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Food Additives and Contaminants



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Reaction of winter wheat (*Triticum aestivum* L.) cultivars to infection with *Fusarium* spp. - mycotoxin contamination in grain and chaff

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Abstract

The aim of this study was to compare susceptibility of winter wheat (Triticum aestivum L.) cultivars to Fusarium Head blight - FHB and accumulation of mycotoxins in kernels and chaff, in different climate conditions in two locations Cerekwica near Poznan (Central West Poland) and Sitaniec, near Zamosc, Lublin region (South East Poland). Very high variation was found in the concentrations of mycotoxins (zearalenone-ZEA, nivalenol-NIV, deoxynivalenol-DON and moniliformin-MON) in examined fractions (Fusarium-damaged kernels-FDK and healthy looking kernels-HLK) and in chaff for individual cultivars in both locations. Moreover, in most cases significantly higher concentrations of investigated toxins were recorded in wheat coming from the area of Lublin than in that from the area of Poznan (p<0.05). The highest *Fusarium* infection rate as well as mycotoxin biosynthesis level were observed in the Lublin location with percentage of the FDK fraction in the range from 8.1 to 81.6. In this region ZEA concentration ($\mu g g^{-1}$) after inoculation with F. culmorum and F. graminearum ranged from 0.02 to 0.48 and 0.32-1.04, respectively. In the Poznan area the toxin concentrations were considerably lower, ranging from 0.01 to 0.10 and from 0.03 to 0.13 for both isolates, respectively. The concentration of DON was significantly higher when compared to ZEA and NIV levels. The levels of MON accumulation ($\mu g g^{-1}$) in the FDK fraction were between 0.14 and 1.73 (Poznan area) and ND - 2.51 (Lublin area). F.

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avenaceum infection rate ranged between 7 and 35% in the samples where the toxin was detected.

Keywords: *Fusarium culmorum, F. graminearum, F. avenaceum, Fusarium* head blight, wheat, zearalenone, deoxynivalenol, nivalenol, moniliformin, HPLC

Introduction

Fusarium species are common necrotrophic pathogens of small grain cereals in most European countries. Three species F. culmorum (W.G. Smith) Sacc., F. avenaceum (Corda ex Fries) Sacc., and F. graminearum Schwabe, have been considered as the most important in Poland and other central European countries, while several other species as less important (Bottalico and Perrone 2002). F. culmorum and F. graminearum have been shown to be frequent and most aggressive to cereals in countries of moderate and colder climates. FHB severity depends on several genetic, agronomic and climatic factors. The disease can result in Fusarium-damaged kernels (FDK), which are smaller, shriveled and discoloured (Kiecana et al. 2004. FHB can also cause economical losses because of reduced kernel weight and size, low grain quality, and accumulation of several mycotoxins (mostly deoxynivalenol - DON and zearalenone - ZEA) in infected kernels, chaff and rachises (Jones and Mirocha 1999). In some regions disease epidemics have been observed, with high yield losses particularly in wheat and other cereals. Observations of disease occurrence showed a lower susceptibility of selected cultivars and breeding lines to most fungal pathogens (Mielniczuk et al. 2004, Wisniewska et al. 2004). Profiles of mycotoxins produced by the above mentioned two species (F. graminearum and F. culmorum) were found to be similar both under laboratory and field conditions, with DON, nivalenol (NIV) and ZEA being the most important (Quarta et al. 2005). The mycotoxin content in infected grains is also genotype-dependent (Chelkowski et al. 2000, Wisniewska et al. 2004).

The major *Fusarium* toxin found in the climatic conditions of Poland is zearalenone, detected in cereals during plant growth and vegetation, as a secondary metabolite of fungi of genus *Fusarium* (Golinski et al. 2002). The amounts of ZEA accumulated in cereals depend on many factors such as temperature, duration of *Fusarium* infection, substrate and strain of fungal species (Bennett and Klich 2003, Nuryono et al. 2005). This toxin is recognized as a phytoestrogen with reported cytotoxic and genotoxic activity. Toxic properties of ZEA are

related to the metabolite binding to estrogen receptors and disrupting the endocrine regulation of the reproductive functions in females (Zinedine et al. 2007).

An important toxin of the group of trichothecenes is deoxynivalenol (DON), being the product of metabolism of such fungi as *F. graminearum* and *F. culmorum*. DON was detected in such cereals as wheat, oat, barley, rye, rice, corn, as well as cereal-based products, e.g. flour, bread or muesli. Accumulation of DON and its derivatives, including NIV, in spring wheat accessions was on average $3.1 \ \mu g \ g^{-1} (0.7-9.0 \ \mu g \ g^{-1})$ and it was lower than in winter wheat accessions, with an average of $6.7 \ \mu g \ g^{-1} (1.6-38.5 \ \mu g \ g^{-1})$ (Goral et al. 1995). DON, similarly to other trichothecenes, has a significant effect on biochemical processes, blocking e.g. protein biosynthesis or elongation of the polypeptide chain. It is assumed that DON, being a highly toxic compound, does not exhibit carcinogenic properties. The most frequently observed DON toxicosis symptoms in animals include vomiting and body weight loss with successive numerous physiological changes in internal tissues (Wisniewska et al. 2002, 2004). Pigs were found to be the most susceptible animals to DON, despite the reported potential detoxication by the intestinal microflora. Intake of feed containing DON may also have a significant effect on reproduction of animals, causing in most cases fatal toxicoses.

