

## Article

# Fusarium Head Blight and Associated Mycotoxins in Grains and Straw of Barley: Influence of Agricultural Practices

Dimitrios Drakopoulos <sup>1</sup>, Michael Sulyok <sup>2</sup>, Eveline Jenny <sup>1</sup>, Andreas Kägi <sup>1</sup>, Irene Bänziger <sup>1</sup>, Antonio F. Logrieco <sup>3</sup>, Rudolf Krska <sup>2,4</sup> and Susanne Vogelgsang <sup>1,\*</sup>

<sup>1</sup> Ecological Plant Protection in Arable Crops, Plant Protection, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland; dimitrios.drakopoulos@agroscope.admin.ch (D.D.); eveline.jenny@agroscope.admin.ch (E.J.); andreas.kaegi@agroscope.admin.ch (A.K.); irene.baenziger@agroscope.admin.ch (I.B.)

<sup>2</sup> Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, 3430 Tulln, Austria; michael.sulyok@boku.ac.at (M.S.); rudolf.krska@boku.ac.at (R.K.)

<sup>3</sup> Institute of Sciences of Food Production, National Research Council, CNR, Via Amendola 122/O, 70126 Bari, Italy; antonio.logrieco@ispa.cnr.it

<sup>4</sup> Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast BT7 1NN, Northern Ireland, UK

\* Correspondence: susanne.vogelgsang@agroscope.admin.ch



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**Abstract:** Fusarium head blight (FHB) is a devastating fungal disease of small-grain cereals that causes significant yield losses and mycotoxin contamination, diminishing food and feed safety worldwide. In contrast to wheat, little is known about the agricultural practices that influence FHB and *Fusarium* mycotoxins in barley. Thus, a nationwide survey was conducted across Switzerland for harvest samples in 2016 and 2017, accompanied with a questionnaire to obtain information about the agricultural practices in each barley field. In total, 253 grain and 237 straw samples were analyzed. In both years, *F. graminearum* was the predominant *Fusarium* species in grains followed by *F. avenaceum* and *F. poae*. Growing maize before barley was associated with increased amount of *F. graminearum* DNA in grains and straw as well as with elevated concentrations of deoxynivalenol in grains of barley. On the other hand, growing pasture before barley resulted in increased incidence of *F. poae* and concentration of numerous mycotoxins in grains (e.g., enniatins) and straw (e.g., beauvericin). Reduced tillage practices were linked to increased incidence of *F. graminearum* and deoxynivalenol content in grains and straw. In contrast, conventional tillage was linked to higher incidence of *F. poae*. Moreover, use of spring barley was associated with decreased amount of *F. graminearum* DNA in grains and straw, but increased incidence of *F. poae* and *F. avenaceum*. Use of the spring variety Eunova was linked to increased concentrations of several *Fusarium* mycotoxins in grains (e.g., enniatins and nivalenol). Furthermore, the application of strobilurin-based fungicides was associated with higher deoxynivalenol and beauvericin contents in grains. The application of plant growth regulators was associated with increased concentration of some *Fusarium* mycotoxins in grains (e.g., culmorin), while absence of growth regulators application was linked to elevated concentration of some other mycotoxins (e.g., nivalenol). We conclude that individual agricultural practices can suppress some FHB causing species and reduce the associated mycotoxins, but can promote others. Hence, integrated control measures combining numerous prevention and intervention strategies should be applied for the sustainable management of mycotoxins in barley.

**Keywords:** barley; Fusarium head blight; mycotoxin; agricultural practice; cropping factor; grain; straw; questionnaire; survey

## 1. Introduction

Barley (*Hordeum vulgare* L.) is a major cereal crop with a global annual production of ~158 Mt [1]. Barley grain is used for animal feed and human food as well as fermentable

material for the production of beer and distilled beverages. Fusarium head blight (FHB) is a devastating disease of small-grain cereals, including barley, which causes significant yield losses and mycotoxin contaminations jeopardizing food and feed safety at a global level. Mycotoxins have carcinogenic, genotoxic, gastrotoxic, nephrotoxic, and hepatotoxic effects causing both acute and chronic diseases [2,3]. For instance, deoxynivalenol, also known as vomitoxin, is frequently responsible for acute gastrointestinal symptoms, e.g., feed refusal, vomiting, anorexia and hemorrhagic diarrhea [4]. The acute effects of deoxynivalenol in animals can be similar to those in humans [5]. Furthermore, zearalenone can affect reproduction through estrogenic effects [6]. However, other understudied *Fusarium* mycotoxins frequently occur in grains and straw of barley [7] and can also cause adverse health effects. For example, beauvericin and enniatins have been associated with cytotoxic effects including a decrease in metabolic activity and damage of mitochondria [8].

