

## Rapid *Fusarium* head blight detection on winter wheat ears using chlorophyll fluorescence imaging

E. Bauriegel, A. Giebel, W.B. Herppich

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### Summary

*Fusarium* infection on wheat is a widespread threat for humans' and animals' nutrition as these fungi are known to produce the highly dangerous mycotoxin deoxynivalenol (DON). Besides this, *Fusarium* also induces head blight, a disease resulting in huge economic losses due to shrivelled and low mass or dead kernels. Early disease detection could help to reduce yield losses and health threats from DON contamination. The potential of chlorophyll fluorescence imaging (CFI) to yield these aims was investigated in laboratory experiments applying a FluorCam 700MF commercial CFI system. Healthy (control) plants as well as plants artificially infected with *Fusarium culmorum* were rated visually according to the stage of development and the degree of disease. Subsequently, a chlorophyll fluorescence image analysis of the potential maximum photochemical efficiency ( $F_v/F_m$ ) was applied to determine the degree and the distribution of the damage of the ears. Between the sixth and eleventh day after artificial inoculation photosynthetic activity of single damaged kernels of diseased ears dropped to zero.

Although this only marginally affected the average maximum photochemical efficiency of entire ears, the infection led to a significant increase in the statistical distribution of  $F_v/F_m$  in the images. Pixelwise integration of  $F_v/F_m$ -values (from low to high) of the fluorescence images allowed a differentiation, in steps of 10%, between ears of different degree of disease of 10% on in the BBCH stage 75. Lowest level of disease detection by CFI corresponded to a visually rated degree of disease of at least 5%. However, the possibility to distinguish between diseased and healthy ears became highly limited with incipient ripening of kernels and concomitant chlorophyll degradation at growth stage 81.

**Abbreviations:** CFI – chlorophyll fluorescence imaging, dai – days after inoculation, DON – deoxynivalenol,  $F_0$  - basic fluorescence emission of a dark-adapted plant,  $F_m$  - maximal fluorescence emission of a dark-adapted plant,  $F_v = F_m - F_0$  - variable fluorescence measured on dark-adapted plant,  $F_v/F_m$  - maximum fluorescence yield of PS II (photosynthetic efficiency), PS II - Photosystem II

### Introduction

During recent years, *Fusarium* infections of crops are of increasing public concern due to resulting health threat by mycotoxine contaminations of food (DELWICHE, 2003; DEHNE et al., 2004). These fungi infect all types of grain and various grasses but also dicotyledonous plants (*F. culmorum*). However, durum wheat, oat and triticale are especially prone to ears' infections (<http://www.lfi.bayern.de/ipz/weizen/08468/>). Head blight is mainly caused by the strains *F. graminearum*, *F. avenaceum* and *F. culmorum* (HEITEFUSS et al., 1993; LIENEMANN et al., 2002; SCHLÜTER et al., 2006). Infections with *Fusarium* do not necessarily occur during bloom, but are assumed to take also place via chlamydospores from the ground or infected plant remains (SCHLÜTER et al., 2006).

*Fusarium* are fastidious pathogens, which deaden the tissue of the

host plants. Infected spikelets of the ears will die, which results in chlorophyll losses, becoming visible as head blight (also called scab, DELWICHE, 2003). Finally the disease spreads over the entire ear.

Beside the often discussed allergy supporting effect of *Fusarium culmorum* (HOFF, 2003), mainly metabolites such as deoxynivalenol (DON), zearalenone and fumonisin produced by the fungi in different amounts are considered to be noxious for humans and animals (DELWICHE, 2003; RAGAB et al., 2005; DLG-WORKING GROUP „MYKOTOXINE“, 2000). Feeding fodder containing DON at concentrations above 1 mg kg<sup>-1</sup> DON (at 88% dry matter) causes vomiting and fodder refusal in non-ruminants like pigs. At high concentrations the oestrogenic mycotoxin zearalenon induces infertility and pseudopregnancy (CURTUI et al., 2001; DLG-WORKING GROUP „MYKOTOXINE“, 2000).

However, visual appearance of head blight does not always reflect the detectable mycotoxin contents (MIEDANER and SCHNEIDER, 2002). Obviously, there are a lot of different influences, which either promote growth of *Fusarium*, or cause the production of the toxins. However, the close correlation between both parameters may form the basis of breed selection. Production of mycotoxins by *Fusarium* starts in the field but, at high humidity and temperatures between 12 and 25 °C, can continue in the granary (LEONARD and BUSHNELL, 2003). Hence, harvest and storage of grains should be done very carefully.

During the last twenty years, an increase of ear *Fusarium* infections was reported (DLG-WORKING GROUP „MYKOTOXINE“, 2000; MIEDANER and SCHNEIDER, 2002; DELWICHE, 2003). Among others, intensification of maize cultivation and a shorter ear crop rotation may be the reasons. To protect consumers, a new ordinance about threshold values for those *Fusarium* toxins had been brought into effect by the EU in June 2005 (Ordinance (EG) Nr. 856/2005 in the European Union, June 2005).