Fusarium avenaceum is reported as a very potent producer of moniliformin (MON), a metabolite in the form of sodium or potassium salt of 3-hydroxycyclobut-3-ene-1,2-dione, is toxic to plant and animal species (Kiecana et al. 2002, Jestoi et al. 2004). MON also is suspected to cause the Keshan disease, a human myocardial impairment reported in rural areas of China and South Africa (Pineda-Valdes and Bullerman 2000). Inhibition of pyruvate dehydrogenese, i.e. the blockage of entry of pyruvate into the Krebs's cycle, and a decrease in mitochondrial respiration are probably responsible for the acute toxicity of the compound (Reams et al. 1996).

The aim of this study was to compare susceptibility of winter wheat (*Triticum aestivum* L.) cultivars to FHB and accumulation of mycotoxins in kernels and chaff, in different climate conditions at two locations in Poland.

Materials and methods

Wheat samples

Material for the study comprised 10 winter wheat cultivars most common in East Europe, differing in their reaction to *Fusarium* pathogens (6 quality cultivars - group A: cv. Tonacja, Zyta, Sukces, Finezja, Ludwig, Rywalka; 3 bread cultivars - group B: cv. Mewa, Nutka, Soraja; 1 forage cultivar - group C: cv. Symfonia) (Table 1). The experiment was performed

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in 2008, in a randomized complete block design in triplicate, at two different locations of Poland: Cerekwica (82 m a.s.l.), Central West Poland - 30 km northwest of Poznan, and Sitaniec (210 m a.s.l.), South East Poland - 90 km southeast of Lublin. Weather conditions for both locations received from Institute of Meteorology and Water Management.

Inoculation procedure

At mid-anthesis (zadoks scale 65) 30 wheat heads of each replication were inoculated individually (by brushing) with the conidial suspension (100 000 spores ml⁻¹) isolates of three *Fusarium* species: *F. graminearum* (KF 2870), *F. culmorum* (KF 846) and *F. avenaceum* (NN 1337), with plot size $1m \times 1m$. During two days after inoculation micro-irrigation was used. Non-inoculated plots of the same genotypes were used as a control. Two weeks after inoculation, disease progress was visually evaluated as the disease score of head (Fi) on a scale of 0-5, where 0 means no infection and 5 denotes the highest infection of the head. Inoculated and control heads were harvested and threshed manually, then kernels were separated into two fractions: *Fusarium*-damaged kernels (FDK) and in healthy looking kernels (HLK). The percentage of *Fusarium*-damaged kernels (%FDK) was calculated as a percentage by weight.

Chemicals and reagents

Mycotoxin standards (ZEA, DON, NIV, MON) were supplied by Sigma-Aldrich (Steinheim, Germany). Acetonitrile and methanol (HPLC grade) were also purchased from Sigma-Aldrich (Steinheim, Germany). Potassium chloride, di-potassium phosphate, *n*-hexane and *t*-butyl-ammonium hydroxide were purchased from POCh (Gliwice, Poland) and water (HPLC grade) was from our own Millipore water purification system.

Mycotoxin analysis

Mycotoxins (ZEA, DON, NIV and MON) were analyzed as well in the two fractions of wheat kernels described above - FDK and HLK, as in the fraction of chaff.

Deoxynivalenol, nivalenol and moniliformin analysis

Finely ground material was extracted with acetonitrile-methanol-water (16:3:1, v/v/v) using 5 ml of solvent per 1 g of sample. Extracts were defatted with *n*-hexane (3×50 ml) and then concentrated. Half of the extract was used for MON analysis, while the remaining part was used for DON and NIV determination. The extract of MON was purified on a Florisil

column according to the method described by Kostecki et al. (1999). To analyze the trichothecenes (DON, NIV), extracts were purified by filtration on a column according to the method described by Tomczak et al. (2002).

DON, NIV and MON were quantified by HPLC using a Waters 501 apparatus (Waters Division of Millipore, Milford, MA, USA) with a C-18 Nova Pak column (3.9×300 mm) and a Waters 486 UV detector ($\lambda_{max} = 224$ nm for DON and NIV, while $\lambda_{max} = 229$ nm for MON). DON and NIV were eluted from the column with a 25% water solution of methanol (flow rate 0.7 ml min⁻¹) with retention times of 11.72 and 7.46 min, respectively. Regarding MON, the acetonitrile:water solvent (15:85, v/v) buffered with 10 ml of 0.1 M K₂HPO₄ in 40% *t*-butyl-ammonium hydroxide in 1 L of solvent (Sharman et al. 1991) was used as a mobile phase (flow rate 0.6 ml min.⁻¹). Retention time of MON was 11.5 min. Detection limits for DON, NIV and MON were 0.01 µg g⁻¹. Positive results (on the basis of retention time) were confirmed by HPLC analysis and by comparison with the relevant calibration curve (correlation coefficients for NIV, DON and MON were 0.9994, 0.9997 and 0.9990, respectively). Recoveries for NIV, DON and MON: 75, 87 and 90%, respectively, were estimated in triplicates by extracting mycotoxins from blank samples spiked with 0.01–0.10 µg g⁻¹ of the compounds. The relative standard deviation (R.S.D.) value was below 5% for DON and NIV, and below 7% for MON.