Several fungal species can cause FHB and contaminate the harvested products with mycotoxins. The geographic region and the annual climatic conditions are important factors influencing the occurrence of FHB causing species and, therefore, the diversity of mycotoxins in grains. In 2013, a study across Umbria in central Italy showed that the predominant *Fusarium* species in malting barley was *F. avenaceum* (a prominent producer of moniliformin and enniatins), followed by *F. graminearum* (a prominent producer of deoxynivalenol and zearalenone), while HT-2 toxin was the most frequently detected mycotoxin, followed by enniatins (B, B1), T-2 toxin, and nivalenol [9]. Moreover, a barley survey across Switzerland in 2013 and 2014 showed that *F. graminearum* was the predominant *Fusarium* species in grains followed by *F. avenaceum*, and deoxynivalenol was the most prominent mycotoxin [10]. Nevertheless, the agricultural practices before and during crop production represent the major driver influencing FHB and mycotoxin accumulation in the harvested products.

For *F. graminearum*, agronomic practices that are most effective against disease development and mycotoxin production are crop residue management with conventional tillage, suitable crop rotation and selection of less susceptible crop varieties [11]. An eight-year survey of wheat in Switzerland indicated that high levels of *F. graminearum* DNA and deoxynivalenol were mainly observed in grain samples from fields with reduced tillage practices, maize as the previous crop and use of *F. graminearum*-susceptible varieties [12]. Moreover, the agricultural practices can greatly affect the diversity of *Fusarium* species and mycotoxins in grains. Most *Fusarium* species are spread via the dispersal of conidia (asexual spores), but *F. graminearum* has a potential epidemiological advantage since it is also able to form perithecia on crop residues resulting in the discharge of ascospores (sexual spores; teleomorph stage: *Gibberella zeae*) [13]. Chemical control with fungicides can provide solutions against FHB and reduce mycotoxins only to some extent, since the efficacy of the product depends on several factors during application, e.g., homogeneity of anthesis and weather conditions [14]. A multivariate meta-analysis across the USA including data from over 100 wheat fields showed that fungicides based on azoles (metconazole, prothioconazole, and tebuconazole) were the most effective in reducing deoxynivalenol [15]. In contrast, strobilurin-based fungicides have frequently been associated with increased FHB severity and deoxynivalenol content in grains [12,16].

The effect of agricultural practices on the diversity and accumulation of *Fusarium* mycotoxins in barley raw materials has been insufficiently studied compared with wheat. In addition, it remains unrevealed how agronomic practices affect the accumulation of *Fusarium* mycotoxins in barley straw, which is often used as a bedding substrate and part of animals' diets. Thus, to elucidate which agricultural practices influence the incidence of FHB causing species and the accumulation of associated mycotoxins in grains and straw of barley, a nationwide survey across Switzerland was conducted analyzing harvest samples from 2016 and 2017. For each sample origin, a questionnaire was included to obtain information about the agricultural practices in the respective barley field.

## 2. Materials and Methods

### 2.1. Sampling Procedure, Sample Origin, and Questionnaire

The contact details of barley growers in Switzerland were obtained from the cantonal plant protection offices. In 2016 and 2017, instruction letters were sent to barley growers regarding the sampling procedure of the grain and straw material. In brief, samples were collected directly after harvest by mixing ten subsamples into one composite sample corresponding approximately to 1000 g for grains and 150 g for straw. In total, 253 grain and 237 straw samples from 18 cantons across Switzerland were received and analyzed (Table S1).

Growers also received a questionnaire to obtain information about the agricultural practices: production system, sowing season (autumn versus spring), barley variety, previous and pre-previous crop, tillage practice, plant height at harvest, grain yield, fungicide type, application of plant growth regulator, and fertilizer type (Table 1). Moreover, growers provided data on grain yield. With respect to production system, ÖLN (“Ökologischer Leistungs-Nachweis” (in German), translated to “proof of ecological performance”) is the minimum standard for an environmentally friendly agriculture. It requires rational use of fertilizers, targeted use of plant protection products by considering potential economic losses, suitable crop rotation, soil protection measures, animal welfare measures, and allocation of an appropriate proportion of ecological compensation areas [17]. Extenso is a production system for small-grain cereals, canola, sunflower, field peas, faba beans, and lupins, which, on top of the ÖLN requirements, prohibits the use of synthetic insecticides, fungicides and plant growth regulators [18]. The organic production system not only prohibits the use of synthetic plant protection products and mineral fertilizers but requires also natural diversity on the farm and ethologically sound livestock management [19]. The varieties Eunova, RGT Planet, Quench, and Sydney were sown in spring, while all other varieties were sown in autumn. With respect to previous and pre-previous crops, small-grain cereals included wheat, barley, rye, triticale, and oat, while “other” included potato, sunflower, onion, and sugar beet. Tillage included soil cultivation and crop residue management techniques prior to barley production, i.e., conventional tillage (moldboard ploughing) and reduced/zero tillage practices. Fungicides were grouped into triazole- and strobilurin-based products. Mineral fertilizer refers to chemically synthesized products.