This raises the pressure on farmers and food producers to detect infected grain and to exclude it from harvest. In conventional agriculture, *Fusarium* infections are preventively treated e.g. by crop rotation, soil turning ploughing after maize cultivation, use of less sensitive species or preventive spraying with azole preparations (MIEDANER and SCHNEIDER, 2002) during periods of climates favouring infections. If *Fusarium* infections occur despite the preventive treatments, their toxins must be determined by pre- and postharvest tests such as serological rapid tests, Fast-DON-ELISA-tests or counting methods (THATE et al., 2008). However, at present, no fully successful prophylaxis by preventive spraying is applicable nor do truly *Fusarium* resistant species exist (LIENEMANN et al., 2002; CHRPOVA et al., 2004).

On-field-detection of *Fusarium* by means of imaging methods may enable a selective harvest directed to highly diseased areas. Imaging methods have been proven to be very promising as they work quickly, efficiently and non-destructively. Furthermore, imaging methods provided a spatial allocation and they can be processed analytically. During recent years, different approaches have been tested. Multispectral image analysis has been applied for disease detection e.g. in the VIS range analysing degradation of chlorophyll (BRAVO et al., 2004; FRANKE et al., 2005; LORENZEN and JENSEN, 1989; POLDER et al., 2009). Furthermore, in the NIR-range, loss of

water and other substances have been used to determine the degree of pathogen infection in crop plants (DELWICHE, 2003; DELWICHE and HARELAND, 2004; SINGH et al., 2007). For the detection of weed, image analysis based on special shape parameters has been used (KLUGE and NORDMEYER, 2009).

In addition, chlorophyll fluorescence imaging (CFI) has been widely applied to evaluate the vitality of plant tissues (HERPPICH et al., 2001; TARTACHNYK and RADEMACHER, 2003). In most cases, based on the ratio of variable fluorescence  $F_v$  (calculated  $F_v = F_m - F_0$ ) to maximum fluorescence  $F_m$ , CFI was successful in detecting diseases or to determine the degree of ripeness in fruits and vegetables (NEDBAL et al., 2000a).

With CFI, metabolic changes of diseased plants can be recorded (VON WILLERT et al., 1995). Thus, it provides information about a potentially maximum and the actual efficiency of photosynthesis, which is often considerably reduced in stressed or damaged plants. With this measurement of the physiological activity symptoms of a disease can be determined at a very early stage.

Thus, the CFI qualifies for the detection of plant tissue damages caused by herbivory (FORSTREUTER et al., 2006) and freezing (NEUNER and BUCHNER, 1999). In addition, there are reports about studies on the degree of ripening in bananas (HERPPICH, 2002), fungi infestation on lemons (NEDBAL et al., 2000a) or brushing on apples (CHEN, 2009).

The development of different fungal grain diseases caused by *Puccinia recondita* and *Blumeria graminis* (KUCKENBERG et al., 2007) as well as the infection by tobacco mosaic virus (DALEY, 1995) or pepper mild mottle virus (CHAERLE et al., 2006) have been investigated. With quantitative CFI, effects of crown rust (*Puccinia corota*) on oat leaves could have been demonstrated already five days after infection (SCHOLLES and ROLFE, 1996). Using  $F_v/F_m$ , FÖRSCHLER et al. (2003) found first signs of a *Venturia inaequalis* infection on leaves of apple seedlings seven days after infection. These examples nicely indicate the sensitivity of this method and its ability for early disease detection.

To our best knowledge, up to now there is no report on the application of CFI to rapidly and quantitatively detect the development of *Fusarium* infection on wheat ears. Hence, it was the aim of this research to apply CFI for the analysis of head blight disease on this important crop. Furthermore, the possible time frame for a successful early detection of head blight should be evaluated and detection accuracy of CFI at different degrees of infection determined. This should support a better understanding of the dynamic of the expansion of *Fusarium* infection and may be incorporated into different modelling approaches.

## Material and methods

### Plant material

In eight pots (18 cm x 18 cm) 16 wheat grains (cv. 'Taifun') per pot (four control pots and four infected pots, respectively) were sown and cultivated in a green house. Plants were inoculated with a germ mixture of the species *Fusarium culmorum* at a spore-density of 250 000 spores per ml on three successive days with beginning of flowering. To guarantee the spread of germs plants were kept at  $20 \pm 2$  °C, high humidity (70%) and an illumination period of 12 h (high-pressure sodium-vapour lamps, SON-T Plus 400 W, Philips GmbH, Hamburg, Germany). Measurements started immediately after inoculation.

The degree of *Fusarium* infection of the laboratory plants was rated three times a week estimating the percental affection of the ears according to WALTHER et al. (2000). Developmental stage of ears was graded according to the BBCH-scale. All measurements were performed on intact plants.

