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Multivariate analysis of image descriptors of common wheat (*Triticum aestivum*) and spelt (*T. spelta*) grain infected by *Fusarium culmorum*

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A b s t r a c t. The response of five spring common wheat and five spring spelt cultivars to the infection of their spikes with *Fusarium culmorum* was examined using the shape and colour analysis of kernel images. The results obtained suggest that there is a significant correlation between the thousand kernel weight (TKW) and the shape descriptors of the kernel image: area, perimeter, length and width. Such a correlation was observed especially for TKW and image area (Pearson's correlation coefficient r ranged from 0. 737 for common wheat to 0. 914 for spelt) as well as for the shape coefficient S_5 calculated on the basis of image length and area (r equalled 0.716 and 0.886, respectively). A significant correlation was also observed for TKW and H (hue), S (saturation) and I (intensity) of colour of the kernel image. The results of cluster analysis performed for the values of H, S, I and S_5 permitted precise differentiation between kernels obtained from control and infected heads of common wheat and spelt. A reliable evaluation of grain infection by *F. culmorum* was possible only when the results of both shape and colour analysis were considered.

K e y w o r d s: *Fusarium* head blight, grain, image analysis, wheat, spelt

INTRODUCTION

Fusarium head blight is one of the most important fungal diseases of cereals (Jones and Clifford, 1983). It occurs in humid and semi-humid cereal-growing areas all over the world. This disease can be caused by 17 species belonging to the genus *Fusarium* sp., especially *F. culmorum, F. graminearum* and *F. avenaceum* (Parry *et al*., 1995). It may result in a significant decrease in the grain yield (above 40%), as well as in low quality of both seed material and grain used for baking bread and for animal feed production (Smith *et al*., 1994; Parry *et al*., 1995; Chełkowski *et al*., 2001). Isolates of *Fusarium* sp. often produce mycotoxins, most importantly trichothecenes and other toxic metabolites *eg* zearalenone, fumonisins moniliformine and other compounds. These substances have been shown to be harmful to human and animal health, some *eg* fumonisins are also considered carcinogenic.

Spelt (*Triticum spelta* L.) is one of the oldest cultivated crops, closely related to common wheat. The growing of this cereal has gained an increasing interest in recent years, because compared to common wheat, spelt grain is usually richer in protein of a higher nutritional value and contains larger quantities of minerals (Abdel-Aal *et al*., 1995). Spelt is also known for its high tolerance to several environmental stressors, particularly to cold and drought, and has relatively low soil quality requirements (Rüegger *et al*., 1990).

The degree of seed infestation with fungi of the genus *Fusarium* is most often expressed as a percentage of kernels with disease symptoms visible to the naked eye *ie* the so called *Fusarium* damaged kernels (FDK). However, this method may be inaccurate and burdened with error since often there are no clear symptoms of infestation even if the mycelium is present in kernel tissues (Chełkowski, 1989). Standard microbiological techniques (isolation and identification of pathogens) are time-consuming, and mycotoxin content determination with chromatographic techniques is very expensive. Therefore, it seems necessary to search for and implement new, reliable techniques permitting rapid evaluation of infestation of cereal grain used as seed material or for the purpose of consumption and animal feed production. One of such techniques is computer image analysis (Ruan *et al*., 1998; Wiwart *et al*., 2001).

The aim of the present study was to determine the shape and colour descriptors of wheat and spelt kernel images that would enable reliable differentiation between healthy kernels and kernels infected by *F. culmorum.*

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MATERIAL AND METHODS

The experimental material comprised five spring wheat cultivars (Kontesa, Torka, Hena, Triso, Zebra) and five cultivars of spring spelt (Roter Sommerkolben, Spelz aus Tzaribrod, Blauer Samtiger, Weisser Grannenspelz, Lohnauer Sommerspelz), and one isolate of *Fusarium culmorum* (W.G. Smith) Sacc. obtained from naturally infected wheat kernels.