Zearalenone analysis

ZEA content was determined by HPLC with a fluorescence detector according to the method described by Visconti and Pascale (1998). The chromatographic system was a Waters 2695 HPLC, with a Waters 2475 Multi λ Fluorescence Detector and a Waters 2996 Array Detector. The excitation and emission wavelengths were 274 and 440 nm, respectively. The reverse-phase column was a C-18 Nova Pak column (3.9 × 150 mm), while the mobile phase was acetonitrile-water-methanol (46:46:8, v/v/v), at a flow rate of 0.5 ml min⁻¹. Quantification of ZEA was performed by measuring the peak areas at the ZEA retention time according to the relevant calibration curve (correlation coefficient r=0.9998). The limit of zearalenone detection was 0.003 µg g⁻¹. The recovery of the compound was measured in triplicates by extracting ZEA from blank samples spiked with 0.005–0.10 µg g⁻¹ of compound. The results of experiments confirmed literatures data and contained in the range from 97 to 99%. The relative standard deviation (R.S.D.) value was below 1%. In order to confirm the presence of ZEA the Photodiode Array Detector was used.

Statistical analysis

The two-way analysis of variance was carried out to determine the effects of locations, cultivars and locations × cultivars interactions on the variability of disease score of head (Fi), the percentage of *Fusarium*-damaged kernels (%FDK), ZEA and DON concentrations in FDK as well as in HLK fractions and in chaff (ZEA in FDK, ZEA in HLK, ZEA in chaff, DON in FDK, DON in HLK, DON in chaff), HLK kernels per spike, FDK kernels per spike, HLK weight per spike, FDK weight per spike and thousand kernels weight (TKW).

The least significant differences (LSD) for each trait were calculated, and on these basis homogeneous groups for the analyzed traits were determined. The relationship between ZEA and DON for different traits (mycotoxin concentration in FDK and HLK fraction and in chaff) as well as Fi, HLK kernels per spike, FDK kernels per spike, HLK weight per spike, FDK weight per spike and TKW were estimated on the basis of correlation coefficients.

Results and discussion

Susceptibility of tested wheat cultivars to the *Fusarium* spp. and mycotoxin biosynthesis depends on pathogen virulence and weather conditions.

Analysis of variance indicated that the main effects of locations (Poznan and Lublin area) were significant for all the traits in the study (Tables 2-4), except for FDK kernels per spike for *F. culmorum* (Table 2), FDK weight per spike for *F. graminearum* (Table 3) and moniliformin concentration in the HLK fraction (MON in HLK) (Table 4); the main effects of cultivars were significant for all the traits, except for TKW for *F. culmorum* (Table 2). The locations × cultivars (L×C) interactions were not significant only for number of as well HLK and FDK kernels per spike, FDK kernels per spike and TKW for *F. culmorum* (Table 2), and for MON in FDK number of HLK and FDK kernels per spike and HLK weight per spike for *F. avenaceum* (Table 4).

Very high variation was found in concentrations of mycotoxins (ZEA, DON, MON) in fractions (HLK and FDK) and in chaff for individual cultivars in both locations (Tables 5-9). Moreover, in the most cases significantly higher concentrations of toxins were found in wheat of Lublin area origin than area of Poznan (p<0.05) (Tables 5-9), what confirms earlier findings (Golinski et al. 1999, 2002).

The highest *Fusarium* infection rate as well as mycotoxin biosynthesis level were observed in the Lublin location with percentage of the FDK fraction in the range from 8.1 to

81.6. In this region the ZEA concentration ($\mu g g^{-1}$) after inoculation with *F. culmorum* and *F. graminearum* ranged from 0.02 to 0.48 and 0.32–1.04, respectively (Tables 5 and 6). In the Poznan area the toxin concentrations were considerably lower, ranging from 0.01 to 0.10 and from 0.03 to 0.13 for both isolates, respectively (Tables 5 and 6).

The differences in climate conditions are in our opinion the reason of differences in ZEA concentrations in both locations (Tables 5 and 6). Inoculation in Cerekwica was performed May 30 and June 5 for all *Fusarium* pathogens isolates with few days of average temperature increase with peaks of the highest temperature May 31, June 6 and 10. At the same time rainfalls level was lower than it was before inoculation. In contrast, inoculation in Lublin area was few days later (June 10) than in Cerekwica which is the reason because of differences in climate and vegetation (cereals flowering). After inoculation - in Lublin area – there were a few days with lower temperature and higher air relative humidity than previously and during inoculation (weather reports for Lublin and Cerekwica). Such a situation explains that lower temperature in Lublin area during disease development induced ZEA contamination of kernels. Such observation was also reported by others (Klix et al. 2008).