### 2.2. Identification of *Fusarium* Head Blight Causing Species with Seed Health Tests

To determine the incidence of FHB causing species in grains, subsamples of 5 g were obtained using a riffle divider (Schieritz & Hauenstein AG, Arlesheim, Switzerland). Seed health tests were conducted with 100 grains per sample as described in Vogelgsang, et al. [20] using half-strength potato dextrose agar (Oxoid Ltd., Basingstoke, UK) with streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA) at 0.1 g liter<sup>-1</sup>, as culture medium. The assessment was based on macroscopic and microscopic observations of the developed fungal colonies [21].

### 2.3. Extraction of *Fusarium graminearum* DNA and Quantitative PCR

The extraction of *F. graminearum* DNA from grains and straw as well as the quantitative PCR were performed as described in Drakopoulos, et al. [22]. In brief, the DNA from 50 mg and 20 mg grain and straw flours, respectively, was extracted following the protocol of NucleoSpin® 96 Plant II Kit (Macherey-Nagel, Düren, Germany). The quantitative PCR method was developed by Brandfass and Karlovsky [23] and was performed with a CFX96™ Real-Time PCR Detection System for in vitro diagnostics (C1000™ Thermal Cycler; Bio-Rad Laboratories, Hercules, CA, USA). The limit of quantification (LOQ) was set at 40 copies per reaction and the limit of detection (LOD) at one tenth of the LOQ. To determine the amount of total DNA in the samples, the Fluorescent DNA Quantitation Kit (BIO-RAD, Switzerland) was used. A Cary Eclipse Fluorescence Spectrophotometer (Varian, Agilent Technologies, Santa Clara, CA, USA) was used for the quantification, which was based on the emitted fluorescence of a serially diluted DNA standard.

**Table 1.** Explanatory variables with the respective levels used for the analysis. The number of observations (n) is provided in brackets for barley grain and straw samples, respectively.

Explanatory Variable	Level and Number of Observations
Production system <sup>1</sup>	ÖLN (138, 129), extenso (94, 89), organic (21, 19)
Sowing season	autumn (235, 221), spring (18, 16)
Barley variety <sup>2</sup>	Meridian (60, 56), Cassia (39, 37), Tonic (33, 32), Semper (33, 31), Hobbit (28, 28), Etincel (13, 9), Eunova (11, 9), Wootan (9, 9), Caravan (8, 8), other (17, 17)
Previous crop <sup>3</sup>	small-grain cereals (156, 145), maize (40, 38), canola (26, 26), pasture (8, 8), other (23, 20)
Pre-previous crop <sup>3</sup>	maize (118, 109), pasture (40, 39), canola (33, 31), small-grain cereals (24, 23), other (37, 34)
Tillage <sup>4</sup>	conventional tillage (130, 120), reduced tillage (123, 117)
Plant height at harvest	short: $x \leq \text{median}$ ; tall: $x > \text{median}$
Grain yield	low: $x \leq \text{median}$ ; high: $x > \text{median}$
Fungicide type	triazole (74, 66), triazole + strobilurin (37, 36), strobilurin (13, 13)
Growth regulator application	yes (129, 120), no (124, 117)
Fertilizer type	mineral (134, 128), mineral + manure (85, 78), manure (31, 29)
Harvest year	2017 (130, 122), 2016 (123, 115)

<sup>1</sup> ÖLN: “Ökologischer Leistungs-Nachweis” (in German; translated to “proof of ecological performance”) requires rational use of fertilizers and plant protection products, crop rotation, soil protection, animal welfare, and ecological compensation areas; extenso: on top of the ÖLN requirements, also prohibits the use of synthetic insecticides, fungicides, and plant growth regulators; organic: on top of extenso, also prohibits the use of all synthetic plant protection products and mineral fertilizers (for more details, see Section 2.1). <sup>2</sup> Other: varieties with less than 8 observations, i.e., Zoom, RGT Planet, California, Quench, Casanova, Lomerit, and Sydney. <sup>3</sup> Maize: silage and grain maize; small-grain cereals: wheat, barley, rye, triticale, and oat; pasture: mainly grass-clover mixtures; other: potato, sunflower, onion, and sugar beet. <sup>4</sup> Conventional tillage: moldboard ploughing; reduced tillage: reduced and zero tillage systems.

