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IDENTIFICATION OF *FUSARIUM* DAMAGED WHEAT KERNELS USING IMAGE ANALYSIS

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Abstract

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Visual evaluation of kernels damaged by *Fusarium* spp. pathogens is labour intensive and due to a subjective approach, it can lead to inconsistencies. Digital imaging technology combined with appropriate statistical methods can provide much faster and more accurate evaluation of the visually scabby kernels proportion. The aim of the present study was to develop a discrimination model to identify wheat kernels infected by *Fusarium* spp. using digital image analysis and statistical methods. Winter wheat kernels from field experiments were evaluated visually as healthy or damaged. Deoxynivalenol (DON) content was determined in individual kernels using an ELISA method. Images of individual kernels were produced using a digital camera on dark background. Colour and shape descriptors were obtained by image analysis from the area representing the kernel. Healthy and damaged kernels differed significantly in DON content and kernel weight. Various combinations of individual shape and colour descriptors were examined during the development of the model using linear discriminant analysis. In addition to basic descriptors of the RGB colour model (red, green, blue), very good classification was also obtained using hue from the HSL colour model (hue, saturation, luminance). The accuracy of classification using the developed discrimination model based on RGBH descriptors was 85 %. The shape descriptors themselves were not specific enough to distinguish individual kernels.

Fusarium, mycotoxin, DON, deoxynivalenol, image analysis, wheat

An important factor influencing food chain safety is a risk of mycotoxins occurrence. Deoxynivalenol (DON), also known as vomitoxin, rank among the most widespread mycotoxins present in cereals. DON is a metabolite with a low molecular weight from the group of trichothecene mycotoxins produced by fungi of the genus *Fusarium*, especially *F. graminearum* and *F. culmorum*. These fungi infect ears of barley and wheat or maize where they induce a disease called *Fusarium* head blight (FHB) on wheat and barley or maize ear rot on maize.

DON causes intestinal disorders and vomiting, skin changes and it is also known for its immunotoxic effect on human and animal organisms. A particular attention is paid to this mycotoxin in agricultural practice because of contaminated feed refusal by pigs.

Cereal kernels infected by the *Fusarium* pathogens differ from healthy kernels in a number of aspects, mainly by their chemical composition resulting in lower grain quality. The changes in chemical composition are accompanied by changes in physical characteristics, e.g. kernel bleaching and weight reducing. Mathur and Cunfer (1993) described wheat kernels infected by *Fusarium* spp. as smaller and shrivelled, with the surface looking like covered with white chalk, often with pink tint. However, variability of these symptoms could be specific for particular variety (Zhang and Jin, 2004) and some of the symptoms can also be caused by other factors than the infection by *Fusarium* spp., for instance, by unfavourable environmental conditions during the growing season. Literature sources demonstrate that there is a relationship between visual symptoms on kernels and DON content in wheat, however, various authors consider it variable (Sinha and Savard, 1997) and differently close, depending on location, genotypes, and year (Beyer

et al., 2007, Liu *et al.*, 1997; Paul *et al.*, 2005; Polišenská and Tvarůžek, 2007). It is also caused, among others, by a structure of *Fusarium* spp. present on the kernel. Some *Fusarium* spp. do not produce DON, but they develop identical symptoms like DON producing species. The occurrence of *Microdochium nivale*, which does not produce any mycotoxins, is also of significance (Nicholson *et al.*, 2003). Despite that, the relationship between higher concentrations of DON in harvested grain and the presence of visually evaluated scabby kernels (VSK) exists (Beyer *et al.*, 2010) and is relatively often used in practice. In Canada, for instance, it is one of the criteria used for classifying grain into quality categories. Results of numerous studies conducted by the Canadian Grain Commission document that samples with the DON content higher than 1 000 μg.kg^{-1} contained on average more than 0.4 % VSK (Dexter and Novicki, 2003). Similar results were also obtained from survey of wheat grain harvested in the Czech Republic in 2003–2005, when the minimum content of VSK for samples with DON content higher than 1 000 μg.kg^{-1} was 0.47 %. A close correlation was found between VSK percentage and DON content in all years; correlation coefficients ranged in individual years from 0.68 in 2005 to 0.92 in 2004 (Polišenská *et al.*, 2007).