NIV was reported only in trace amounts in the FDK fraction of two cultivars after inoculation with *F. culmorum* in the Lublin region. The concentration of DON was significantly higher when compared to ZEA and NIV levels. In the Poznan region contamination with DON did not exceed 80.8 μ g g⁻¹ in FDK fraction (Tables 7 and 8). It was also noticed that the infection was higher after inoculation with *F. culmorum* both in the Poznan and Lublin area, where the DON concentration in FDK fraction was in the range from 21.6 up to 114.6 μ g g⁻¹ (Table 7).

It is worth noting that in several samples especially in Lublin area, but also in Cerekwica, the level of DON was higher in kernels of FDK fraction than in chaff (Table 8). We assume that dry summer in both locations was responsible for the fungus penetration to inner parts of kernels (where humidity and water content is higher), followed by the disease development and higher yield of the mycotoxins biosynthesis and/or their transport with nutrients into the kernels centre, what effected in higher the metabolites concentration level than it was in outer parts - in chaff temperature had to be higher (sun operation) in a contrary to low moisture. To some extend this concept is similar not only to the results indicating penetration of DON into kernels of cereals with FHB symptoms and pathogen development but also in asparagus spears without fusariosis symptoms but more often MON and fumonisins positive than diseased ones. This phenomenon was elucidated as follows: mycotoxins accumulated in overgrown with pathogenic fungus and/or fungi crowns are

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transported with nutrients to developing spears. In fact the mycotoxins presence is definitely related to abundance of pathogen(s) in crown and not in situ of chemical analysis (spears) (Waskiewicz at el. 2010). It is necessary to point out the differences in the structure of kernels and spears, differences resulting in only limited penetration of mycotoxins in hard tissue.

Moreover, a significant positive correlation for F. culmorum were observed between FDK weight per spike and FDK kernels per spike in both locations as well as between HLK weight per spike and TKW in Lublin (Table 9). The negative significant correlation was observed only between HLK weight per spike and Fi (Table 9). As mentioned above metabolite penetration into the kernels might be limited when humidity is high, mycotoxins accumulate - in such situation - mostly on the kernels surface. The literature and our earlier findings (Young and Miller 1985, Perkowski et al. 1990) indicate that usually the outer parts chaff, husk are higher in the mycotoxin contamination levels, which is an interesting finding with practical implications. During kernel threshing, hulling, dry and wet cleaning (washing), grinding and milling significantly reduce the concentration levels of the mycotoxins not only in kernels but also in such final products as flour, groats and flakes. In turn, for F. graminearum a significant relationship was observed between FDK weight and kernels per spike and HLK weight and kernels per spike in both locations as well as between DON in HLK and ZEA in HLK, HLK weight per spike and TKW in Poznan (Table 10). In Lublin, HLK kernels per spike was correlated with FDK kernels per spike and FDK kernels per spike (Table 10). Edwards (2009) observed that the relationship between DON and ZEA concentrations was not stable between years. The ZEA distribution was a similar pattern to DON which is to be expected as they are produced by the same *Fusarium* species.

In studies conducted in Austria, kernel samples of durum wheat predominantly infected by *F. graminearum* and with a lower incidence of *F. culmorum* contained high amounts of DON (up to 8.2 μ g g⁻¹) together with low levels of ZEA (up to 0.33 μ g g⁻¹) (Adler et al. 1995), while in other studies DON was found in all tested wheat samples, ranging from 0.2 to 4.1 μ g g⁻¹ (Berthiller et al. 2009). In Germany DON, together with ZEA and NIV, was found in 87% of wheat samples at very high levels, mean being 3.96 μ g g⁻¹ and the highest 43.4 μ g g⁻¹ (Lepschy von Gleissenthal et al. 1989). The occurrence of DON (up to 1.18 μ g g⁻¹) was reported in Finland (Hietaniemi and Kumpulainen 1991) and in the Netherlands (up to 0.5 μ g g⁻¹) together with ZEA (De Nijis et al. 1996). In France, Bakan et al. (2001) analyzed strains of *F. culmorum* isolated from wheat grain, which produced ZEA (1700 μ g g⁻¹), DON (up to 51.9 μ g g⁻¹) and NIV (up to 12.0 μ g g⁻¹), whereas in Italy isolates of *F. culmorum* produced DON up to 1700 μ g g⁻¹ (Quarta et al. 2005). In Poland high amounts of DON (2.30-

15.32 μ g g⁻¹) related with a high incidence of *F. culmorum* and *F. graminearum* were observed, whereas DON concentration was in most samples higher than those of NIV (0.01-0.74 μ g g⁻¹) (Chelkowski et al. 2002). Analysis of mycotoxins of six winter wheat genotypes inoculated with *F. graminearum* revealed DON content ranging from 0.89 up to 1.53 μ g g⁻¹ and a very low level of NIV (Ittu et al. 2000). In other studies the highest contamination with DON of inoculated wheat was observed in Polish cultivars, at 38.5 and 35.7 μ g g⁻¹ for cv. Olimpia and Henika, respectively (Wisniewska et al. 2004).