Inoculation was performed at the full flowering stage (BBCH 65, Growth stages … 2001). Thirty heads of each variety, chosen randomly, were treated with aqueous suspension of *F. culmorum* conidia (at a concentration of 500 000 spores cm^{-3}) obtained from 14-day old cultures grown on a PDA medium (Merck®). The inoculated heads were immediately covered with polyethylene bags for 48 h. The reduction in the thousand kernel weight (TKW) calculated for the inoculated heads versus non-inoculated ones (the control) was used as a measure of the response to infection. The field experiment was performed in three replications.

Digital image analysis was conducted on a Pentium III PC, equipped with measuring software MultiScan® 8.01 (CSS Warsaw, Poland), connected to an HP ScanJet 4500c scanner. 50 kernels were selected randomly from each replication, and placed so that their furrows touched the scanner screen. Colour 24-bit images at a resolution of 300 dpi were recorded in the BMP format. Shape analysis was performed following yellow filtration (Y) and the application of a non-linear median filter (3x3 in one replication) to reduce background noise. After binarization at a threshold of 55, 1-bit images were subjected to shape analysis. The area, perimeter, length and width were determined for the image of each kernel represented by a single blob, and the shape factors $S_1 \div S_{10}$ were calculated according to the following formulas (Costa and Cesar, 2001; Shouche *et al*., 2001; Wiwart *et al*., 2006):

$$S_1 = 2\left(\sqrt{\frac{A}{\pi}}\right), \tag{1}$$

$$S_2 = \frac{P}{\pi}, \tag{2}$$

$$S_3 = \frac{P}{2\sqrt{\pi A}}, \tag{3}$$

$$S_4 = \frac{4\pi A}{P^2}, \tag{4}$$

$$S_5 = \frac{L}{A}, \tag{5}$$

$$S_6 = \frac{A}{L_3}, \tag{6}$$

$$S_7 = \frac{A}{\pi(0.5L)^2}, \tag{7}$$

$$S_8 = \frac{A}{\pi(0.5L)(0.5W)}, \tag{8}$$

$$S_9 = \frac{P-\sqrt{P^2-4\pi A}}{P+\sqrt{P^2-4\pi A}}, \tag{9}$$

$$S_{10} = \frac{P^2}{A}, \tag{10}$$

where: A – blob area (mm^2); P – blob perimeter (mm); L – blob length (mm); W – blob width (mm).

The significance of differences between means of all descriptors for control and inoculated objects was estimated using Student's t-test.

During the colour analysis, the original 24-bit images were filtered using red, green and blue filtration, and mean values of *R, G*, and *B* were determined for the image of each kernel. These values were next used to calculate *H* (hue), *S* (saturation) and *I* (intensity) *ie* three other components of colour. *R, G, B* to *H, S, I* (Gonzalez and Woods, 2002):
if

$$0<(R;\ G;\ B)<1,$$

then

$$H = \cos^{-1}\left\{\frac{\frac{1}{2}[(R-G)+(R-B)]}{\left[(R-G)^2+(R-B)(G-B)^{1/2}\right]}\right\}, \tag{11}$$

$$S = 1-(\min(R,G,B)), \tag{12}$$

$$I = \frac{R+G+B}{3}. \tag{13}$$

A hierarchical cluster analysis involving Euclidean distances and Ward's linkage for selected image descriptors was applied to estimate the similarity of images of kernels derived from control (non-inoculated) and inoculated heads, and then dendrograms were plotted using SYSTAT®11 software.

RESULTS

The infection caused a significant decrease in TKW – on average by 26.26% for common wheat and by 39.89% for spelt, compared to non-inoculated control heads. Among the common wheat cultivars the weakest response was noted for Torka and Zebra, whereas among the spelt cultivars for Lohnauer Sommerspelz (Table 1).