### Chlorophyll fluorescence imaging

Chlorophyll fluorescence imaging was performed with a modular system (FluorCAM 700MF, PSI, Brno, Czech Republic) measuring sequences of fluorescence images with a user-defined timing of set points, measurement intervals and irradiance (NEDBAL et al., 2000b; HERPPICH 2001, 2002). Basic fluorescence  $F_0$  was induced by two sets of 345 super-bright orange light emitting diodes ( $\lambda_{\max} = 620$  nm). Photosynthesis was driven by continuous actinic irradiation provided by the LED panels. Maximum fluorescence ( $F_m$ ) was measured by short-term (1 s) closure of photosystem II as induced by saturation light pulses (max. 2500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) generated by a electronic shutter-equipped halogen lamp (250 W). A CCD camera with a F1.2/2.8-6 mm objective and a short-pass filter recorded fluorescence images (12-bit, 512x512 pixel; maximal frequency 50 images  $\text{s}^{-1}$ ) synchronously with the weak, non-actinic measuring-light pulses. The system was controlled by the FluorCAM 6 software (PSI, Brno, Czech Republic).

All measurements were performed on dark-adapted (at least 10 min) plants (6 ears per treatment). The duration of the measurement of  $F_0$  and  $F_m$  was 4s. For the analysis of the development of head blight and its distribution on the wheat ears the parameters  $F_v/F_m$  and  $F_v/F_0$  were calculated from the above fluorescence signals. The ratio  $F_v/F_m$  ( $F_v = F_m - F_0 =$  variable fluorescence) is a good indicator of the potential maximum photochemical efficiency of PSII, and, thus, a valuable tool to determine both capacity and stability of photosynthesis (VON WILLERT et al., 1995). On the other hand,  $F_v/F_0$  is assumed to reflect the maximum quantum yield of fluorescence (BUSCHMANN et al., 1999).

To compare the CFI results and the visually rated degree of disease, both  $F_v/F_m$  and  $F_v/F_0$  values of each fluorescence image were pixel-wise accumulated (from low to high) and divided by the total amount of pixel per image. These cumulative relative fluorescence parameters were class wise analysed further.

### Grain yield and mycotoxin determination

The kernels per ear were counted, weighted and visual rated regarding disease degree. Due to the low sample numbers only three disease classes and one control class were defined. For the estimation of the mycotoxin loads, the ELISA-Test (*r-Biopharm*, RIDASCREEN<sup>®</sup>FAST DON) was chosen. Three repetitions of control plants and two classes (0-5% and 5-30% degree of disease) of the infected plants were investigated. After drying (48 h at 60 °C) the samples (5 g in double estimation) were ground with a mill (GRINDOMIX GM200, Retsch GmbH, Haan, Germany) for 25 s at 10000 rpm. The photometric calibration ranges between 0.2 and 6 ppm (200-6000  $\mu\text{g kg}^{-1}$  DON).

### Statistical analyses

The distribution curves of all  $F_v/F_m$ -values of the measured ears were calculated as spikelet density-curves. To explore the highest correlation between rating and the proportion of cumulative values of  $F_v/F_m$  at 0.3, the SPEARMAN-index was used. For the analysis of the correlation between mean cumulative proportion of  $F_v/F_m$ -values at 0.3 and mean degree of disease rating, 15 ears were incorporated, which were measured on three different days.  $F_v/F_m$ -values and disease rating were weighted regarding the time duration, because there were non-uniform spaces. The WILCOXON-rank sum test was applied for the statistical evaluation of the differences between control and infected ears during the progressive infection. All statistical analyses were performed by SAS 9.13 (SAS Institute Inc., Cary, NC, USA).

## Results

### Differentiation of the degree of infection at the BBCH 75 (medium milk)

At the time of medium milk, diseased and healthy spikelets or tissue areas of the ears can be well distinguished by chlorophyll fluorescence imaging (Fig. 1). The photochemical efficiency clearly assigns the respective areas within the ears (indicated in the false-colour image as blue = low and red = high). Under optimal conditions,  $F_v/F_m$  of healthy undamaged plant parts can obtain a maximum of 0.84 (VON WILLERT, 1995). However, under the conditions of the experiments mean maximum photochemical efficiency of healthy control plants was 0.76 (Fig. 1, first row). The associated kernel-density-curve shows a clear and relatively narrow single peak of high  $F_v/F_m$ -values. At the beginning of the disease (up to 3% infection), the  $F_v/F_m$ -distribution still has one peak and reflects a high degree of efficiency, i.e. 99.87% of all  $F_v/F_m$ -values are in the range of 0.5 to 0.75.

With progressing infection, the distribution of  $F_v/F_m$  broadened. At a degree of infection of 20%, there were areas of highly reduced fluorescence indicating dead ears beside ears that are completely healthy and undamaged (Fig. 1, second row). Hence, the contribution of  $F_v/F_m$  in the range of 0 to 0.5 increased in the distribution plot. At the same time, the maximum  $F_v/F_m$  decreased to 0.71. This can be estimated from the considerable shift of the  $F_v/F_m$ -values.