In Cerekwica the levels of MON accumulation ($\mu g g^{-1}$) in HLK were between not detectable level (ND) and 0.13, while in the FDK fraction from 0.14 and 1.73, and in chaff at levels between ND and 1.07 (Table 11). *F. avenaceum* infection rate ranged between 7 and 35% in the samples where the toxin was detected. In the Lublin region the MON content ($\mu g g^{-1}$) in the HLK fraction ranged between ND and 0.11, in FDK in the range of ND-2.51, while in chaff between 0.03 (cv. Rywalka) and 0.48 (cv. Sukces) (Table 11). In this area the infection rate with *F. avenaceum*, in the analyzed cultivars varied, from 3 to 41%. For *F. avenaceum* we observed positive correlation between number and weight of HLK and FDK kernels per spike, FDK kernels and weight per spike, MON in FDK and MON in HLK in both locations (Table 12). Additionally, MON in chaff was correlated with MON in HLK and MON in FDK as well as HLK weight per spike with TKW in Lublin (Table 12).

The achieved results indicate that MON content depends on the susceptibility of particular genotype to infection with *F. avenaceum*, what is confirmed as well by studies in Norway, where moniliformin was found in 76% tested wheat samples (with the highest toxin concentration 0.95 μ g g⁻¹) (Uhlig et al. 2004) as studies conducted in Finland by Jestoi et al. (2004), where significant correlation between *F. avenaceum* contamination level and the level of MON (0.03-0.10 μ g g⁻¹) was observed. In Poland, wheat samples with *F. avenaceum* inoculum contained the toxin in the range from 0.01 to 0.35 μ g g⁻¹ (Chelkowski et al. 2002) and 1.72 μ g g⁻¹ (Tomczak et al. 2002).

In cereals four types of plant resistance mechanisms to fusariosis have been suggested: resistance of the host plant to infection with pathogenic fungi (mechanism I); resistance of the host to pathogen spread in the tissue (II); ability of the plant to degrade mycotoxins (III) and tolerance of the host tissue to a high concentration of toxic metabolites (Kiecana et al. 2002). In the district of Lublin, with suggestion to the other parts of Poland two cultivars Sukces and Tonacja are recommended for general purpose kernels while cv. Symfonia as a feed - component. In our present studies cultivars Ludwig and Finezja were of the lowest

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susceptibility to *F. avenaceum* inoculation and MON accumulation (Mewa, Ludwig and Finezja in Lublin; Rywalka, Ludwig and Finezja in Poznan) while cv. Sukces exhibited the lowest susceptibility in both locations. After inoculation with *F. culmorum* the lowest susceptibility and the mycotoxin formation were observed for cv. Nutka, Zyta and Rywalka for both localities (Finezja and Soraja in Lublin; Rywalka in Poznan), while the highest for cv. Symfonia and Sukces (Soraja in Poznan and Mewa and Symfonia in Lublin). Cultivars Tonacja and Nutka (Finezja and Ludwig in Lublin and Symfonia and Zyta in Poznan) were the lowest susceptibility to *F. graminearum* inoculation and with the lowest toxic metabolite biosynthesis while cv. Mewa (Soraja in Lublin and Mewa in Poznan) of the highest.

Recorded results indicates on winter wheat cultivars with low susceptibility to infection under different climate conditions, therefore experiments described in this paper should be continued to select winter wheat cultivars of the lowest susceptibility to FHB, mycotoxin formation and the most suitable for different climate and cultivation conditions in Poland.

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Cultivar	Technological value/group	Frost resistance	Lodging resistance	Soil acidity tolerance	Protein and gluten contents
Finezja	quality/A	medium	medium	medium	high
Ludwig	quality/A	low/medium	low	medium	high
Mewa	bread/B	high	low	medium	high
Nutka	bread/B	low	high	medium	low/medium
Rywalka	quality/A	medium	low	medium	high
Soraja	bread/B	medium	medium	medium	medium
Sukces	quality/A	low	high	high	high
Symfonia	forage/C	high	medium	medium	medium
Tonacja	quality/A	high	high	medium	high
Zyta	quality/A	low	medium	medium	high
				v Only	

		Mean squa	ares	
Trait	Cultivars	Locations	Cultivars × Locations interaction	Residual
Degrees of freedom	9	1	9	38
Fi	0.873***	85.049***	0.786***	0.161
ZEA in HLK	0.149***	1.830***	0.150***	0.002
ZEA in FDK	0.051***	0.537***	0.518***	0.001
ZEA in chaff	268.058***	1258.871***	266.055***	2.456
DON in HLK	1.065***	3.353***	1.304***	0.253
DON in FDK	780.700*	24265.900***	797.100*	359.800
DON in chaff	2037.300***	2575.300**	3122.700***	211.700
HLK kernels per spike	77.570**	4005.480***	33.600	22.610
FDK kernels per spike	19.878*	16.537	12.642	7.495
HLK weight per spike	0.112**	10.660***	0.080*	0.035
FDK weight per spike	0.019**	0.071***	0.014*	0.005
TKW	185.000	3721.200***	163.500	172.600
* - significant at 0.05 level				
** - significant at 0.01 lev	rel			
*** - significant at 0.001 l	evel			