The results of the shape analysis indicated differences in the area, perimeter, length and width of kernel images. The kernels of common wheat and spelt coming from inoculated heads were characterized by significantly lower values of

T a b l e 1. Average values of a thousand kernel weight (g) in control and inoculated heads of common wheat and spelt cultivars

Parameter	Common wheat					
	Kontesa	Torka	Hena	Triso	Zebra	Mean
Inoculated	25.45	34.17	25.37	28.27	30.77	28.81^{b}
Control	37.36	41.91	39.07	39.45	37.56	39.07^{a}
Mean	31.43^{c}	36.94^{a}	34.02^{b}	33.73^{b}	33.21^{b}	–
	Spelt					
	RS	SaTB	BS	WG	LS	Mean
Inoculated	20.84	21.46	28.00	28.90	29.91	25.47^{b}
Control	41.09	46.63	39.86	43.66	40.62	42.37^{a}
Mean	36.26^{b}	39.20^{a}	36.09^{b}	38.83^{a}	36.95^{b}	–

a, b, c – homogenous groups, according to multiple Student Newman-Kuels test for each treatment separately, at $p<0.01$. RS – Roter Sommerkolben, SaTB – Spelz aus Tzaribrod, BS – Blauer Samtiger, WG – Weisser Grannenspelz, LS – Lohnauer Sommerspelz.

these parameters (Tables 2, 3), and the response of common wheat was weaker than that of spelt. The most considerable decrease was observed in the area (on average by 7.95% for common wheat and by 13.75% for spelt, in relation to the control) and in the width of kernel images (by 6.81 and 10.87%, respectively), whereas the slightest – in their length. The differences in the above parameters between particular cultivars were small. The high and significant values of the Pearson's coefficient of correlation between TKW and the area and width of kernel images, especially for spelt (Table 3), suggest that there is a clear positive interdependence between these parameters. The differences in the values of the majority of the shape factors obtained for common wheat kernels coming from control and inoculated heads were significant, except for the factors S_2, S_4 and S_6. In the case of spelt kernels, the above differences were found to be significant only for S_1, S_2 i S_5 (Table 4). In both cereals the correlations between the shape factors and TKW were significant for the factors S_1 and S_5 (absolute r values were higher than 0.699). The factor S_5, reflecting the relationship between image length and area, was found to be highly correlated with TKW in both wheat and spelt, while the negative value of r indicated that the kernel image becomes elongated as TKW decreases.

T a b l e 2. Area, length, width, and perimeter of images of common wheat kernels derived from inoculated and control heads and values of Pearson's correlation coefficient for these descriptors and TKW

Cultivar	Area (mm^2)	Length (mm)	Width (mm)	Perimeter (mm)
		Inoculated		
Kontesa	5.60	3.68	1.77	9.33
Torka	5.97	3.90	1.81	9.72
Hena	5.67	3.71	1.77	9.43
Triso	5.66	3.70	1.80	9.37
Zebra	5.44	3.61	1.75	9.16
Mean	5.67^{a}	3.72	1.78^{a}	9.40
SD	0.192	0.108	0.024	0.204
		Control		
Kontesa	6.14	3.80	1.90	9.68
Torka	6.57	3.97	1.96	10.07
Hena	6.13	3.80	1.90	9.71
Triso	6.07	3.78	1.90	9.60
Zebra	5.91	3.70	1.88	9.44
Mean	6.16^{b}	3.81	1.91^{b}	9.70
SD	0.245	0.098	0.030	0.232
$r^{\dagger}$	0.737**	0.564*	0.678**	0.653*

† correlation coefficient for TKW and each shape descriptor separately; a, b – difference between mean values for inoculated and control grain significant at $p<0.01$; *, ** – value of r significant at $p<0.05$ or $p<0.01$, respectively.