A further clear shift to a two-peaked distribution pattern of the photochemical efficiency was visible at an infection rate of 50%. However,

only damaged or dead kernels had a very low  $F_v/F_m$  close to 0 (0-0.04). In contrast, the undamaged tissue retains high  $F_v/F_m$ -values in the range of 0.46 to 0.74 (Fig. 1, third row). This means that *Fusarium* does not only inhibit photosynthetic performance of infected kernels but causes irreversible damage to photosystem II, and the entire tissue. The progression of the disease is easily visible from both the false-colour images and the value-distribution. Completely dead ears again showed a one-peaked distribution curve (not shown), which now concentrated in the range of low  $F_v/F_m$  (0-0.2).

Very similar conclusions can be obtained by the analysis of the maximum yield of fluorescence ( $F_v/F_0$ ). Hence, a pixel wise accumulation of  $F_v/F_m$ - and  $F_v/F_0$ -values (from low to high) of the fluorescence images should allow the effective differentiation between healthy or weakly infected and strongly infected ears with many completely dead spikelets (Fig. 2). In a cumulative plot,  $F_v/F_m$ -values of plants with degrees of disease between 2 and 100% were classified in steps of 0.05 and cumulated accordingly. Indeed, both parameters showed similar curves at respective degrees of disease although  $F_v/F_m$ -values seemed to allow a slightly better differentiation. An optimal detection and distinction, respectively, of the disease was obtained with cumulative  $F_v/F_m$ -value of 0.3 (Fig. 2). Here, the SPEARMAN-correlation value between the degree of disease and single  $F_v/F_m$ -classes was 0.94. Hence, the threshold for the distinction of areas with damaged tissues was set at 0.3. The highest correlation with  $F_v/F_0$  was found at 0.6.

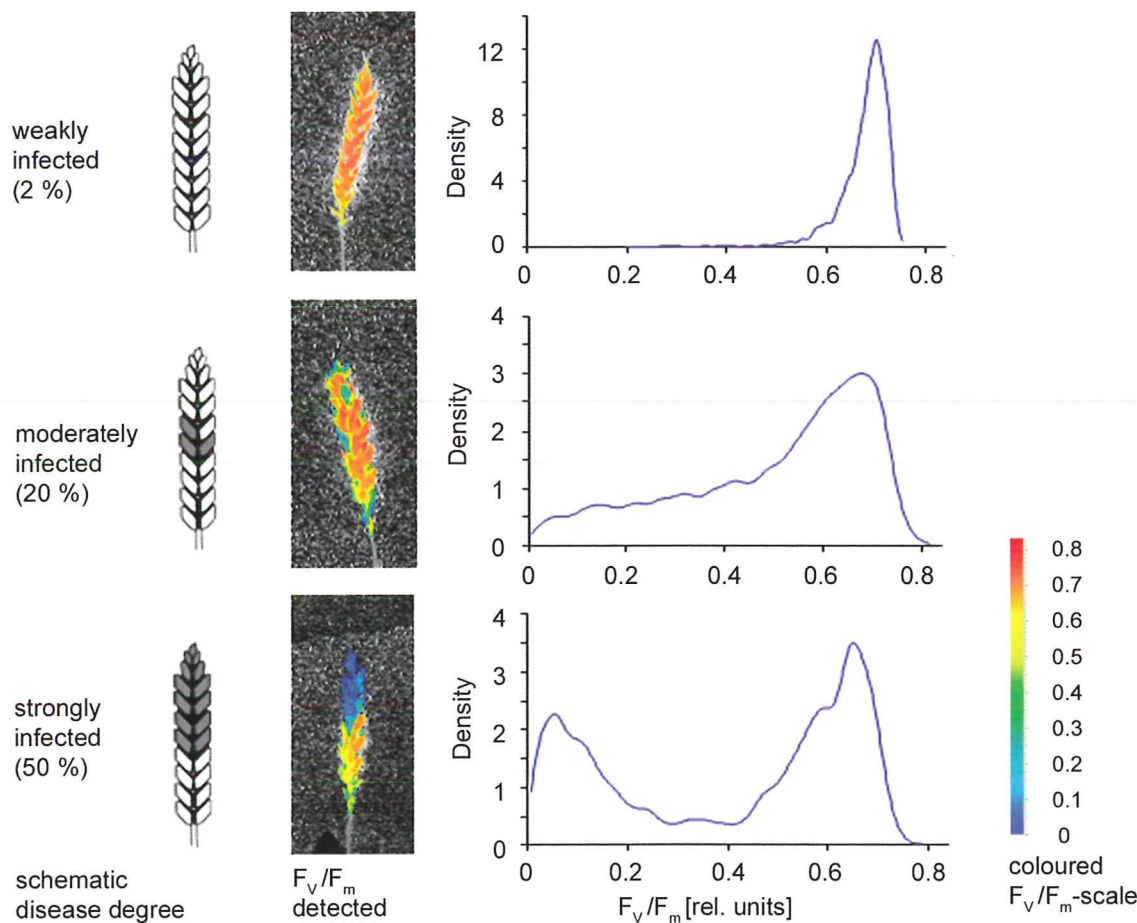


Fig. 1: Schematic depiction of different disease degrees (adapted from OLDENBURG, 2004) and the respective photochemical efficiency of a weakly, a moderately and a strongly infected ear: chlorophyll fluorescence images and density of  $F_v/F_m$ .

