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CONCENTRATION OF FUNGAL BIOMASS AND TRICHOTHECENES IN DIFFERENT PARTS OF EINKORN

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Abstract: Analyses of ergosterol (ERG), adenosine-5'-triphosphate (ATP) and groups A and B trichothecenes were performed in three *Triticum monococcum* cultivars registered in Germany (Albini, Terzino and Tifi), grown in the organic system. The experiment was carried out on two dates: the first – in the final phase of flowering (BBCH 69) and the second – in the phase of full ripeness (BBCH 89). The analyses were performed on shanks, glumes, grain and awns. Concentrations of analyzed metabolites in different parts of *T. monococcum* plants varied significantly. Mean ERG concentration in the first term was more than 30 times higher than immediately before harvest, whereas for group B trichothecenes it was 4 times higher. Contents of ATP and concentrations of group A trichothecenes were similar at both times. When analyzing parts of the spike, the highest amount of metabolites was recorded in shanks (ERG – 114 mg/kg, ATP 900,000 RLU, group A and B trichothecenes – 0.07 and 0.20 mg/kg, respectively), while the lowest in grain (ERG – 5 mg/kg, ATP 55,000 RLU, group A and B trichothecenes – 0.03 and 0.08 mg/kg, respectively). A higher ERG concentration was found in awns (65 mg/kg) than in glumes (41 mg/kg), whereas for ATP and group A and B trichothecenes by contrast higher concentrations were recorded in glumes (160,000 RLU, 0.06 and 0.029 mg/kg, respectively) than in awns (77,000 RLU, 0.05 and 0.014 mg/kg, respectively). Recorded results indicate a potential occurrence of trichothecenes in shanks, awns and glumes already during flowering, when grain has not yet developed. In these parts of plants, after harvest, the highest amounts of microorganisms and *Fusarium* toxins were found, which pose a threat for farmers and workers employed in the cereal industry.

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INTRODUCTION

The human nutrition guidelines presented in the form of the food pyramid are based on cereals. The most important among the cultivated species is bread wheat. Apart from that crop, a growing interest may be observed on the part of breeders in such species as *Triticum spelta* L. (spelt), *Triticum dicoccum* L. (emmer) or *Triticum monococcum* L. (einkorn). They are increasingly appreciated by consumers of cereals due to their obvious health-promoting value. To-date these cereals have not been thoroughly investigated in terms of their susceptibility to diseases, including *Fusarium* head blight (FHB), particularly under conditions of organic farming, in which no mineral fertilisers or chemical pesticides are used.

An incentive for the undertaking of the presented study was the fact that einkorn is a species of wheat, in relation to which there is very little reliable information concerning its response to ear infection with pathogens from genus *Fusarium*, and thus there are practically no data on the

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infection process itself, its intensity and phenomena connected with the biosynthesis of *Fusarium* toxins in grain and other parts of the plant [24].

In view of the numerous theories presented in literature, connected with etiology of ear blight as well as pathogenicity and toxin productivity of pathogens causing the disease in bread wheat, it seems to be of interest and significance to determine to what degree these theories may be valid in relation to einkorn. In bread wheat, the highest amounts of mycoflora and the highest concentrations of toxic fungal metabolites are found in glumes, and several times more in relation to grain, in cereal dust found in the agricultural environment. It has extensive implications for workers employed in this branch of the economy [16, 27].

To-date, in relation to einkorn, there have been no available data referring to the distribution of mycoflora or trichothecenes within a single plant. Information on the subject may prove to be particularly useful in view of the hazards presented above, and the cultivation of this cereal becoming increasingly popular in Europe [5].

The aim of the study was to determine the amount of fungal biomass through the analysis of ergosterol and total microflora (fungal and bacterial) through the analysis of ATP present in different parts of *T. monococcum* plants coming from organic plantations [26]. Moreover, at two dates (at the end of anthesis BBCH 69 and at the complete maturity phase BBCH89) analyses of contents of trichothecenes groups A and B were performed. Results of these analyses were to answer the question whether the formation of *Fusarium* toxins in plants occurs already during blooming, or whether they are accumulated during biosynthesis in the course of grain ripening and whether it is affected by physico-chemical properties of trichothecenes. On the basis of recorded results it was also attempted to clarify the manner of microorganism distribution within the plant and the possible translocation of metabolites formed by these microorganisms.

MATERIALS AND METHODS

Materials. The analysed material comprised shanks, glumes, grain and awns of three *T. monococcum* cultivars: Albini, Terzino and Tifi. Albini and Tifi are winter cultivars and cv. Terzino is a wniter-and-spring form. The material originated from the Plant Breeding Station at Darzau near Hamburg (Germany), where all the cultivars were grown on seed plantations, on which BIO certified seed material was reproduced. No chemical plant protection or mineral fertilizers were used.

Samples were collected in 5 replications for each of the 2 adopted dates: one at the end of anthesis (BBCH 69) and the other – at full grain ripeness (BBCH 89). Ears were collected with a 20-cm section of the shank, and in the next step the material was divided into shanks, glumes, grain and awns. All the tested cultivars were characterised by similar resistance to fungal infestation and similar yielding.