Table 2. Mean squares from analysis of variance for F. culmorum

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		Mean squa	res	
Trait	Cultivars	Locations	Cultivars × Locations interaction	Residual
Degrees of freedom	9	1	9	38
Fi	0.741***	5.424***	0.546***	0.104
ZEA in HLK	0.099***	0.554***	0.100***	0.001
ZEA in FDK	2.583***	12.520***	2.524***	0.027
ZEA in chaff	47.739***	1801.983***	60.470***	5.131
DON in HLK	0.932***	2.212***	1.510***	0.081
DON in FDK	460.800*	13320.600***	481.800**	160.500
DON in chaff	1801.360***	867.540**	1843.910***	89.610
HLK kernels per spike	48.620	2856.140***	61.190*	26.760
FDK kernels per spike	18.583*	49.625*	20.061*	7.581
HLK weight per spike	0.111*	10.394***	0.185**	0.051
FDK weight per spike	0.026*	0.027	0.030*	0.012
TKW	60.710***	1516.270***	39.220**	11.630
* - significant at 0.05 lev	vel			
** - significant at 0.01 l	level			
*** - significant at 0.00	1 level			
C				

		Mean squa	ares	
Trait	Cultivars	Locations	Cultivars × Locations interaction	Residua
Degrees of freedom	9	1	9	38
Fi	1.050***	9.025***	0.657***	0.045
MON in HLK	0.006***	0.000	0.004*	0.001
MON in FDK	2.167***	1.580**	0.407	0.192
MON in chaff	0.596***	1.908***	0.285***	0.042
HLK kernels per spike	20.092**	110.433***	7.378	5.310
FDK kernels per spike	3.806**	26.268***	2.268	1.284
HLK weight per spike	0.132*	17.997***	0.087	0.051
FDK weight per spike	0.044***	1.311***	0.030**	0.009
TKW	65.590**	506.630***	43.380*	20.210

Table 4. Mean squares from analysis of variance for F. avenaceum

* - significant at 0.05 level

** - significant at 0.01 level

el vel *** - significant at 0.001 level

Cultivor _		ZEA in HLk	K		ZEA in FDk	K	,	ZEA in chaff		
	Lublin	Poznan	Means	Lublin	Poznan	Means	Lublin	Poznan	Means	
Finezja	0.085	0.000	0.043g	0.415	0.009	0.212c	5.862	0.022	2.942bcd	
Ludwig	0.528	0.002	0.265d	0.188	0.036	0.113e	4.279	0.002	2.141cd	
Mewa	0.863	0.000	0.432a	0.036	0.072	0.054f	46.810	0.097	23.454a	
Nutka	0.095	0.000	0.048fg	0.439	0.035	0.237bc	4.016	0.016	2.016cd	
Rywalka	0.067	0.005	0.036g	0.217	0.029	0.123de	4.343	0.008	2.175cd	
Soraja	0.259	0.017	0.138e	0.480	0.103	0.292a	6.247	0.014	3.130bc	
Sukces	0.605	0.007	0.306c	0.424	0.068	0.246b	5.004	0.056	2.530cd	
Symfonia	0.791	0.002	0.397b	0.196	0.101	0.148d	9.124	0.045	4.585b	
Tonacja	0.188	0.006	0.097ef	0.023	0.047	0.035f	3.926	0.018	1.972cd	
Zyta	0.045	0.000	0.023g	0.034	0.061	0.047f	2.286	0.005	1.145d	
Means	0.353A	0.004B		0.245A	0.056B	RI.	9.190A	0.028B		

C: 0.050, L: 0.023, C×L: 0.071 C: 0.031, L: 0.014, C×L: 0.043 C: 1.831, L: 0.819.1, C×L: 2.590

Table 5. The average values for ZEA in FDK, ZEA in HLK and ZEA in chaff (µg g⁻¹) for *F. culmorum*

Means with the same letter are not significantly different

NON/

LSD_{0.05}

Culting		ZEA in HL	K		ZEA in FD	K		ZEA in chaff		
Cultivar -	Lublin	Poznan	Means	Lublin	Poznan	Means	Lublin	Poznan	Means	
Finezja	0.091	0.0000	0.046cd	0.315	0.032	0.173c	14.925	0.130	7.527b	
Ludwig	0.119	0.0001	0.060c	0.598	0.003	0.300c	22.455	0.020	11.238a	
Mewa	0.115	0.0002	0.057c	1.037	0.025	0.531b	10.994	0.040	5.517b	
Nutka	0.189	0.0000	0.094b	0.563	0.026	0.295c	4.015	0.044	2.029d	
Rywalka	0.040	0.0022	0.021d	0.468	0.007	0.237c	4.152	0.014	2.083d	
Soraja	0.912	0.0003	0.456a 🗸	4.617	0.065	2.341a	10.990	0.037	5.514b	
Sukces	0.068	0.0101	0.039cd	0.442	0.032	0.237c	10.609	0.084	5.347bc	
Symfonia	0.205	0.0009	0.103b	0.387	0.126	0.257c	14.321	0.173	7.247b	
Tonacja	0.130	0.0004	0.065c	0.687	0.018	0.352bc	5.591	0.002	2.797cd	
Zyta	0.068	0.0000	0.034cd	0.401	0.043	0.222c	12.240	0.172	6.206b	
Means	0.194A	0.0014B		0.951A	0.038B	C1	11.029A	0.072B		