T a b l e 3. Area, length, width, and perimeter of images of spelt kernels derived from inoculated and control heads and values of Pearson's correlation coefficient for these descriptors and TKW

Cultivar[#]	Area (mm^2)	Length (mm)	Width (mm)	Perimeter (mm)
		Inoculated		
RS	6.17	4.71	1.58	11.03
SaTB	6.21	4.78	1.56	11.20
BS	6.34	4.51	1.67	10.76
WG	6.45	4.49	1.69	10.80
LS	6.56	4.56	1.72	10.86
Mean	6.35[a]	4.61	1.64[a]	10.93[a]
SD	0.163	0.128	0.070	0.183
		Control		
RS	7.60	4.98	1.85	11.83
SaTB	7.74	5.09	1.85	12.04
BS	6.92	4.68	1.78	11.16
WG	7.38	4.63	1.90	11.26
LS	7.17	4.72	1.84	11.27
Mean	7.36[b]	4.82	1.84[b]	11.51[b]
SD	0.329	0.203	0.043	0.396
r[†]	0.914**	0.514*	0.954**	0.650*

[#]Spelt cultivars as in Table 1. Explanations as in Table 2.

T a b l e 4. Average values of ten shape factors (S_1-S_{10}) of images of common wheat and spelt kernels derived from control and inoculated heads and values of Pearson's correlation coefficient for these descriptors and TKW

Parameter	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
					Common wheat					
Inoculated	2.686[a]	2.993	1.114[a]	0.806	0.656[a]	0.110	0.522[a]	1.090[a]	0.388[a]	15.598[a]
Control	2.801[b]	3.088	1.102	0.779	0.618[b]	0.112	0.541[b]	1.079[b]	0.408[b]	15.268[b]
r[†]	0.699**	0.196	-0.658*	0.065	-0.886**	0.350	0.558*	-0.716**	0.694**	-0.654*
					Spelt					
Inoculated	2.842[a]	3.479[a]	1.224	0.668	0.727[a]	0.065	0.381	1.067	0.270	18.848
Control	3.061[b]	3.664[b]	1.197	0.638	0.655[b]	0.066	0.404	1.055	0.292	18.007
r[†]	0.745**	0.419	-0.333	-0.235	-0.716**	-0.011	0.224	-0.123	0.331	-0.333

Explanations as in Table 2.

The results of the colour image analysis revealed greater differences in the colour of control and infected kernels in common wheat, as compared with spelt (Table 5). This may be related to the impact of hulling on pathogen spread following infection, which could lead to a different than in *T. aestivum* pattern of disease development. Infected kernels of both cereals had higher values of *H* and *I* (the hue was closer to yellow while the colour was brighter) and lower values of *S* (the colour was less saturated and paler than in control kernels). Particular attention should be paid to the very strong correlation between colour components and TKW: the values of *r,* recorded in common wheat and spelt respectively, were -0.858 and -0.936 for *H,* 0.780 and 0.801 for *S*, and 0.726 and 0.626 for *I*.

A hierarchical cluster analysis was performed based on the results of the shape and colour analysis, including all three variables of the *H S I* model as well as the shape factor S_5. The fact that in both cereals the shape factor assumed

T a b l e 5. Values of colour components *H S I* obtained for images of kernels derived from inoculated and control heads of common wheat and spelt

Grain		H		S		I	
		Inoculated	Control	Inoculated	Control	Inoculated	Control
Common wheat	Kontesa	31.16	30.49	0.76	0.86	1.28	0.96
	Torka	30.15	28.25	0.81	0.85	1.17	0.97
	Hena	32.10	29.68	0.75	0.85	1.30	1.00
	Triso	30.76	30.76	0.80	0.80	1.16	1.16
	Zebra	31.03	30.03	0.80	0.86	1.22	1.03
	mean	31.04*	29.84	0.78**	0.84	1.23**	1.02
	SD	0.709	0.417	0.027	0.011	0.063	0.036
Spelt[#]	RS	30.42	27.51	0.81	0.84	1.15	0.92
	SaTB	30.61	26.60	0.80	0.82	1.12	1.12
	BS	31.04	29.90	0.80	0.82	1.17	1.08
	WG	30.66	30.23	0.79	0.82	1.14	1.12
	LS	30.75	30.08	0.81	0.81	1.12	1.13
	mean	30.70	30.24	0.80	0.81	1.14	1.13
	SD	0.227	0.808	0.008	0.013	0.021	0.068