Though the determination of an accurate level of DON content in a certain cereal batch based on the evaluation of VSK occurrence cannot be supposed, using rapid and unambiguous methods could prevent subjectivity at evaluating VSK and thus identify contaminated lots. Samples would be taken from such lots using a method specified by legislation and subjected to accurate chemical analyses. However, visual screening is a labour-consuming way and may lead to inconsistencies coming from low repeatability and high subjectivity of such an evaluation.

The use of a rapid method on the basis of digital image analysis for individual kernels can also, in combination with a sorter, remove infected kernels, and thus to reduce mycotoxin content in grain. In the imaging studies dealing with FHB, kernel morphology and colour characteristics were the primary features used to distinguish damaged from healthy kernels, with the *Fusarium* damage characterized by kernels having a white or pinkish colour and being shrivelled (Delwiche *et al.*, 2005). However, the symptoms of the disease can be sometimes hardly noticeable by naked eye. Digital imaging, when combined with appropriate statistical methods such as artificial neural networks can produce more accurate determination of VSK percentage than the human expert panel, as concluded by Ruan *et al.* (1998).

This study was aimed at using digital image analysis and statistical methods to develop a model for identifying wheat kernels exhibiting *Fusarium* disease symptoms. We assumed that the *Fusarium* infected kernels are distinguishable from the healthy ones on the basis of such symptoms and image analysis will increase the accuracy of discrimination.

MATERIALS AND METHODS

Winter wheat kernels used in this study were taken from two field experiments: a) variety Ludwig – artificial inoculation, different fungicidal treatments (four fungicides and three application times), and b) variety Sulamit – natural infection, different agronomical practices (three pre-crops – pea, lucerne, maize, and four tillage practices). Due to a different FHB level of individual experimental variants, samples with different VSK percentages were available. The harvested grain was examined visually and kernels were classified into the three groups:

1. kernels with no infection symptoms (VSK0),
2. whitish, pinkish kernels of normal size (VSK1),
3. whitish, pinkish, strongly shrivelled kernels (VSK2).

Forty kernels were selected deliberately to cover equally both varieties (20 + 20) and healthy/infected kernels – 19 of which were characterized as VSK0, 16 as VSK1 and 5 as VSK2. All symptomatic kernels were evaluated as one group for discriminant analysis. DON content was assessed in individual kernels using an ELISA method (R-Biopharm, 2007) at the limit of quantification of 0.2 mg·kg^{-1} and assessment uncertainty of 25 %. The amount of extraction agent was adjusted depending on the weight of meal from one kernel, maintaining the extraction ratio specified by the ELISA kit manufacturer.

Images of individual kernels were obtained using a digital reflex camera Canon EOS 20D with a macro lens and a ring flash in manual mode. Imaging was performed on contrast (dark) background under stable conditions, i.e. at minimized effects of ambient light, and recorded in the form of a digital image in the RGB model. Colour and shape characteristics were obtained by image analysis using software Longboard™ 7.4 (IMT i-Solution, Inc., USA). In the image, the area representing the kernel was selected. Basic colour and shape descriptors were determined for each object (Tab. I). Colour descriptors represent average values calculated for the whole area.

Statistical analysis was carried out using software Statistica 8.0 (StatSoft, Inc., USA). Statistical significance was assessed at a level

I: *A list of the descriptors used*

Term	Description
R, G, B	Red, green, and blue
H, S, L	Hue, saturation, and luminance
Length, width, perimeter, area	Basic shape parameters
$S_1 - S_{10}$	Shape parameters derived from basic ones (Suchowilska and Wiwart, 2006)

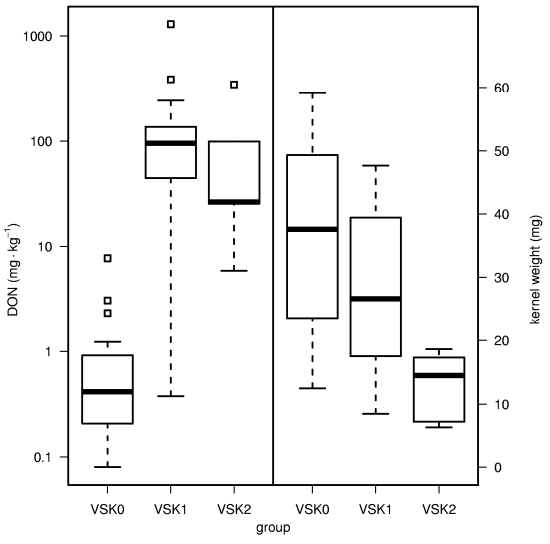
of 0.05. Relationships between samples were described using multivariate statistical methods (hierarchical cluster analysis, principal component analysis). Various descriptors were tested to develop a discrimination model by means of linear discriminant analysis. Both primary colours (RGB) and derived descriptors of the HSL colour model were employed. The method of linear discriminant analysis established decision criteria in the form of discrimination equations when based on obtained descriptors it was decided whether the kernel is infected or healthy.