Analysis of trichothecenes. Plant material was analyzed for the presence of trichothecenes according to Perkowski *et al.* [19]. Briefly, sub-samples (10 g) were extracted with acetonitrile/water (82:18) and cleaned-up on a charcoal column [Celite 545/charcoal Draco G/60/ activated alumina neutral 4:3:4 (w/w/w)]. Trichothecenes group A (H-2 toxin, T-2 toxin, T-2 tetraol, DAS) were analysed as TFAA derivatives. The amount of 100 µl trifluoroacetic acid anhydride was added to the dried sample. After 20 min the reacting substance was evaporated to dryness under nitrogen. The residue was dissolved in 500 µl of isooctane and 1 µl was injected onto a gas chromatograph-mass spectrometer. Trichothecenes group B (DON, NIV, 3-AcDON, 15-AcDON, FUS-X) were analysed as TMS (trimethylsilylsilyl ethers) derivatives. The amount of 100 µl TMSI/TMCS (trimethylsilyl imidazole/ trimethylchlorosilane v/v 100/1) mixture was added to the dried extract. After 10 minutes, 500 µl of isooctane were added and the reaction was quenched with 1 ml of water. The isooctane layer was used for the analysis and 1 µl of the sample was injected on a GC/MS system. The analyses were run on a gas chromatograph (Hewlett Packard GC 6890) hyphenated to a mass spectrometer (Hewlett Packard 5972 A, Waldbronn, Germany), using an HP-5MS, 0.25mm × 30 m capillary column. The injection port temperature was 280ºC, the transfer line temperature 280ºC, and the analyses performed with programmed temperature, separately for group A and B trichothecenes. Group A trichotecenes were analysed using the following programmed temperatures: initial 80ºC held for 1 min., from 80–280ºC at 10ºC/min, the final temperature being maintained for 4 min. For group B trichothecenes, the initial temperature of 80ºC was held for 1 min, from 80–200ºC at 15ºC/min was held for 6 min, and from 200–280ºC at 10ºC/min, the final temperature being maintained for 3 min. The helium flow rate was held constant at 0.7 ml/min. Quantitative analysis was performed in the single ion monitored mode (SIM) using the following ions for the detection of STO: 456 and 555; T-2 tetraol 455 and 568; T-2 triol 455 and 569; DAS 402 and 374; HT-2 455 and 327; T-2 327 and 401. DON: 103 and 512; 3-AcDON: 117 and 482; 15-AcDON: 193 and 482; FUS X: 103 and 570; NIV: 191 and 600. Qualitative analysis was performed in the SCAN mode (100 – 700 amu). Recovery rates for the analyzed toxins were as follows: STO 82.3±3.8; T-2 triol 88.0±4.0; T-2 86±3.8%; T-2 tetraol 88±4.0%; HT-2 91±3.3%; DAS 84±4.6%; DON 84±3.8%; 3AcDON 78±4.8%; 15 AcDON 74±2.2%; FUS X 87±5.9% and NIV 81±3.8%. The limit of detection was 0.001 mg/kg.

Determination of ATP. Adenosine-5'-triphosphate (ATP) measurement was carried out using the luciferase enzyme. ATP was extracted from 1.00 g grain samples using 8 ml boiling Tris buffer (0.1 molar solution, pH – 7.75). The amount of 100 µl of this extract was mixed with 100 µl reagent containing luciferin and luciferase (LuminATE

(QM), Celsis, Netherlands). The emitted light was quantified by a Lumac Biocounter M 1500 luminometer, and read as Relative Light Units (RLU). The emitted light correlates with the amount of ATP in the sample.

Chemical analysis of ergosterol. Ergosterol was determined by HPLC, as described by Young [28], with modifications [17, 19]. A detailed evaluation of the method was given in a study by Perkowski *et al.* [17]. Samples containing 100 mg of ground grains were placed into 17 ml culture tubes, suspended in 2 ml of methanol, treated with 0.5 ml of 2M aqueous sodium hydroxide, and tightly sealed. The culture tubes were then placed within 250 ml plastic bottles, tightly sealed and placed inside the microwave oven (Model AVM 401/1WH, Whirlpool, Sweden), operating at 2,450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s and after about 5 min for an additional 20 s. After 15 min, the contents of the culture tubes were neutralized with 1M aqueous hydrochloric acid, 2 ml MeOH were added and extraction with pentane (3 × 4 ml) was carried out within the culture tubes. The combined pentane extracts were evaporated to dryness in a nitrogen stream. Before analysis, samples were dissolved in 4 ml of MeOH, filtered through 13-mm syringe filters with a 0.5 mm pore diameter (Fluoropore Membrane Filters, Millipore, Ireland) and evaporated to dryness in a N2 stream. The sample extract was dissolved in 1ml of MeOH and 50 µl were analyzed by an HPLC. Separation was performed on a 150 × 3.9 mm Nova Pak C-18, 4-mm column, and eluted with methanol/acetonitrile (90:10) at a flow rate of 0.6 ml/min. Ergosterol was detected with a Waters 486 Tunable Absorbance Detector (Milford, MA, USA) set at 282 nm. The presence of ERG was confirmed by a comparison of retention times and by co-injection of every tenth sample with an ergosterol standard.