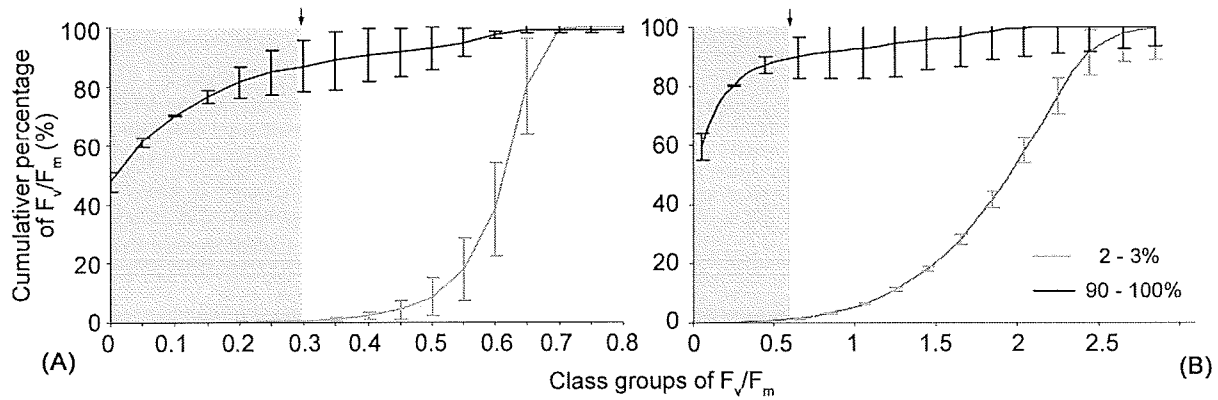


Fig. 2: Cumulative percentage of  $F_v/F_m$  (A) and  $F_v/F_0$  (B) of wheat ears of different classes of disease degrees.

The analysis of the cumulative  $F_v/F_m$  at 0.3 of ears with different degree of disease indicated that wheat ears of lowest disease class (2-3% disease degree) showed only a very minor proportion (0.68%) of this low photochemical efficiency class (Fig. 3). The proportion increased to 10.4% in ears of 10-20% and to 33.0% in ears of 40-60% disease degree. This clearly points out that under these conditions the ears contain both fully active as well as totally inactive, damaged spikelets. In ears visually rated as nearly completely dead (>90% disease degree), on average 85% of the spikelets were identified as damaged.

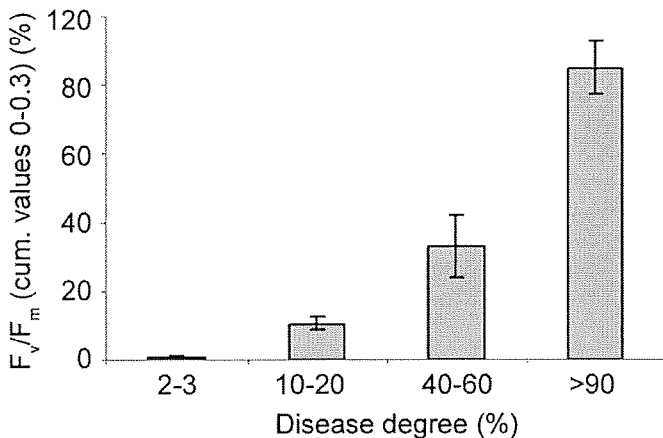


Fig. 3: Classes of cumulative values of  $F_v/F_m$  at 0.3 (%) at different degree of disease of the ears ( $n=2-6$ ).

A plot of the degrees of infection, as rated according to WALTHER et al. (2000) and the proportion of cumulative values of  $F_v/F_m$  at 0.3 (Fig. 4) emphasises the close correlation between both parameters ( $R^2 = 0.978$ ). This close correlation points out that the fluorescence parameter used may allow to successfully qualify and quantify infected ears. This interrelationship yielded the equation  $y = 4.00256 + 1.0731529 * x$ . For a future application of the method of disease detection, the degree of infection  $y$  can be determined using the regression equation and the  $F_v/F_m$ -values (cumulative portion at 0.3) as  $x$ -values.

However, the plot also indicates that the assignment of a low degree of infection is hardly possible. Only above an infection threshold of 5%, the clear relationship allows a statistically sound interpretation. This is also indicated by a root mean square error (RMSE) of 5.32%. Hence, this approach may cause a slight over- and underestimation

of about 6%, respectively, of the infection. However, this interferes only in the low-infection classes and mostly at the very beginning of infection.