RESULTS AND DISCUSSION

Figure 1 illustrates a comparison of DON contents and kernel weights along with classifying into individual groups based on visual evaluation. It is apparent that the kernels visually evaluated as healthy (VSK0) and infected (VSK1 + VSK2) differ significantly in DON concentration – 1.06 and 160.54 mg·kg^{-1}, respectively (Tab. II). Sinha and Savard (1997) detected DON in about 50 % of the normal-looking and shrivelled kernels (typically 5 ppm or less), all tombstone kernels contained DON, but he concentration varied considerably (from less than 1 ppm to 600 ppm). Kernel weight was markedly reduced by infection (in average by 35 %, from 36.6 mg to 24.0 mg); but there is a large overlap between VSK0 and VSK1, so it cannot be generally considered as specific implication of FHB effects. Results of correlation analysis are given in Tab. III. Significant correlations were found between kernel weight and shape descriptors, especially the width (r = 0.877) and area (r = 0.849), which corresponds to weight reduction. DON concentration only weakly

II: *Summary statistics of the basic descriptors of healthy and infected kernels*

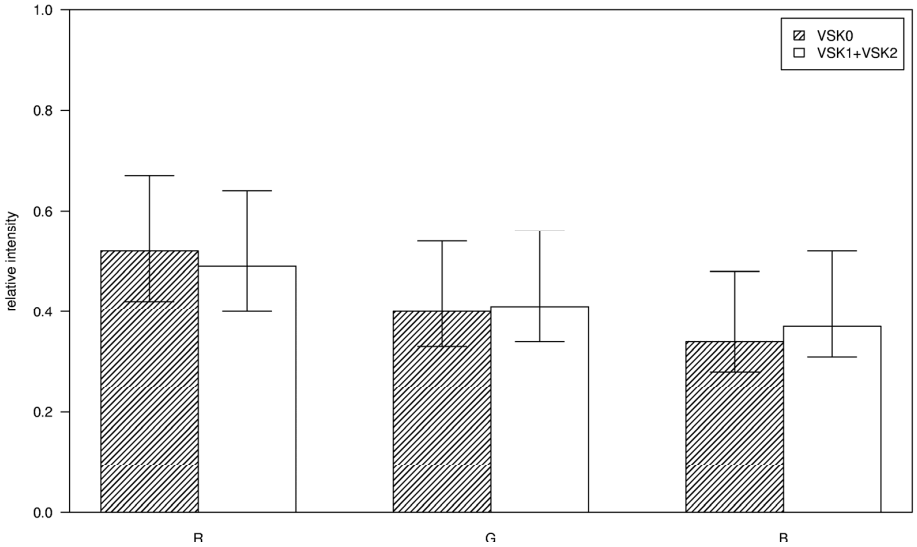
	DON (mg·kg^{-1})	Weight (mg)	Length (mm)	Width (mm)	Perimeter (mm)	Area (mm^2)	R	G	B
VSK0 (n = 19)									
min	0.08	12.5	5.70	2.34	13.79	9.97	0.42	0.33	0.28
max	7.73	59.1	7.61	3.80	19.25	22.59	0.67	0.54	0.48
mean	1.06	36.6	6.47	3.08	16.43	15.79	0.52	0.40	0.35
median	0.42	37.6	6.47	3.14	16.65	16.24	0.49	0.38	0.33
SD	1.80	14.5	0.47	0.52	1.47	3.73	0.07	0.06	0.06
VSK1 + VSK2 (n = 21)									
min	0.38	6.4	5.98	2.21	14.54	9.71	0.40	0.34	0.31
max	1290.70	47.7	7.58	3.66	18.61	20.46	0.64	0.56	0.53
mean	160.54	24.0	6.69	2.91	16.70	15.29	0.49	0.41	0.37
median	87.38	21.9	6.69	2.95	16.75	15.63	0.48	0.40	0.36
SD	279.33	12.8	0.41	0.44	1.14	3.16	0.06	0.06	0.05