Statistical analysis. Results recorded in the course of the conducted chemical analyses were subjected to statistical analysis with the use of STATISTICA v 8.0 software. In order to compare contents of individual metabolites in samples, Tukey's multiple comparison procedure was used, with identical letters in rows or columns denoting a lack of differences at the significance level α=0.05.

RESULTS

A lack of reliable information on the response to ear blight caused by fungi from the genus *Fusarium* and resulting processes such as infection and biosynthesis of toxins in different parts of plants, was an incentive to undertake research on this subject. An additional aspect of the study was to collect samples at 2 dates – at the end of anthesis and at the full grain ripeness phase. Thus, on the first date, kernels were not there to be analysed together with the other elements of plants. Recorded results are presented jointly in Table 1. However, for improved clarity they were also

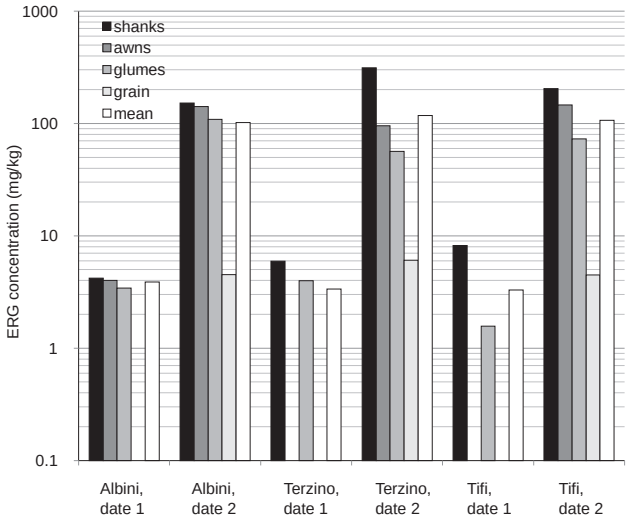

Figure 1. ERG (mg/kg) concentration in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

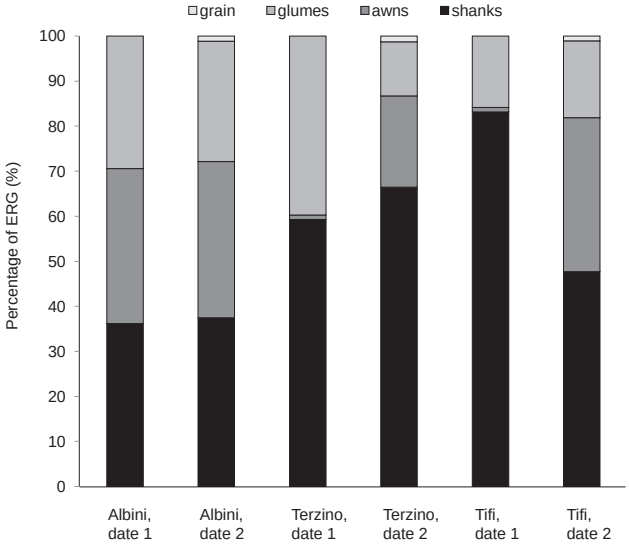

Figure 2. Percentage of ERG in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

given in figures. This was also caused by the fact that in the course of statistical analysis it was observed that among the investigated cultivars no significant differences were found within all the analysed traits, at both dates. In parts of plants, significant differences were only recorded for shanks in the course of analyses of ERG and ATP. In turn, significant differences were found between the vegetation dates and it was both in case of the analyses of ERG and those for group A and B trichothecenes. However, these calculations are not given in this paper, since they result from the presented figures.

Figure 1 presents mean concentrations of ERG in different parts of *T. monococcum* plants, while the percentage of this metabolite is given in Figure 2. The highest ERG concentration was found in shanks of mature plants,

where it was a max. of 312.71 mg/kg, while it was much lower in awns (max. 146.57 mg/kg) and in glumes (max. 109.07 mg/kg). The concentration of this metabolite in grain turned out to be lowest and ranged from 4.49–6.07 mg/kg. Recorded results showed that ERG found in grain accounted, on average, for only approx. 1% entire amount of ERG found in the analysed parts of plants. At anthesis, the highest percentage was recorded for ERG found in shanks (mean 59.5%), followed by glumes (mean 28.3%) and awns (mean 12.1%). At full grain ripeness, the amount of mycoflora was many times higher than at anthesis, as is indicated by the approx. 30 times higher mean ERG concentration.

