Detection and quantification of *Fusarium*-specific disease symptoms in maize ears by spectral imaging

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Abstract

In today's maize breeding, resistance against ear fusariosis is an important challenge. Breeders need reliable methods for quantifying *Fusarium*-specific disease symptoms on maize ears. 20 maize varieties were inoculated with conidia of two predominant pathogenic *Fusarium* species at the stage of female flowering. After harvest, the ears were cut vertically into halves. Spectral images were taken of the inner side of the halves. A supervised classification procedure was applied to quantify the areas with *Fusarium*-specific disease symptoms. Results were compared to the visual estimation of an expert. The figures from the image analysis were slightly higher than the expert's estimation but they may be used to predict the expert estimation with $r^2 = 0.81$.

Key words: maize production, maize breeding, ear fusariosis, disease control, automatic image analysis

1. Introduction

The prevention of *Fusarium* infections and *Fusarium* toxin contaminations in maize ears is an important challenge in agricultural production to fulfill the requirements of European quality standards for harvested products. An effective measure to reduce the infection risk with *Fusarium* pathogens is the use of maize varieties being resistant against ear fusariosis. It is therefore of special interest to maize breeders to obtain reliable methods for quantifying *Fusarium*-specific disease symptoms on maize ears for genotype selection purposes. Current methods to assess maize ear fusariosis are based on the visual estimation of the infected area on the outer surface of the whole dehusked ear (Enerson & Hunter, 1980) or of the number of infected kernels (Reid & Sinha, 1998). The severity of infection symptoms is estimated as a percentage of the whole ear surface or the total number of kernels, respectively. Often, the rating is done by using a small number of classes, defined by a range of the severity percentage. Enerson and Hunter (1980) and CIMMYT (1985) use 5 classes, Silva et al. (2007) use 6 classes, Reid and Sinha (1998), Papst et al. (2007) and Afolabi et al. (2007) use 7 classes. However, the accuracy of these evaluations is mainly dependent on the qualification and experience of the individual assessing person.

As automatic image analysis offers innovative options to gain more precise, repeatable and comparable results in estimating differently coloured areas of objects, Chungu et al. (1997) tried to assess the disease severity of maize ears after inoculation with *Fusarium graminearum* by image analysis. Due to the inability of the image analyzer to distinguish between infected and healthy kernels, outlines of the infected areas were drawn manually onto clear acetate sheets. These sheets could be analyzed automatically, so the image analysis was only partially automated.

Pearson and Wicklow (2006) and Del Fiore et al. (2010) showed the ability of spectrometers and spectral imaging systems to discriminate corn kernels infected by fungi from healthy corn kernels. Spectral signatures in the NIR (near infrared) are better suited for the
discrimination between infected and healthy corn kernels than the visible wavelength range because of the different pigmentation of corn genotypes (Del Fiore et al., 2010).

In this report, we present first results of a new approach by detecting and quantifying *Fusarium*-specific disease symptoms in maize ears by spectral imaging technology.

2. Material and Methods

Image analysis was based on 2000 maize ears from 20 varieties cultivated under field conditions at a location near Braunschweig (Germany) in 2010. To promote ear fusariosis, the ears were inoculated at the stage of female flowering (BBCH 65, Meier 2001) with conidia of *Fusarium graminearum* or *F. culmorum*, which are predominant pathogenic species producing mycotoxins in European maize (Logrieco et al., 2002). The ears were manually removed from the experimental field at the stage of kernel maturity (BBCH 89). After harvest, the ears were dehusked and cut vertically into halves by use of a ribbon saw. This allows an assessment of the inner *Fusarium*-specific disease symptoms in the rachis showing greyish-brownish or pink discolouration of the pith, which is usually easier to evaluate than the outer signs due to interferences with other fungal pathogens developing on the surface of the ear. An experienced expert visually evaluated the disease incidence on the cut surface of the halves and estimated the size of the visibly infected area as a percentage of the total area of the inner surface of the rachis.

![FIGURE 1: Inner side of a cut maize ear (top) and segmentation (bottom) into its central pith (white), woody ring (lignified parenchyma cells, grey), chaff (residual parts of florets, dots) and kernels (stripes).](image)

Spectral images were taken from the inner side of the ear halves with a Zeutec spectral imaging system that includes a Specim ImSpector V10E spectrograph, a Zeutec LuxVis CMOS camera, and a rotating mirror. Each image contained 940 wavelength images in the wavelength range from 460 nm to 1130 nm. Each image was spectrally calibrated using a 60% reflectance standard (Spectralon). All images were low-pass filtered by a two-dimensional Gaussian filter.

Each image contained 2 ear halves, so in total 1000 images were acquired. 42 images that contained fusariosis symptoms were selected manually for training purposes. Within these images, areas with and without visible fusarium symptoms were manually selected, separately for the four area subtypes pith, woody ring, chaff, and kernels (Fig. 1). Thus, we defined eight segmentation classes in the training data.