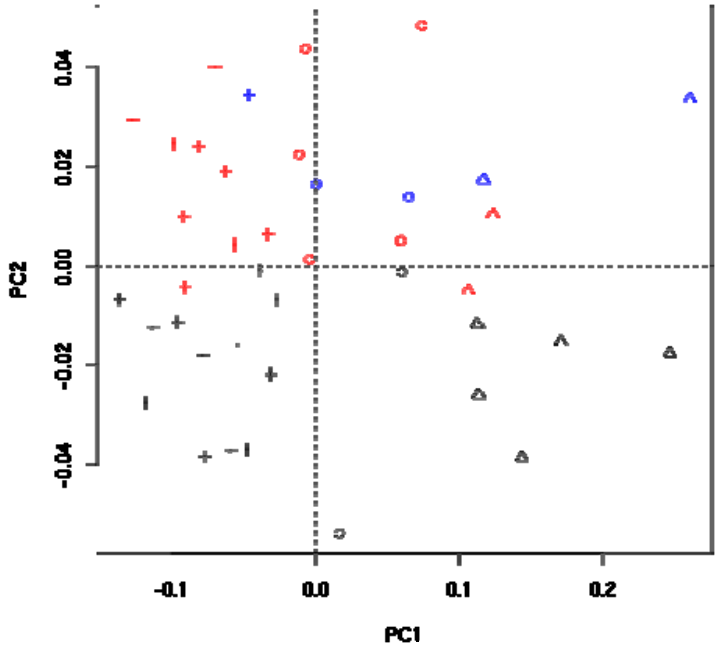
1: *Deoxynivalenol (DON) content and kernel weight in individual groups of visually scabby kernels (VSK0 – kernels without visual symptoms, VSK1 – whitish, pinkish kernels of normal size, VSK2 – whitish, pinkish, shrivelled kernels). Each group is represented by the extreme of the lower whisker, the lower hinge, the median, the upper hinge and the extreme of the upper whisker. Whiskers are limited to distance of 1.5×inter quantile range from appropriate hinge.*

III: *Correlations (r) between the selected descriptors and DON concentration and kernel weight (n = 40). The values in bold are statistically significant (p < 0.05).*

Descriptor	DON concentration	Kernel weight
Length	0.186	**0.387**
Width	–0.066	**0.877**
Perimeter	0.094	**0.670**
Area	–0.028	**0.849**
R	–0.089	**–0.427**
G	0.005	**–0.595**
B	0.196	**–0.722**
L	0.201	**–0.564**
H	**0.359**	–0.004
S	–0.218	–0.145



2: *A comparison of average, minimum and maximum values of red (R), green (G) and blue (B) components for asymptomatic (VSK0) and symptomatic (VSK1 + VSK2) kernels*



3: *Results of principal component analysis (PCA) in the RGB colour model. Symptomatic kernels (VSK1 + VSK2) (red and blue) and asymptomatic kernels (VSK0) (black) are distinguished by colour. A shape symbols distinguish three groups differing in average brightness (luminance) according to results of hierarchical cluster analysis.*

correlated with kernel weight (r = –0.329) and hue (r = 0.359).

The analysis of colour descriptors showed a level of average differences in values of red, green and blue components (Fig. 2). The least difference is in a green colour, whose average value corresponds to average intensity of all three components (R/3 + G/3 + B/3). In infected kernels exhibiting noticeable symptoms, in accordance with findings reported by Wiwart *et al.* (2001), an increase in total intensity can be found, which is associated with colour changes caused by the disease (whitening). With regard to geometric parameters, the changes were expressed most (statistically significant differences) in criteria using simultaneously the area and perimeter (S_3, S_4, S_9, S_{10}) or the area and length (S_6) (Tab. IV). The correlation between shape parameters and kernel weight indicates a tendency toward lengthening kernels with weight decreasing (Suchowilska and Wiwart,

IV: *Average values of ten shape factors of kernel images and p value of their differences (t test)*

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
VSK0	4.454	5.229	1.179	0.723	0.427	0.058	0.474	0.998	0.314	17.48
VSK1 + VSK2	4.389	5.317	1.217	0.681	0.454	0.051	0.433	0.992	0.282	18.68
p	0.685	0.516	0.040	0.042	0.325	0.013	0.066	0.568	0.047	0.040

2006). However, the differences were not significant for four basic dimensions.