The amount of ATP in the analysed samples is presented in Figure 3 and its percentage distribution in different parts of plants is given in Figure 4. This index, corresponding to the total amount of microbial biomass in different parts of plants, had approx. two-fold higher values at full ripeness of grain than at anthesis; similarly, as was observed for ERG. The amount of microbial-origin ATP in grain ranged from 16,842–95,296 RU, while in shanks the maximum value of 1,234,051 RU was recorded at full ripeness of grain. ATP found in shanks accounted to 42–88% of the entire amount of this compound determined in plants. In grain, ATP was found in the smallest amounts (on average only approx. 3.5%) (Fig. 4), while in glumes it was found at approx. 10% (except for cv. Albini at the phase of anthesis), whereas in awns it was approx. 7%. Figures 5–8 contain results concerning concentrations of total trichothecenes of group A (Fig. 5), group B (Fig. 6) and the percentage content of group A toxins (Fig. 7) and group B toxins, respectively (Fig. 8). The mean concentration of group A trichothecenes decreased with plant development and at anthesis amounted on average to 0.084 mg/kg, while at full ripeness it was 0.036 mg/kg (Fig. 5). The biggest drop in concentration was observed in shanks - from 0.116–0.036 mg/kg. A decrease in the percentage content of total group A trichothecenes found in awns occurred at the expense of their formation in grain, in which at the last maturity phase their level was as high as approx. 20% of these toxins. An increase in the concentration of these metabolites was found only in glumes, and only in case of cv. Albini and Tifi.

Mean concentration of group B trichothecenes was presented in Figure 7, while their percentage distribution in plants is given in Figure 8. On average, it was 3 times high-

Table 1. Concentrations of ERG, ATP and total trichothecenes of groups A and B in different parts of plants in 3 cultivars of *T. monococcum* at phases of anthesis and full grain ripeness.

Cultivar	Development phase	Analysed metabolite	Parts of plants				
			Shanks	Awns	Glumes	Grain	Mean
Albini	Anthesis	ERG (mg/kg)	4.22	4.01	3.43	–	3.89
		ATP (URL)	265,941	44,562	326,018	–	212,174
		Total trichothecenes group A (mg/kg)	0.059	0.017	0.029	–	0.035
		Total trichothecenes Group B (mg/kg)	0.102	0.091	0.078	–	0.090
	Full grain ripeness	ERG (mg/kg)	152.55	141.34	109.07	4.53	101.87
		ATP (URL)	1,234,051	100,367	280,300	95,296	427,504
		Total trichothecenes group A (mg/kg)	0.053	0.001	0.043	0.009	0.026
		Total trichothecenes group B (mg/kg)	0.162	0.024	0.076	0.050	0.078
Terzino	Anthesis	ERG (mg/kg)	5.97	0.10	4.00	–	3.36
		ATP (URL)	569,912	62,463	96,478	–	242,951
		Total trichothecenes group A (mg/kg)	0.116	0.084	0.158	–	0.119
		Total trichothecenes group B (mg/kg)	0.110	0.076	0.063	–	0.083
	Full grain ripeness	ERG (mg/kg)	312.71	95.30	56.39	6.07	117.62
		ATP (URL)	1,149,795	89,665	109,285	52,442	350,297
		Total trichothecenes group A (mg/kg)	0.036	0.006	0.017	0.047	0.027
		Total trichothecenes group B (mg/kg)	0.251	0.016	0.492	0.076	0.209
Tifi	Anthesis	ERG (mg/kg)	8.24	0.10	1.57	–	3.30
		ATP (URL)	986,881	67,551	68,101	–	374,178
		Total trichothecenes group A (mg/kg)	0.105	0.162	0.027	–	0.098
		Total trichothecenes group B (mg/kg)	0.144	0.093	0.060	–	0.099
	Full grain ripeness	ERG (mg/kg)	204.01	146.57	73.00	4.49	107.02
		ATP (URL)	1,223,201	96,526	71,677	16,842	352,062
		Total trichothecenes group A (mg/kg)	0.033	0.026	0.110	0.047	0.054
		Total trichothecenes group B (mg/kg)	0.570	0.564	0.952	0.111	0.549

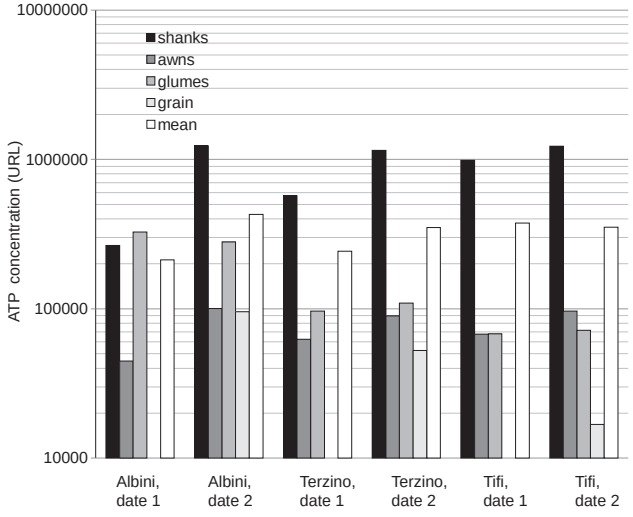

Figure 3. ATP (URL) concentrations in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