[#]Spelt cultivars as in Table 1. Explanations as in Table 2.

significantly different values for infected and healthy kernels, and was strongly correlated with TKW, was also taken into account. The results of this analysis, presented in the form of dendrograms in Fig. 1, indicate the possibility of obtaining two separate groups, one for control grain and the other for grain infected by *F. culmorum.*

DISCUSSION

Head blight is a dangerous disease causing considerable deterioration of the quality of both raw material and products, due to the accumulation of toxic compounds, especially deoxynivalenol (DON). The results reported by Perkowski (1999) show that in wheat grain infected under natural conditions, kernels with diameters below 2.5 mm contain on average as much as 88% of the total quantity of this toxin. Although mechanical separation of this fraction is possible and relatively easy to perform, toxins may be also present in apparently normally developed kernels, showing no visual symptoms of infection. The results obtained show that TKW coming from control heads is by 20.45 to 53.98% higher, compared to the inoculated samples, which indicates grain damage and high pathogenicity of the applied isolate. Therefore, it seems recommendable to search for a technique that would make the health screening of kernels faster and, most importantly, more objective than visual observations.

It has been known since the mid 1990s that the computer imaging technology was sufficiently advanced so that it could be proposed to discriminate between wheat classes and varieties by image analysis (Sapirstein, 1995; Shouche

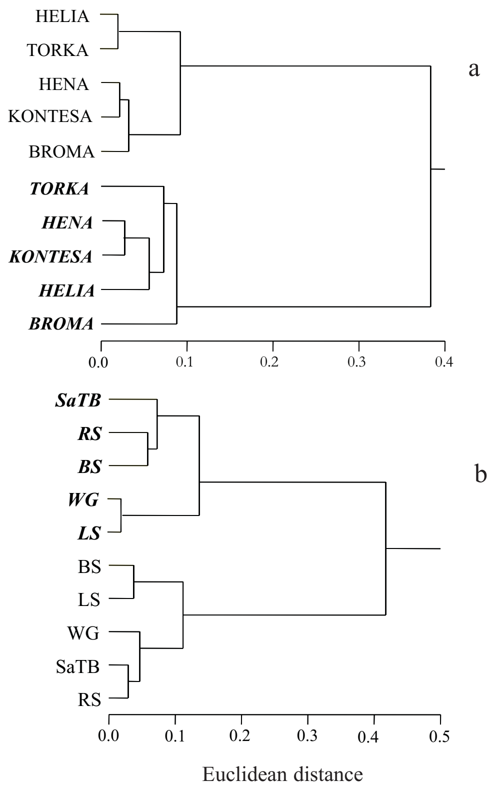

Fig. 1. Dendrograms illustrating the similarities between the images of kernels infected by *F. culmorum* (italics) and kernels obtained from non-inoculated heads (upright). Cluster analysis was performed including all three colour components *H S I* and shape factor S_5 $\left(S_5 = \frac{L}{A}\right)$. Spelt cultivars as in Table 1. a – common wheat, b – spelt.

et al., 2001; Schaafsma *et al*., 2003). Luo *et al*. (1999) used machine vision to distinguish between healthy and damaged wheat kernels, whereas Kokko *et al*. (1999) reported the detection of FDK using image analysis and neural networks on individual seeds. An interesting computer technique for estimating the degree of grain infestation with pathogenic species of *Fusarium* is colour image analysis. Ruan *et al*. (1998) applied four-layer back propagation artificial neural networks to analyse the colour components of images of *Fusarium* scabby wheat kernels. A trained neural network produced an excellent estimation of the FDK percentage, much more accurate than that by a panel of experts. The maximum and absolute errors recorded for this technique were 5.14 and 1.93%, respectively.