Prior to discriminant analysis, the correlations between variables (descriptors) and objects (kernel images) were assessed using multivariate techniques – principal component analysis (PCA) and hierarchical cluster analysis (HCA). In spite of that it is not apparent from the average value of colour descriptors (Fig. 2), the results of PCA (Fig. 3) already show clear separation of symptomatic and asymptomatic kernels based on the second factor that takes colour dispersion into consideration. The first factor, as well as HCA, reflects differences in total colour intensity, which are not specific for individual groups examined.

To develop the discrimination model, linear discriminant analysis was employed. Various combinations of individual shape and colour descriptors were tested during the development of the model. The shape descriptors themselves were not specific enough to distinguish individual kernels. This mainly concerns basic dimensions (length, width, area and perimeter). Larger differences were found using calculated shape descriptors (S_1 – S_{10}).

In addition to basic characteristics of the colour model (RGB), very good classification was also obtained using other colour descriptors of the HSL model, saturation (S) and especially hue (H). Infected kernels (VSK1 + VSK2) were characterized by higher values of H and lower values of S than healthy kernels, which corresponds to published data (Suchowilska and Wiwart, 2006; Wiwart *et al.*, 2001). The classification accuracy of 85 % using the developed discrimination model based on RGBH descriptors is apparent from Tab. V. Increase of model complexity by including shape factors did not lead to adequate increase in discrimination ability, so such models were not further considered.

The results presented herein as well as earlier reported data confirm that digital image analysis could be useful to distinguish wheat kernels infected by *Fusarium* spp. from healthy kernels, especially based on colour changes. As these parameters are affected by other factors (variety and environment), to obtain robust and accurate results at applying the method, it is necessary to develop a calibration mode for a certain reference object and validate the model by processing data from a larger number of measurements that would comprise kernels of defined origins focusing on possible influencing factors (variety, infection by a certain species of *Fusarium*, location, etc.). The method could be used, for instance, in combination with a sorter for removal of infected kernels during a cleaning process, and thus to decrease the content of *Fusarium* mycotoxins.

V: *Classification matrix of the discrimination model for classifying kernels infected by Fusarium pathogens using four colour descriptors (R, G, B, H). VSK – kernel groups based on visual screening: VSK0 – kernels without visual symptoms, VSK1 – whitish, pinkish kernels of normal size, VSK2 – whitish, pinkish, strongly shrivelled kernels.*

Actual group	Predicted group		Agreement
	VSK0	VSK1 + VSK2	
VSK0	16	4	80 %
VSK1 + VSK2	2	18	90 %

SUMMARY

The aim of the present study was to develop a discrimination model to identify wheat kernels infected by *Fusarium* spp. using digital image analysis and statistical methods.

Winter wheat kernels from field experiments with a gradated level of FHB infection were evaluated visually and classified into the three groups: kernels with no infection symptoms (VSK0), whitish, pinkish kernels of normal size (VSK1) and whitish, pinkish, strongly shrivelled kernels (VSK2). DON content of 40 kernels was determined using an ELISA method. For discriminant analysis, all kernels exhibiting infection symptoms were evaluated as one group (VSK1 + VSK2). Images of individual kernels (RGB colour model) were produced using a digital reflex camera Canon EOS 20D on contrast (dark) background. Colour and shape characteristics were obtained by image analysis using software Longboard™ 7.4 (IMT i-Solution, Inc., USA). Basic colour and shape descriptors of the area representing the kernel were determined for each object. Statistical analysis was performed using software Statistica 8.0 (StatSoft, Inc., USA).

Groups VSK0 and VSK1 + VSK2 differed significantly in DON content (1.06 and 160.54 $mg \cdot kg^{-1}$, respectively) and kernel weight (36.6 and 24.0 mg, respectively). Significant correlations were found between kernel weight and shape descriptors, particularly the width ($r = 0.877$) and area ($r = 0.849$), which corresponds to weight reduction. In the group of infected kernels (VSK1 + VSK2) the increasing of total intensity (brightness) was found, which is related to colour changes caused by the disease

(whitening). With regard to geometric parameters, the changes were most expressed for criteria using simultaneously the area and perimeter (S_3, S_4, S_9, S_{10}) or the area and length (S_6). Various combinations of individual shape and colour descriptors were examined during the development of the model using linear discriminant analysis. In addition to basic descriptors of the RGB colour model, very good classification was also obtained using hue (H) from the HSL colour model. Infected kernels (VSK1 + VSK2) were characterized by higher values of H in comparison with healthy kernels. The accuracy of classification using the developed discrimination model based on RGBH descriptors was 85 %. The shape descriptors themselves were not specific enough to distinguish individual kernels.

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