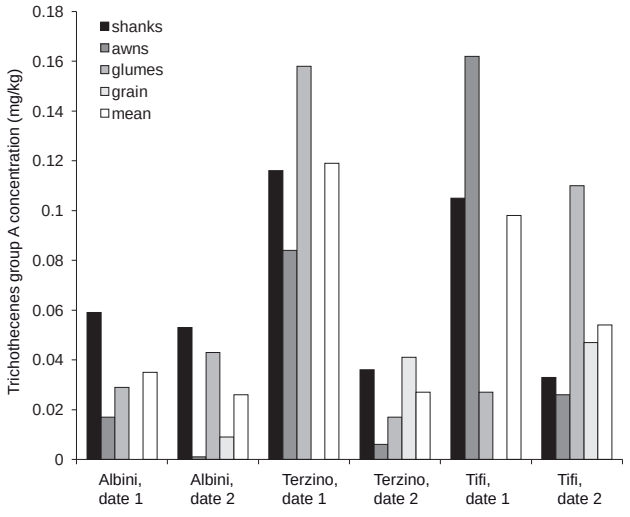

Figure 5. Concentrations of total trichothecenes group A (mg/kg) in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

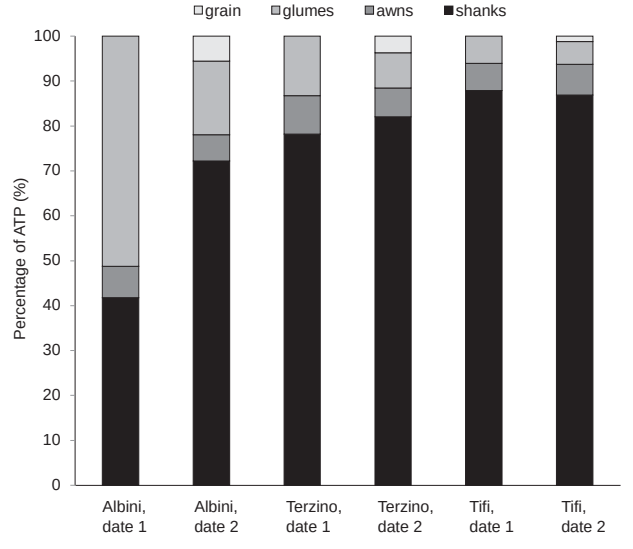

Figure 4. Percentage content of ATP in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

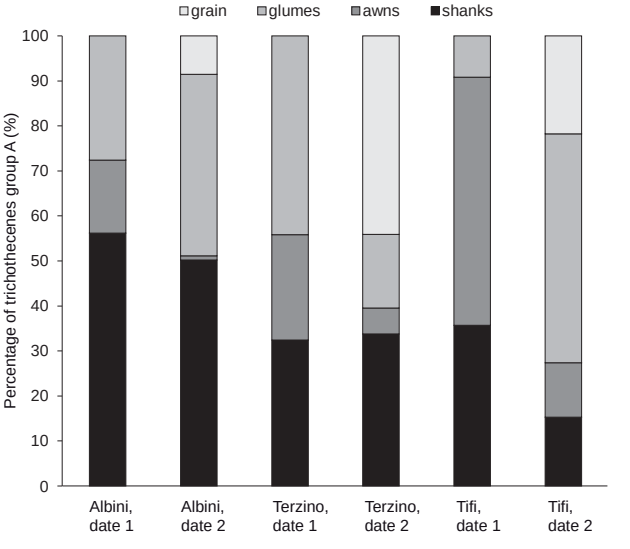

Figure 6. Percentage content of total trichothecenes group A in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

er at full grain ripeness (for all examined parts of plants mean 0.279 mg/kg) than in case of anthesis (mean 0.091 mg/kg), with the highest concentration found for cv. Tifi (0.952 mg/kg in glumes, 0.564 mg/kg in awns, 0.570 mg/kg in shanks, and only 0.111 mg/kg in grain). The share of group B trichothecenes in grain was only 10%. At full grain ripeness an approx. 2-fold increase in their content was observed in glumes (in comparison to anthesis), while in awns their concentration decreased 2-fold at that time. The decrease in their concentration was also found in shanks, although it was to a much lower degree.

The distribution in shanks, awns, glumes and in grain for individual trichothecenes of group A (scirpentriol (STO), T-2 teraol, T-2 triol, DAS, and HT-2) and group B (DON, fusarenon X (Fus-X), 3-AcDON, 15-AcDON and NIV) is presented on Table 2. The highest concentrations of DON and NIV were found in glumes of cv. Tifi (0.370 mg/kg and 0.386 mg/kg, respectively). Slightly lower amounts of these toxins were observed in awns (0.257 mg/kg and 0.139 mg/kg, respectively), while in shanks their concentration was 0.110 mg/kg and 0.369 mg/kg, and in grain it was only 0.082 mg/kg and 0.015 mg/kg. Among group A trichothecenes, the biggest number of samples analyses showed the presence of STO (max. 0.083 mg/kg in glumes) and T-2 tetraol (max. 0.091 mg/kg in awns). The presence of DAS in most samples was recorded only during anthesis, and the maximum concentration of this metabolite was 0.069 mg/kg in awns of cv. Tifi.