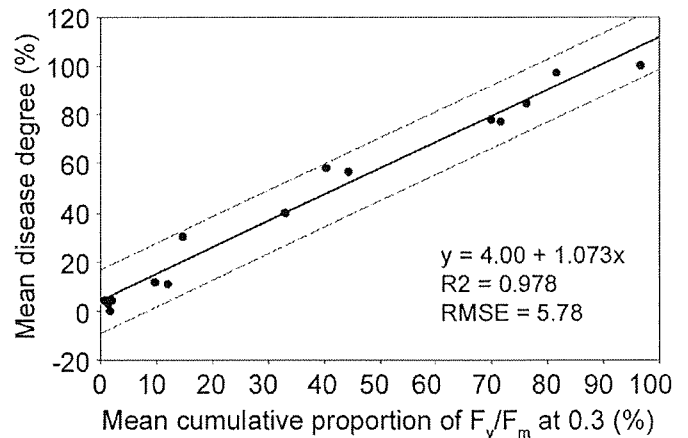


Fig. 4: Regression between mean cumulative proportion of  $F_v/F_m$ -values at 0.3 and mean degree of disease rated according to WALTHER et al. 2000 (15 ears, measured at 3 different dates).

#### Differentiation of the degree of disease during progressive infection

The measurements started one day after completion of inoculation. Ears with an infection larger than 5% on the 11th day after infection (dai) were classified as highly infected (Fig. 5, squares,  $n = 4$ ). Two ears (black lines) were classified as weakly infected (Fig. 5, weak symptoms) while the controls (Fig. 5, diamonds,  $n = 6$ ) were rated free of any infection.

Applying the cumulative portion of  $F_v/F_m$ -values at 0.3, it was successfully possible to identify diseased ears with a degree of infection of higher than 5% (Fig. 5). Ears with strong symptoms showed an average cumulative portion of  $F_v/F_m$  of 2.8%, even on the first day of measurement (6th dai). In contrast, weakly infected plants had a mean cumulative portion of  $F_v/F_m$  of 0.4%, and controls that of 0.2%.

On the 11th dai, the average difference between controls and plants with strong symptoms increased to 7.4%. There were no differences between control and diseased plants at a degree of infection below 5%. The statistical examination by means of the WILCOXON-rank sum test showed that the class of the strong diseased ears significantly

differs from the control in all measurements ( $p \leq 0.019$ ). A significant separation in the class of the weakly diseased ears could not be verified.

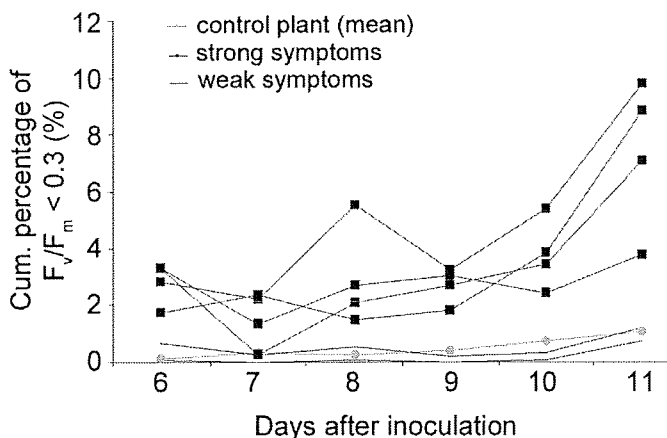


Fig. 5: Cumulative portion of  $F_v/F_m$ -values at 0.3 of controls (diamonds) and weakly (<5% disease degree, black lines) and strongly diseased plants (5-10% disease degree, squares) during progression of infection.

### Effects of infection on yield and the mycotoxin content of grains

#### Grain yield

As expected, the mass of harvested grains was the lower the higher the degree of infection. Compared to ears of the low infection class (0-5%), grain mass was reduced by about one third (0.45 g to 0.3 g) in plants of the strongest-infection class. However, this difference was statistically not significant.

The grain mass of the control plants was nearly the same as that of the infection class 5-10% and therefore relatively low. This may be due to the fact that stronger plants of the cultivated batch were inoculated while the control included some weaker plants.

#### Proportion of sterile grains and grains infected with mycelium

Some flower primordia have been seriously affected by *Fusarium* infection resulting in sterile grains. Furthermore, some of the grains have also been coated with a pink-reddish mycelium. The proportion of the so called shrunk grains, i.e. the sterile grains and those covered with the reddish mycelium increased with the degree of disease. In the class of weakly diseased ears, the proportion of shrunk grains was approx. 10%, in the class of strongly diseased ears (20-30%) it was twice as high. As expected, control plants were completely free of symptoms. Furthermore, the number of spikelet per ear and the ear length did not show any differences between control and infected plants.

Tab. 1: DON-contents of grains from infected and control plants (control 2 was removed because of mildew infection).

Sample	Mean DON content ( $\mu\text{g kg}^{-1}$ )
control	107
control	48
control	0
diseased, 0 - 5%	3583
diseased, 5 - 30%	> 6300

### Contents of mycotoxin

In addition to external symptoms of head blight disease, the infected plants were also massively contaminated by DON. As expected, untreated control plants did not contain any DON or showed only a minimum and neglectable content of DON (Tab. 1). However, the artificially infected plants were highly contaminated with DON and considerably exceeded the limits legal in the EU ( $1250 \mu\text{g kg}^{-1}$ ). Therefore, it could be verified that successful infection with *Fusarium* inevitably results in mycotoxin contamination. Hence, the results of the degree of infection obtained by CFI may be directly related to the mycotoxin content of the grains.