The spectral feature set was built, starting with 135 calibrated reflectance values at wavelengths from 460 nm to 1130 nm with a step of 5 nm. After interactive analysis of the spectra of the eight classes, some additional combined features were defined (Table 1).

Linear discriminant analysis was used to calculate classification criteria for the segmentation of all spectral images. The GNU R package was used for all statistical calculations.
First, a measure $ES$ for the error of separation of two classes in a n-dimensional feature space was defined: Normal distribution was assumed for both classes. The class mean points $\mu_1$ and $\mu_2$ and the Fisher discriminant function $\alpha$ were calculated as described by Wehrens (2011). If both classes have equal variances, the “discriminant point” between two classes is $\mu = (\mu_1 + \mu_2) / 2$. In cases of differing variances, this point might not always be the ideal discriminant point. In this paper, the discriminant point $\delta$ was defined in the feature space on the line between $\mu_1$ and $\mu_2$, such that it had the same Mahalanobis distance from $\mu_1$ and from $\mu_2$ with the resp. Mahalanobis metric of each class. The Fisher discriminant value $F(x) = \alpha^T x$ was calculated for the discriminant point $F(\delta)$, for the class means $F(\mu_1)$ and $F(\mu_2)$, and for all samples $F(x_i)$. The error $e_i$ was calculated for all samples $x_i$: For correctly classified samples $x_i$, the error was $e_i = 0$. For all misclassified samples $x_i$ in both classes $c = 1, 2$, the error $e_i$ was calculated as $e_i = (F(x_i) - F(\mu_c)) / (F(\delta) - F(\mu_c))$. The measure of the error of separation $ES$ was calculated as the square sum of all errors: $ES = \Sigma e_i^2$.

The first step of the discriminant analysis was to compare all segmentation classes and also pairs of “similar” classes (neighbouring areas with identical symptomatic appearance) in order to find one class or class pair that can best be discriminated from the total of all other classes. For each class or pair of classes, all training samples were divided into two sample groups, one group with all samples within the selected class(es) and the second group with all other samples. For both of the sample groups, normal distribution was assumed. The separation error measure $ES$ for the separation of these two groups with the complete feature set was calculated. This was done independently for all classes and class pairs. The best separable class or class group (minimum $ES$ value) was selected as first resp. next class or class group for a step-wise classification. The samples of that class or class pair were removed from the total of samples and this step was repeated until all classes were put into the classification order.

Each time after selecting one class or class pair as the next one in the classification order, a second discriminant analysis pass was applied in order to select an optimal subset of three

<table>
<thead>
<tr>
<th>Generic feature name</th>
<th>Calculation of the feature</th>
<th>Investigated features</th>
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</thead>
<tbody>
<tr>
<td>REFLXXX</td>
<td>Calibrated reflectance at a wavelength of XXX nm</td>
<td>135 (REFL460 - REFL1130)</td>
</tr>
<tr>
<td>DYYY_XXX</td>
<td>Difference REFLYYY - REFLXXX</td>
<td>D530_480, D630_570, D640_580, D650_550, D660_580, D900_850, D920_740, D1100_900, D1130_1030</td>
</tr>
<tr>
<td>DDGSXXX</td>
<td>Value of the reflectance spectrum at wavelength XXX after filtering the spectrum with a second order derivative Gaussian filter.</td>
<td>DDGS677, DDGS970, DDGS975, DDGS1030</td>
</tr>
<tr>
<td>DEPRYYY</td>
<td>Depression of the spectrum at wavelength YYY. Distance in the spectrum between the point of the spectral reflectance at XXX and the connecting line between the reflectances at two other wavelengths XXX and ZZZ. XXX &lt; YYY &lt; ZZZ.</td>
<td>DEPR677 (XXX=605; ZZZ=720)</td>
</tr>
</tbody>
</table>
features for the segmentation of the resp. class or class pair from the total of all other (not yet classified) classes. In a full exhaustive search, all possible combinations of three features were examined, and the separation error measure $E_S$ was calculated for the resp. classification task and the resp. feature set. The feature set with the minimum $E_S$ was selected and the Fisher discriminant function $\alpha$, the discriminant point $\delta$ and the Fisher discriminant value $F(\delta)$ were calculated for later use in the image segmentation.

All images were segmented by stepwise classification. For each pixel, the classes or class pairs were checked in the previously described order and with the respective feature set, calculating the Fisher value $F(x_i)$ and comparing it to the Fisher discriminant value $F(\delta)$ until the Fisher criterion was fulfilled for one of the classes or class pairs.