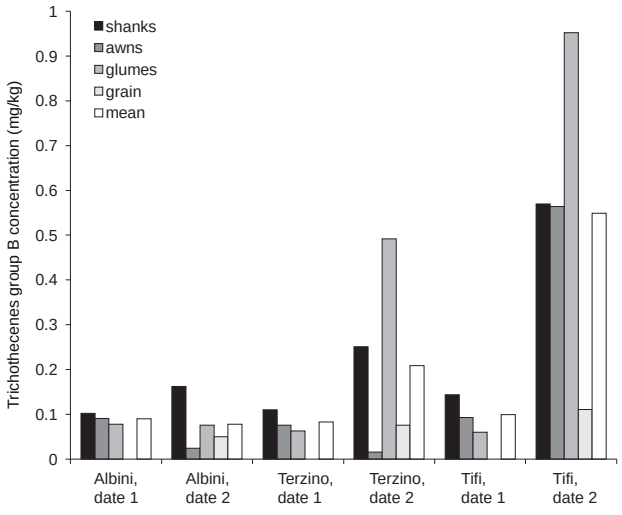

Figure 7. Concentrations of total trichothecenes group B (mg/kg) in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

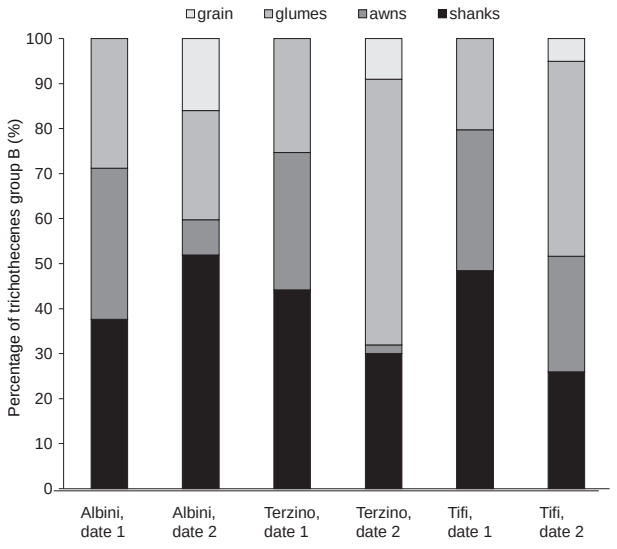

Figure 8. Percentage contents of total trichothecenes group B in different parts of plants in three einkorn cultivars: Albini, Terzino and Tifi.

Table 2. Concentrations of trichothecenes groups A and B (mg/kg) in different anatomical parts of 3 einkorn cultivars: Albini, Terzino and Tifi at anthesis (1) and full grain ripeness (2).

Cultivar	Part	Date	Trichothecenes group A (mg/kg)							Trichothecenes group B (mg/kg)		
			Scirpentriol	T–2 Tetraol	T–2 Triol	DAS	HT–2	DON	FUS–X	3–AcDON	15–AcDON	NIV
Albini	Awns	1	0.001	0.015	0.000	0.001	0.000	0.010	0.000	0.000	0.022	0.059
	Grain		–	–	–	–	–	–	–	–	–	–
	Shanks		0.002	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.094
	Glumes		0.000	0.028	0.001	0.000	0.000	0.015	0.000	0.021	0.019	0.023
	Awns	2	0.001	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.001	0.010
	Grain		0.004	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Shanks		0.008	0.042	0.002	0.001	0.000	0.084	0.000	0.012	0.023	0.043
	Glumes		0.000	0.043	0.000	0.000	0.000	0.022	0.000	0.000	0.045	0.009
Terzino	Awns	1	0.001	0.067	0.000	0.005	0.011	0.034	0.000	0.013	0.027	0.002
	Grain		–	–	–	–	–	–	–	–	–	–
	Shanks		0.000	0.079	0.000	0.037	0.000	0.010	0.000	0.024	0.031	0.045
	Glumes		0.000	0.084	0.010	0.064	0.000	0.025	0.002	0.010	0.024	0.002
	Awns	2	0.000	0.006	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
	Grain		0.001	0.041	0.000	0.004	0.001	0.022	0.000	0.000	0.045	0.009
	Shanks		0.034	0.001	0.000	0.001	0.000	0.105	0.022	0.028	0.011	0.085
	Glumes		0.013	0.005	0.000	0.000	0.000	0.155	0.003	0.131	0.138	0.065
Tifi	Awns	1	0.002	0.091	0.000	0.069	0.000	0.031	0.000	0.050	0.011	0.001
	Grain		–	–	–	–	–	–	–	–	–	–
	Shanks		0.005	0.086	0.002	0.012	0.000	0.054	0.000	0.028	0.062	0.000
	Glumes		0.001	0.026	0.000	0.000	0.000	0.049	0.000	0.000	0.001	0.010
	Awns	2	0.006	0.016	0.000	0.000	0.004	0.257	0.000	0.046	0.122	0.139
	Grain		0.045	0.000	0.002	0.000	0.000	0.082	0.003	0.004	0.007	0.015
	Shanks		0.011	0.022	0.000	0.000	0.000	0.110	0.026	0.023	0.042	0.369
	Glumes		0.083	0.023	0.000	0.003	0.001	0.370	0.073	0.052	0.071	0.386

DISCUSSION

At present, we may observe a renaissance in the cultivation of glumed wheat, such as spelt or einkorn, as a result of which it is necessary to conduct studies aiming at the determination of both their sensitivity to fungal diseases and the analysis of contamination found in different parts of plants. This paper presents results of investigations on the contents of mycoflora expressed by the concentration of ergosterol in different parts of plants in the case of 3 cultivars of einkorn, which are registered in Germany and grown practically only in the organic farming system. Recorded results indicate that the cultivars do not differ significantly in terms of the accumulation of fungal metabolites in different parts of plants, and due to the applied organic farming system this result was not influenced either by chemical plant protection or the level of mineral fertilization. The division of samples into 4 fractions (shanks, glumes, awns and grain) resulted from the numerous pieces of information coming from farms and the individuals involved in the harvesting, threshing, storage and processing of grain, during which disease symptoms are frequently observed which could have been caused by metabolites produced by mould fungi [11, 25].