### Discussion

Main *Fusarium* infection occurs via asco- and conidiospores, respectively, from mid ear/panicle emergence until end of flowering (OLDENBURG, 2004). Under humid conditions, ascospores and conidia rapidly propagate on the soil or on plant residues and may be spread over the leaves by plashing rain. From there they easily reach the ears' surface (OBST und GEHRING, 2002; OLDENBURG, 2004). The fungi gradually penetrate into the host's tissue and cells, block the vascular bundles, and disturb nutritional supply and metabolic processes (KANG and BUCHENAUER, 2000). This is accompanied by a complete loss of chlorophyll, which finally leads to a bleaching of the ears as typical for head blight. Besides, above the point of infection, grains often shrunk (OLDENBURG, 2004). Hence, *Fusarium*-caused head blight is characterised by the occurrence of both healthy and diseased spikelet within one ear but finally all grains of the ear may die (HEITEFUSS et al., 1993). The dynamics and the extent of damage development mainly depend on environmental conditions favouring fungi growth and on resistance capability of cereal cultivars.

Owing to this dynamic, disease evolution is highly reflected by an increasingly disturbed and inhibited photosynthetic activity of the individual spikelet. Therefore, chlorophyll fluorescence analysis may be a very helpful tool to non-invasively monitor infection development. However, conventional portable glass fibre fluorimeters measure only an average chlorophyll fluorescence value on a distinct spot of the investigated objects. In a study on the recognition of tulip breaking virus (TBV) on three tulip cultivars with diverging colour patterns, POLDER et al. (2009) used the average  $F_v/F_m$  to identify the TBV-infection as accurately and as early as possible under laboratory conditions. The results of the average  $F_v/F_m$  differed by up to 46% from the disease degree rated by ELISA-tests. The authors referred this large disagreement to hail damages of the tulip leaves. However, it may also be due to the fact that averaging of the fluorescence parameter may level most disease-related differences.

Visual disease inspection is also an important source of uncertainty. POLDER et al. (2009) reported a deviation of visual rating results from those of the ELISA-test of up to 28%. In contrast, our rating errors were considerably less (9.6%). This higher accuracy may be due to the fact that rating of cereal is an often applied and evaluated method, quite in contrast to that of TBV-damaged coloured tulip leaves.

Instead of average chlorophyll fluorescence values spatial variability of disease development was analysed by using chlorophyll fluorescence images in the presented study. This approach allowed the evaluation of the changes of the infection pattern typical for head blight. During the course of disease development, increasing areas of the ears with dead spikelets and, hence, low  $F_v/F_m$  (<0.3), alternates with healthy spikelets showing high  $F_v/F_m$  (>0.3). Hence, the spatial distribution pattern, and not the average chlorophyll fluorescence, is used to estimate the disease degree (BAURIEGEL et al., 2009).

Recent literature (HAUSER-HAHN, 2005; KUCKENBERG et al., 2007) let us assume that variations in photosynthetic activity and, concomitantly, in chlorophyll fluorescence may occur even in the early phase of infection. Therefore, a daily recording was chosen during

the first week after infection. However, the measurements revealed that early changes at cellular level had minor effects on PSII. Only if the integrity of the host plant's cellular structures was destroyed by fungal hyphae, the photosynthetic system was damaged. This interpretation agrees with the findings of KANG and BUCHENAUER (2000). By means of light and electron microscopy, these authors showed that cellular changes such as degeneration of cytoplasm and of cell organelles, the disintegration or digestion of cell walls and deposition of material on vessel walls of the diseased ears occurred only 4-5 d after infection.

The presented analysis indicate that infection by *Fusarium culmorum* on less resistant wheat cultivars did obviously not induce any defence reactions in the spikelet tissues, i.e. *Fusarium* fungi destroy their host perthophytically. In contrast, crown rust infection development on oat leaves (SCHOLLES and ROLFE, 1996) results in pronounced defence reactions including a short-term upregulation of photosynthetic activity and, hence, increase of  $F_m$  in „green islands“ immediately surrounding the area of sporulation. Nevertheless, BUCHENAUER and KANG (2002) suggest that high resistant wheat cultivars may have developed defence mechanisms against *Fusarium* at the cellular level, e.g. by the depositions of distinct cell wall materials and the formation of papillae.

The capability of chlorophyll fluorescence analyses or imaging to rapidly and precisely detect tissue infection in general strongly depends on the object investigated. Its effectiveness may also vary in time. As shown by MATOUŠ et al. (2006), *Pseudomonas syringae* on *Arabidopsis thaliana* is detectable within few hours after infection. In contrast, first symptoms of infection caused by *Venturia inaequalis* on apple seedling leaves can only be recognised 7 d after infection (FÖRSCHLER et al., 2003). Furthermore, identification of *Chrysomyxa rhododendri* evoked needle rust on Norway spruce seedlings by chlorophyll fluorescence analysis (BAUER et al., 2000) is only possible 3 weeks after infection.