3. Results and Discussion

The spectral reflectance of areas with *Fusarium*-specific disease symptoms (fusariosis areas) differed from the reflectance of areas without symptoms (healthy areas). Differences were found in the visible and in the near infrared wavelength ranges. First analyses of the spectra led to the definition of some combined spectral features (Table 1).

![Figure 2](image.png)

**FIGURE 2**: Inner side of cut maize ears with heavy fusariosis symptoms. a) Photograph. b) Segmentation result of spectral image analysis. The lines show the automatically detected borders of the pith, the rachis (pith + woody ring + chaff), and the total ear. The detected fusariosis areas are shown in dark grey (pith fusariosis area) and light grey (woody ring + chaff fusariosis area). 22 % of the pith area (sum of both ears) and 23 % of the total rachis were found to show fusariosis symptoms. The human expert rated each ear separately with values of 15 % (left ear) and 30 % (right ear), so the expert's average value for both ears was 22,5 % (of the rachis).

The discriminant analysis showed that the class pair woody ring + chaff could be separated with a smaller error than the individual classes woody ring and chaff. Therefore, these classes were combined into the classes i) woody ring + chaff with fusariosis and ii) healthy woody ring + chaff. In Table 2, the result of the discriminant analysis is presented. The sequence of classes does not give a final answer to the question which features are the most relevant ones for discrimination between fusariosis and healthy areas. But the features at order number 4 are at least candidates for the answer, because they separate best between the last fusariosis class and the two remaining healthy classes. Remarkably, the same feature set was already used in order number 1.
TABLE 2: Result of the discriminant analysis: In this order of classes, each pixel is tested whether it more likely belongs to that class or to the group of the remaining other classes. Each decision is based on a subset of 3 features.

<table>
<thead>
<tr>
<th>Order</th>
<th>Class</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pith of rachis, visible fusariosis</td>
<td>D630_570, D900_850, D1030_920</td>
</tr>
<tr>
<td>2.</td>
<td>Kernels, visible fusariosis</td>
<td>REFL740, REFL750, REFL820</td>
</tr>
<tr>
<td>3.</td>
<td>Kernels, healthy</td>
<td>REFL500, REFL760, DDGS1030</td>
</tr>
<tr>
<td>4.</td>
<td>Woody ring + chaff*, fusariosis</td>
<td>D630_570, D900_850, D1030_920</td>
</tr>
<tr>
<td>5.</td>
<td>Woody ring + chaff*, healthy</td>
<td>REFL1050, DEPR677, D630_570</td>
</tr>
<tr>
<td>6.</td>
<td>Pith of rachis, healthy</td>
<td>None needed</td>
</tr>
</tbody>
</table>

*Chaff = residual parts of florets.

One sample image and its segmentation result is shown in Fig. 2. The segmentation result includes two types of information: The ear is segmented into its constituents: kernels, woody ring + chaff, and pith. The parts pith and woody ring + chaff are divided into healthy and fusariosis areas. The further evaluation was restricted to the rachis, so the kernels and their division into healthy and fusariosis areas were ignored.

The final results of the spectral image analysis were calculated by dividing the pixel count of the found fusariosis areas in the rachis (pith + woody ring + chaff) by the total pixel count of the rachis. The results were calculated for each image (containing two ears) and compared to the average of the two ears' visual expert estimations. Additionally, the image analysis results were also calculated separately for the pith area and for the woody ring + chaff area.

FIGURE 3: Visual rating of fusariosis areas vs. spectral imaging analysis results. Left side: Image analysis classes for the total rachis. Right side: Image analysis classes for the pith.

Linear regression was calculated between the image analysis results and the visual expert estimations. The intercept of the linear regression was less than 1% in each case, so all regressions were done with a fixed zero intercept (R model formula: $Y \sim X - 1$). The image analysis results were found to be well correlated with the visual estimation. The expert’s estimation was done for the total rachis, so it was expected that the image analysis results for the total rachis had the best correlation. This was found as expected but there was also a very high correlation between the image analysis results for the pure pith areas and the expert estimation (Fig. 3) Exclusion of the pith and comparing the image analysis results for the
woody ring + chaff area to the expert estimation led to a lower correlation ($r^2 = 0.62$).

The spectral image analysis yielded slightly higher figures for the percentage of fusariosis areas than the expert estimation. The regression coefficients of the visual estimation vs. image analysis were 0.86, 0.81, and 0.75 for the image analysis results of the total rachis, the pith, and the woody ring + chaff, respectively.

The techniques described in this paper are a promising approach for the quantitative assessment of inner *Fusarium*-specific disease symptoms in the rachis of maize ears. Next steps in the project will include further combined spectral features, a higher number of features in the classification, and separate data for training and testing the classificator. While in this paper, the validation of the image analysis results could only be done by comparing them to the visual expert estimation, it is also intended to measure the mycotoxin content of infected maize ears and to compare both, the expert estimation and the image analysis results to the mycotoxin contents.

References