The date of sampling was connected with the decision to obtain information, crucial for the ear blight process, whether the count of microorganisms and the concentration of fungal metabolites are dependent on the development phase of the plant. In literature on the subject, the term "toxin accumulation in grain" is frequently used, which would indicate a continuous process, with linear kinetics of formation of these metabolites [13].

However, to-date there have been no definite data concerning the accumulation of fungal metabolites during the entire period of formation, development and ripening of kernels, as well as the kinetics and distribution of *Fusarium* toxins [7]. Studies conducted in recent years by the authors of this paper have not provided clear-cut solutions in this respect, and have indicated several deviations from the hypothetical linear course.

Results described in this study are obviously interesting and particularly reliable, especially that for the 3 analysed cultivars very similar dependencies were recorded. No significant differentiation was observed among cultivars in terms of the concentrations of ERG, ATP and group A trichothecenes. In the case of the concentration of group B trichothecenes, the only significant difference was that between cv. Albini, accumulating in the shank the lowest amount of these toxins, and cv. Tifi, in which this concentration was highest.

Ergosterol. The concentration of ergosterol, which is found only in the cell walls of fungi, is correlated with the content of mycoflora in plant tissues, thus it is its good and reliable indicator, as proved by several studies conducted recently [17]. To-date, its concentration in grain has not been standardised and the suggested value for grain is 3 mg/kg, proposed by Schnürer and Jansson [21]. Maupetit *et al*. [14] proposed for healthy grain a range of ergosterol concentrations from 1–9 mg/kg.

However, the course of biosynthesis of this metabolite in other parts of plants, apart from glumes, has not been investigated. Results presented in this study indicate that similarly to glumes, its concentration increases both in awns and shanks. On average, a 30-fold increase in the amount of this metabolite from anthesis to full grain ripeness clearly proves a significant increment of fungal biomass in ear tissues in the course of plant vegetation. What is interesting is that the mean ERG concentration in shanks, awns and glumes at anthesis is comparable with the concentration in grain at full ripeness, which was on average approx. 5 mg/kg. This is a higher value in comparison to the value determined for wheat in case of studies conducted on a large population of different samples [16]. When analysing ERG content in the other parts of the ear, its concentration needs to be considered very high, since in case of cv. Tifi it was as high as 312.71 mg/kg in shanks, up to approx. 150 mg/kg in awns, and 110 mg/kg in glumes. Results similar to those reported in this paper, referring to ERG content in glumes, have been reported in other studies conducted by the authors [3]. A surprisingly high ERG concentration was found for shanks, which shows its high susceptibility to microbial infestation. We also need to stress here the potential translocation of this metabolite within the plant. The analysis of mycoflora content during the biosynthesis of ergosterol, which is a qualitative indicator of mycoflora, conducted within the framework of earlier investigations performed by this team of researchers, confirmed that this concentration increases linearly in time. This phenomenon, recorded earlier in several independent studies [21], was also confirmed in this study.

ATP. As in the case of ERG concentration, being an indicator of the amount of mycoflora, similarly as in case of ATP as an index of the total count of microbial cells, we did not observe significant differences depending on the applied cultivar and date of observation. It was only observed that in the shanks of a mature plant there is more ATP in comparison to those of a plant at anthesis. Moreover, a tendency was also recorded for the occurrence of its smaller amount in grain in relation to other parts of plants. The percentage of ATP determined in grain was only 3.5% of the amount of this compound in relation to the entire detected amount. It is difficult to confront the recorded result with similar observations in this respect, since to date no such studies have been conducted. Nevertheless, it may be stated that among the examined parts of einkorn plants the biggest amounts of ATP were found in shanks (from 70–80%), while the percentage of ATP found in glumes was approx. 10%. Thus it is possible that during plant maturation the amount of adenosine triphosphate it contains increases slightly, with shanks being the source of its pool [1, 23].

