chemicals (in German). Doctoral dissertation, University of Kiel, Kiel, Germany. Forschungsbericht Agrartechnik, VDI-MEG 399

Vondricka J, Schulze Lammers P (2009) Real-time controlled direct injection system for precision farming. Precis Agric 10:421–430

West J, Bravo C, Oberti R, Lemaire D, Moshou D, McCartney HA (2003) The potential of optical canopy measurement for targeted control of field diseases. Annu Rev Phytopathol 41:593–614

Zhang L, Dickinson M (2001) Fluorescence from rust fungi: a simple and effective method to monitor the dynamics of fungal growth in planta. Physiol Mol Plant P 59(3):137–141