Table 6. The average values for ZEA in FDK, ZEA in HLK and ZEA in chaff (µg g⁻¹) for *F. graminearum*

LSD_{0.05} C: 0.026, L: 0.011, C×L: 0.036

C: 0.194, L: 0.087, C×L: 0.274

C: 2.658, L: 1.189, C×L: 3.759

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Means with the same letter are not significantly different

Cultivor	DON in HLK			Ι	DON in FDK			DON in chaff		
Cultival	Lublin	Poznan	Means	Lublin	Poznan	Means	Lublin	Poznan	Means	
Finezja	0.47	0.54	0.505cd	84.8	43.5	64.14abc	67.5	41.4	54.45b	
Ludwig	1.36	0.21	0.785bcd	114.6	41.3	77.94ab	9.4	33.0	21.20d	
Mewa	0.91	1.11	1.010abc	68.7	44.1	56.38bc	7.1	62.2	34.68cd	
Nutka	1.37	1.26	1.315ab	101.1	54.2	77.66ab	13.3	37.2	25.25d	
Rywalka	1.22	1.92	1.570a	90.3	21.6	55.96bc	43.9	21.7	32.79cd	
Soraja	0.85	0.38	0.615cd	88.4	80.8	84.62a	14.6	97.6	56.09b	
Sukces	2.63	0.29	1.460a	78.6	28.3	53.45c	115.9	45.6	80.75a	
Symfonia	2.10	0.55	1.325ab	60.0	51.8	55.92bc	14.2	71.2	42.73bc	
Tonacja	0.27	0.44	0.355d	84.0	29.1	56.53bc	33.2	37.6	35.44cd	
Zyta	1.15	0.90	1.026abc	82.0	55.5	68.77abc	22.8	25.4	24.07d	
Means	1.23A	0.76B		85.2A	45.0B		34.2B	47.3A		
LSD _{0.05}	C: 0.59,	L: 0.26, C	C×L: 0.83	C: 22.17,	L: 9.91, C	×L: 31.35	C: 17.01,	L: 7.61, C	×L: 24.05	
Means with	h the same	e letter are	not significa	antly differ	rent					

Cultivor		DON in H	ILK	Ľ	ON in FDF	X	DON in chaff		
Cultival	Lublin	Poznan	Means	Lublin	Poznan	Means	Lublin	Poznan	Means
Finezja	0.21	0.69	0.45bcde	71.2	36.3	53.76ab	12.5	41.9	27.19cd
Ludwig	0.24	0.13	0.19e	43.5	18.2	30.86e	45.2	13.3	29.27cd
Mewa	0.08	0.95	0.52bcde	58.7	42.6	50.62abc	60.0	102.3	81.16a
Nutka	0.22	1.27	0.75b	54.2	27.1	40.62bcde	19.7	35.7	27.70cd
Rywalka	0.30	0.22	0.26de	77.4	21.4	49.40abcd	35.9	19.0	27.44cd
Soraja	0.32	0.31	0.32cde	62.7	56.6	59.65a	68.9	16.9	42.90b
Sukces	0.15	2.97	1.56a	52.6	29.1	40.84bcde	31.8	55.2	43.50b
Symfonia	0.76	0.29	0.53bcd	41.8	33.6	37.67cde	18.4	22.5	20.45d
Tonacja	0.77	0.44	0.61bc	60.2	14.2	37.23de	60.5	9.7	35.12bc
Zyta	0.51	0.13	0.32cde	71.2	16.4	43.84bcde	48.6	8.8	28.72cd
Means	0.36B	0.74A		59.3A	29.5B		40.1A	32.5B	
$LSD_{0.05}$	C: 0.3	3, L: 0.15,	C×L: 0.47	C: 14.81,	L: 6.62, C>	<l: 20.94<="" td=""><td>C: 11.06,</td><td>L: 4.95, C×</td><td>L: 15.65</td></l:>	C: 11.06,	L: 4.95, C×	L: 15.65
Means wi	th the sa	me letter a	re not signific	antly differ	ent				

Table 8. The average values for DON in FDK, DON in HLK and DON in chaff ($\mu g g^{-1}$) for *F. graminearum*

 Food Additives and Contaminants

	Fi	ZEA in HLK	ZEA in FDK	DON in HLK	DON in FDK	HLK kernels per spike	FDK kernels per spike	HLK weight per spike	FDK weight per spike	TKW
Fi	1.00	0.30	-0.26	0.22	0.05	-0.25	0.04	-0.66*	-0.16	-0.38
ZEA in HLK	0.34	1.00	0.45	-0.16	0.05	-0.24	0.15	-0.39	-0.09	-0.21
ZEA in FDK	-0.38	-0.18	1.00	-0.24	0.43	-0.38	0.25	-0.27	0.12	-0.11
DON in HLK	-0.16	0.46	0.27	1.00	-0.11	-0.24	-0.03	-0.09	-0.02	0.11
DON in FDK	0.09	-0.26	0.20	0.10	1.00	-0.18	0.24	-0.09	0.10	0.13
HLK kernels per spike	-0.42	-0.11	0.15	-0.20	0.14	1.00	-0.10	0.54	-0.14	-0.33
FDK kernels per spike	0.49	0.42	-0.17	0.35	0.32	-0.16	1.00	-0.12	0.84**	-0.02
HLK weight per spike	-0.05	0.29	-0.12	0.05	-0.03	0.58	0.19	1.00	0.16	0.55
FDK weight per spike	0.23	0.29	-0.11	0.34	0.54	0.09	0.77**	0.14	1.00	0.29
TKW	0.09	0.37	-0.29	0.30	0.05	-0.03	0.26	0.63*	0.37	1.00
* significant a	t 0.05	level, ** si	gnificant a	t 0.01 leve	I					