The Canadian company Maztech Micro Vision Ltd. (www.maztech.com) offers a fully automated system for objective determination of grade and non-grade factors in grains and seeds, the SPY Grain Grader®. On a per kernel basis, this system provides a rapid screening tool for verifying the presence or absence of *Fusarium* damaged kernels (FDK), and for quantifying the amount of sound kernels. Commercialization of machine vision for FDK classification is straightforward with the use of the image analysis system called Acurum® (DuPont Canada Inc.) (www.acurum.com) which samples a large number of individual kernels within seconds. Hinz Technologies markets a flatbed vision machine under the name of TrueGrade® (www.hinztechnologies.com/truegrade.html) which is currently available to grade lentils, hay and noodles. The company Foss Tecator designed an apparatus called 2312 GrainCheck® (www.foss.dk/c/p/solutions/products/graincheck_2312.pdf) in which digital image analysis is performed with the use of a CCD camera, a computer system equipped with a Pentium processor, and software based on the technique of Artificial Neural Networks. This apparatus, characterized by capacity of up to 500 objects min^{-1}, tests the purity of the grain sample (such as wheat, barley, rye, oats, corn, and rice) as well as classifies damaged, broken and immature kernels and kernels with symptoms of ergot and fusariosis (Foss Tecator, patent no 470 465). The results of our previous studies (Wiwart *et al*., 2001; Wiwart *et al*., 2002) also confirmed the possibility of evaluating the health status of triticale and wheat grain using colour image analysis: high and significant values of the Pearson's coefficient of correlation between the decrease in the kernel number and the kernel weight per spike as well as TKW, relative to the values of saturation and intensity, indicate that there is a close relationship between the degree of infection with *F. culmorum* and the colour of the seed-fruit coat of grain.

The results presented in this paper indicate significant differences in the four main shape descriptors (area, perimeter, length and width) of images of kernels obtained from non-inoculated and inoculated heads. This confirms the suitability of computer shape analysis for estimating the degree of wheat grain infestation with *F. culmorum*. However, these parameters are varietal features, so it would be difficult to determine the response of wheat to pathogens on the basis of their absolute values. Therefore, it seems necessary to calculate shape factors, constituting a link between them. These parameters, although burdened with certain errors, allow to describe the shape of various objects in a synthetic way, which in turn permits their comparison. The factors S_1 and S_5 can be especially recommended for this purpose.

The results of the colour image analysis, presented here, correspond to those obtained previously for triticale (Wiwart *et al*., 2001) and common wheat (Wiwart *et al*., 2002). In both these cereals the colour of infected kernels was characterized by a decrease in *S* and an increase in *I*, whereas the differences in *H* levels were inconsiderable. The presence of the pathogen inside the kernel manifests itself by changes in the colour and shape of the seed-fruit coat. Nevertheless, it seems that shape analysis cannot be employed as an independent research tool for grain health diagnostics, although it allows to distinguish between infected and pathogen-free kernels. This is due to the fact that the size and shape of kernels are affected by a variety of environmental (fertilization, soil and climate conditions, agricultural practices) and genotypic (cultivar) factors, which makes it really difficult to determine the health status of grain. It would be only possible if there existed a point of reference (non-inoculated control) for each object under analysis (an infected kernel), which never happens under production conditions on a plantation.

CONCLUSIONS

1. It may be concluded that the use of colour and shape analysis and incorporating colour and shape descriptors into the multivariate analysis enables to perfectly distinguish between infected and control grain samples.

2. Further investigations in the field of interest are expected to provide the basis for designing a fully automated system of computer image analysis aimed at estimating kernel health status.

3. It should be stressed, however, that a better measurement of FDK levels in a grain sample will result in more accurate grading, but will still allow for only an approximate estimate of the DON levels.

4. Image analysis of grain may be called a 'first contact' method. This technique is very rapid, convenient and relatively inexpensive, but standard instrumental methods should be always used for accurate determination of mycotoxin content.

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