Trichothecenes. Trichothecenes are formed by fungi from the genus *Fusarium*, which are the most commonly found toxin-forming fungi in the agricultural environment in Poland [20]. In terms of their chemical structure, these metabolites are divided into 4 groups (A, B, C and D). The most important, due to their presence in the naturally infested cereal grain, are those belonging to groups A and B. The former includes trichothecenes containing a functional group other than a ketone at the C8 position (e.g. T-2 toxin, HT-2 toxin, DAS, T-2 tetraol, STO), while group B comprises those with the carbonyl function at the C8 position (e.g. DON, NIV, FUS-X 3-AcDON, 15-AcDON). However, in terms of their solubility they were divided differently [2]. Group A comprises non-polar trichothecenes, readily soluble in organic solvents such as ethyl acetate, acetone, chloroform, methylene chloride and diethyl ether (T-2 toxin, HT-2 toxin and DAS). In turn, group B includes DON, NIV FUS-X, 3-AcDON, 15-AcDON, STO and T-2 tetraol, which are highly hydroxylated and relatively polar, being soluble in methanol, acetonitryle and ethanol (EMAN, Turkey, He).

Trichothecenes exhibit diverse physico-chemical properties. In view of the presented results, it is solubility which is particularly important for their translocation in the plant, since most of them, to a greater or lesser extent, are soluble in water. Some of the analysed group A trichothecenes (DAS, HT-2 or T-2) are less readily soluble in water than group B trichothecenes, which limits their potential translocation in plants [4].

Recorded results constitute a very interesting illustration of theories presented in literature concerning ear blight and its implications for plant resistance to this disease. At present, we may distinguish 5 types of this resistance, i.e. resistance to the initiation of infection (type I), to the spread of the pathogen (type II), capacity to degrade mycotoxins (type III), tolerance towards high concentrations of mycoxins (type IV) and resistance to grain infection (type V) [15]. Among different types of resistance, we may point to the resistance to the formation of trichothecenes in grain and their potential degradation during plant vegetation, as indicated by Lemmens *et al.* [12] and Boutigny *et al.* [6].

When analysing total concentrations of group A trichothecenes we may observe their presence already at anthesis, both in shanks, awns and glumes. Their amount turned out to be significant, since its maximum concentrations were 0.116 mg/kg, 0.162 mg/kg and 0.158 mg/kg, respectively. What is interesting, at full grain ripeness in the same parts of the ear, these concentrations were only 0.036 mg/kg, 0.026 mg/kg and 0.017 mg/kg, at the simultaneous translocation of toxins, the presence of which was found in the formed kernels, and in 2 cases their concentration in glumes increased. The occurrence of such translocation and degradation is suggested by the percentages of these metabolites (Fig. 6), although this does not concern all toxins (Tab. 2). The concentration of T-2 tetraol and DAS (i.e. toxins very rarely detected in naturally contaminated grain) decreased significantly, as could be evidenced by the process of their degradation. What is interesting is that an increase was observed for the concentration of STO, a toxin best soluble in water [8].

At the phase of full grain ripeness, in comparison to anthesis, both in shanks and in glumes a considerable elevation was found for the concentration of all group B trichothecenes, as indicated by the accumulation of these toxins. Concentrations of NIV and DON, being the most common toxins in grain, increased markedly, while an increase was lower for two acetyl DON derivatives (3-AcDON, 15-AcDON). Distribution of these 2 most important *Fusarium* toxins is as follows: during anthesis their highest concentrations were recorded in shanks, followed by awns and glumes. At the full ripeness phase, when on average approx. 10% these toxins were found in grain, their 40% share were recorded in glumes, 35% in shanks and approx. 15% in awns. This again proves their translocation in plants [18]. Moreover, results reported in this study make it possible for us to conclude that when performing only the analysis of contents for the toxin most commonly found in grain, i.e. DON, excluding other trichothecenes of this group, biased or erroneous conclusions may be drawn. This is evident from data given in Tab. 8. An additional and important aspect of the statement that other parts of the ear, apart from grain, contain the biggest amounts of microflora and toxins, confirms the existence of a serious hazard for workers employed in the cereal sector. This is especially evident e.g. for cv. Tifi. Moreover, it needs to be stressed that in shanks, awns and glumes, as many as 10 toxins were detected with different degrees of toxicity and an unknown summary synergistic effect. In combination with a very high amount of microorganisms found in these parts of plants, this effect may be enhanced. Such a statement is justified in this sense, that trichothecene transformation may be based on many types of reactions, e.g. alkalization, oxidation, reduction, hydrolysis, hydratation and conjugation [9, 10], particularly that numerous examples of microbial transformation of trichothecenes have already been presented. These examples show that the process of biotransformation and accumulation, described in our paper, is highly probable, although this type of a process occurring in naturally infested plants has not yet been discussed in the presented context.

CONCLUSIONS

These investigations supplement information concerning the contents of mycoflora and trichothecenes, both in grain and in other parts of the ear and shanks in *Triticum monococcum*. The highest content of the investigated metabolites was recorded in shanks, glumes and awns, while their lowest concentration was found in grain. A significant variation of toxin content was observed in the phases of anthesis and full grain ripeness. Similar to the case of ERG, and to a lesser extent ATP, their amounts and translocation were

observed to increase within the analysed parts of plants. This result is particularly valuable since such studies in naturally infested einkorn grown in the organic farming system have not been conducted so far. They also show that other parts of cereals, apart from grain, contain the biggest amounts of microflora and toxins. The determination of as many as 10 different toxins with varying toxicity, and a probable strong synergistic effect, offers grounds for the conclusion that they pose a potential hazard for farmers and workers employed in the cereal sector.

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