Moreover, turnip mosaic virus infection of stem mustard did not at all affect the maximum photochemical efficiency of PSII ( $F_v/F_m$ ) but slightly reduced non-photochemical quenching and increased the contribution of alternative electron sinks (GUO et al., 2005). The latter indicates an over-excitation of the photochemical systems, but surprisingly no obvious inhibition of photosynthetic competence occurred.

In wheat spikelets, HÄUSER-HAHN (2005) found no changes in the amount of chlorophyll after three days of *Fusarium* infection. Hence, early physiological effects of fungal infection include a decline in total photosynthetic activity and the maximum quantum efficiency of photosynthesis rather than a rapid degradation of photosystems as indicated by chlorophyll reduction.

In the present study, chlorophyll fluorescence analysis could clearly differentiate diseased and health ears after 5 dai. This nicely reflects the results of KANG and BUCHENAUER (2000), who identified *Fusarium* infection of spikelet tissue within 4 dai by means of microscopic analysis of cytological preparations. The short time gap of 1 to 2 d may be due to the time needed for the development of external fungi mycelia visible on the plant surface.

KUCKENBERG et al. (2007) proposed that in chlorophyll fluorescence imaging analyses, the  $F_v/F_0$  ratio should yield more precise information about rust development of winter wheat leaves than the maximum photochemical efficiency  $F_v/F_m$ . In their study,  $F_v/F_0$  declined 2 to 3 d before occurrence of any visual symptoms. However, in this present study no advantage of  $F_v/F_0$  could be verified.

$F_v/F_0$  is assumed to reflect the maximal quantum yield of fluorescence (BUSCHMANN et al., 1999). Empirically it is rated as an indicator of PSII competence. This parameter has also been used to estimate the transportation of energy from PSII to PSI at 692nm and -196 °C. (KITAJIMA and BUTLER, 1975). However, no clear physiological meaning could be ascribed to  $F_v/F_0$  up to now (MATOUŠ et al., 2006).

In contrast,  $F_v/F_m$  indicates the PSII photochemical efficiency by dark adapted plants with full open PSII-reaction centres (VON WILLERT et al., 1995; MAXWELL and JOHNSON, 2000) as early shown by KITAJIMA and BUTLER (1975).

Anyway, the presented study clearly indicated that important basic prerequisites for chlorophyll fluorescence measurements have to be observed to yield a close correlation between visual rating and  $F_v/F_m$  or  $F_v/F_0$ , respectively. Both the chlorophyll fluorescence signal itself and photosynthesis are highly susceptible to variations in irradiation, e. g. by changes of the distance to light source, direct sun light or shadow effects. For meaningful maximum fluorescence signals, a complete uniform illumination of the object is particularly important. However, rating errors or bad edge regions recognition on infertile spike heads may further complicate the exact comparison.

Despite these problems, the presented approach should rapidly yield valuable information on the degree of *Fusarium* infection on wheat and hence on the risk of its mycotoxin contamination. On the other hand, it has been questioned that visual appearance of head blight always reflects detectable mycotoxin contents (MIEDANER and SCHNEIDER, 2002). In the present study, all infected ears had measured mycotoxin contents of almost three-fold (disease degree <5%) to more than six-folds (disease degree >30%), respectively, of the relevant EU-proposed threshold for DON (1250 µg kg<sup>-1</sup>). Hence, it clearly exceeded the acceptable critical concentration, presumably non hazardous to health. It is still controversially discussed whether the fungal development directly or via yet un-identified toxic metabolic products released by the fungi may induce the dieback of plant tissue. Furthermore, it has not yet been proven, how far the production and the segregation of mycotoxins, respectively, can simultaneously damage the host tissue. It is clear, that DON mycotoxins and nivalenols inhibit the protein synthesis by binding to ribosomes (KANG and BUCHENAUER, 1999). It may be possible that DON and nivalenol can inhibit the defence responses of the host cell thus relieving the penetration of *Fusarium* hyphae (OBST and GEHRING, 2002).

On the other hand, MIEDANER and SCHNEIDER (2002) and BARTELS and RODEMANN (2003) assumed that the disease degree does not necessarily reflect a certain mycotoxin content because concentration of mycotoxin may be highly variable in response to various external factors. Nevertheless, under controlled conditions, a close correlation between mycotoxin content and the degree of disease as obtained by visual rating could always be obtained. Certainly, additional measurements will be necessary to confirm and reinforce the close correlation between mycotoxin content, degree of disease and the results of image analyses. The possibility of a direct inference from imaging to mycotoxin content would be an important progress for practical applications.

To conclude, by chlorophyll fluorescence imaging, it is possible to very early (from 6<sup>th</sup> dai) and also very exactly (10% RMSE) identify *Fusarium* infection on ears of winter wheat plants. The chlorophyll fluorescence parameter  $F_v/F_m$  proved to be a helpful indicator of the damage of photosynthetic activity caused by *Fusarium culmorum*. Nevertheless, for field application of this method, further investigations are necessary.

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Address of the authors:

E. Bauriegel, W.B. Herppich, A. Giebel, Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Max-Eyth-Allee 100, D-14469 Potsdam, Germany