	Fi	ZEA in HLK	ZEA in FDK	DON in HLK	DON in FDK	HLK kernels per spike	FDK kernels per spike	HLK weight per spike	FDK weight per spike	TKW
Fi	1.00	-0.23	0.20	-0.14	0.56	-0.42	0.34	-0.61	0.12	-0.45
ZEA in HLK	0.40	1.00	-0.02	0.72*	-0.02	-0.12	0.15	-0.09	0.28	-0.01
ZEA in FDK	-0.01	0.20	1.00	-0.09	0.47	-0.29	0.19	-0.28	0.33	-0.09
OON in HLK	0.16	0.39	0.30	1.00	0.09	-0.44	0.43	-0.33	0.47	-0.19
OON in FDK	0.09	-0.38	0.04	0.14	1.00	-0.52	0.50	-0.58	0.42	-0.39
HLK kernels per spike	-0.31	-0.28	-0.06	0.11	-0.12	1.00	-0.59	0.81**	-0.54	0.39
FDK kernels per spike	0.20	0.03	0.27	-0.10	0.21	-0.72*	1.00	-0.51	0.82**	-0.33
HLK weight per spike	-0.20	-0.16	0.09	0.20	-0.16	0.93***	-0.62	1.00	-0.24	0.71*
FDK weight per spike	0.19	-0.06	0.32	-0.11	0.25	-0.63*	0.93***	-0.53	1.00	0.02
TKW	0.16	0.09	0.48	0.16	0.00	-0.04	0.25	0.27	0.45	1.00

Table 10 Th . •. diad in D d I uhlin (hal 4: 1) for E1 . • . 1 1. raminearum

* significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level

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Cultivor]	MON in HLI	K		MON in FDI	X	MON in chaff		
Cultival -	Lublin	Poznan	Means	Lublin	Poznan	Means	Lublin	Poznan	Means
Finezja	0.000	0.017	0.008d	0.023	0.216	0.120de	_	0.019	0.019b
Ludwig	0.000	0.000	0.000d	0.037	0.142	0.090e	_	0.147	0.147b
Mewa	0.000	0.132	0.066b	0.000	1.245	0.623bcd	_	0.000	0.000b
Nutka	0.076	0.050	0.063b	0.392	1.155	0.774bc	0.097	0.017	0.057b
Rywalka	0.038	0.000	0.019cd	0.119	0.445	0.282cde	0.035	0.144	0.090b
Soraja	0.038	0.020	0.029bcd	0.060	0.476	0.268cde	0.037	1.069	0.553a
Sukces	0.110	0.108	0.109a	2.510	1.728	2.119a	0.481	0.825	0.653a
Symfonia	0.050	0.022	0.036bcd	0.799	0.874	0.837b	0.176	1.066	0.621a
Tonacja	0.050	0.058	0.054bc	0.122	0.506	0.314cde	0.074	0.079	0.077t
Zyta	0.061	0.050	0.056bc	0.626	1.146	0.886b	0.226	0.428	0.327a
Means	0.042A	0.046A		0.469A	0.793B		0.161B	0.379A	

-1) E TT 1 1 1 TT f. MONL'S EDV. MONL'S HEV

Means with the same letter are not significantly different

Table 12. The correlation matrix for the traits studied in Poznar	(above diagonal) and Lublin (below diagonal) for F. avenaceum

	Fi	MON_HL	K MON_FDK	HLK kernels per spike	FDK kernels per spike	HLK weight per spike	FDK weight per spike	TKW
Fi	1.00	0.03	0.14	-0.29	0.06	-0.34	0.11	-0.12
MON_HLK	-0.29	1.00	0.78**	-0.32	0.40	-0.31	0.29	0.09
MON_FDK	-0.34	0.71*	1.00	-0.58	0.70*	-0.55	0.62	0.19
HLK kernels per spike	-0.29	-0.16	0.04	1.00	-0.51	0.81**	-0.54	-0.13
FDK kernels per spike	0.26	0.15	-0.08	-0.54	1.00	-0.51	0.95***	0.12
HLK weight per spike	-0.32	-0.08	0.10	0.93***	-0.50	1.00	-0.48	0.18
FDK weight per spike	0.20	0.22	-0.02	-0.44	0.97***	-0.39	1.00	0.11
TKW	-0.35	0.22	0.28	0.33	-0.24	0.63*	-0.11	1.00

* significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level