#### 2.4. Analysis of *Fusarium* mycotoxins with LC-MS/MS

For the analysis of *Fusarium* mycotoxins in grains, subsamples of 150 g were obtained using a riffle divider and ground with a mill (Cyclotec™ 1093; Foss Tecator, Sweden; 1 mm mesh size). The straw samples were cut to approximately 5 cm pieces with a chopper device (Wintersteiger Hege 44, Ried im Innkreis, Austria) and then ground with a mill (Retsch SM100; Retsch GmbH, Haan, Germany; 1 mm mesh size). The extraction of *Fusarium* mycotoxins was done for 90 min on a rotary shaker using acetonitrile/water/acetic acid (79/20/1) at a ratio of 20 mL per 5 g for grain samples and 40 mL per 2.5 g for straw samples. The detection and quantification were performed as described in Sulyok, et al. [24] with a QTrap5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source and an 1290 Series UHPLC System (Agilent Technologies, Waldbronn, Germany). The measured analytes as well as the respective LODs are provided in Table S2. Details of the analysis are provided in Drakopoulos, et al. [7].

#### 2.5. Data Analysis

The explanatory and response variables used for the analysis are provided in Tables 1 and 2, respectively. The descriptive statistics (i.e., minimum–maximum concentrations, median and mean values) of all identified *Fusarium* metabolites can be found in Drakopoulos, et al. [7]. The number of positive samples for each *Fusarium* mycotoxin included in the current study is provided in Table S2.

**Table 2.** Response variables used for the analysis.

Incidence (%) of <i>Fusarium graminearum</i> , <i>F. avenaceum</i> , <i>F. poae</i> , and <i>Microdochium</i> spp. in barley grains
Amount of <i>F. graminearum</i> (FG) DNA (copies of FG DNA per ng total DNA) in grains and straw of barley
<i>Fusarium</i> mycotoxin content ( $\mu\text{g kg}^{-1}$ ) in grains and straw of barley: antibiotic Y, apicidin, aurofusarin, beauvericin, butenolide, culmorin, deoxynivalenol, enniatins <sup>1</sup> , equisetin, moniliformin, nivalenol, and zearalenone

<sup>1</sup> Enniatins: sum of enniatin A, A1, B, B1, B2, and B3.

The response variables were split into two categories, i.e., “below or equal to the median” and “above the median”. Prior to the calculation of the median for the mycotoxins content and the amount of *F. graminearum* DNA, the observations with lower values than the LOD were removed from the dataset. Likewise, for the incidence of FHB causing species in grains, the observations with values equal to zero were removed from the dataset. That way, the influence of agricultural practices was evaluated for samples from fields under favorable conditions for FHB development and mycotoxin accumulation [12]. Moreover, the explanatory variable “plant height at harvest” was split into two categories: “short” and “tall” for values “below or equal to the median” and “above the median”, respectively. Likewise, “grain yield” was split into “low” and “high”. To determine which associations between the explanatory and response variables were statistically significant, a cross tabulation analysis followed by Pearson’s chi-square statistics was performed using pooled data from the harvest years 2016 and 2017. The response and explanatory variables were used as row and column variables, respectively. The observed and expected counts as well as the residuals for each cell category were calculated. The standardized residual was used to determine which variables had the largest difference between the expected and the observed counts relative to sample size. When Pearson’s chi-square test was significant ( $\alpha = 0.05$ ), the Bonferroni method was employed for the post hoc comparisons. The statistical analysis was performed with the program, SPSS® Statistics (Version 24; IBM Corporate, Armonk, NY, USA).

To explore the relationships between the response variables, a two-tailed Spearman’s correlation study was conducted using the entire dataset from both harvest years (2016 and 2017). The strength of correlations was evaluated according to Asuero, et al. [25]. The correlation study focused on the relationships between the incidence of FHB causing species and *Fusarium* mycotoxins as well as between the amount of *F. graminearum* DNA in grains and in straw. The correlations between the detected fungal metabolites in grain and straw matrices were investigated in Drakopoulos, et al. [7]. The correlation study and figures were done with Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

### 3. Results

#### 3.1. Incidence of *Fusarium* Head Blight Causing Species in Grains and Correlations

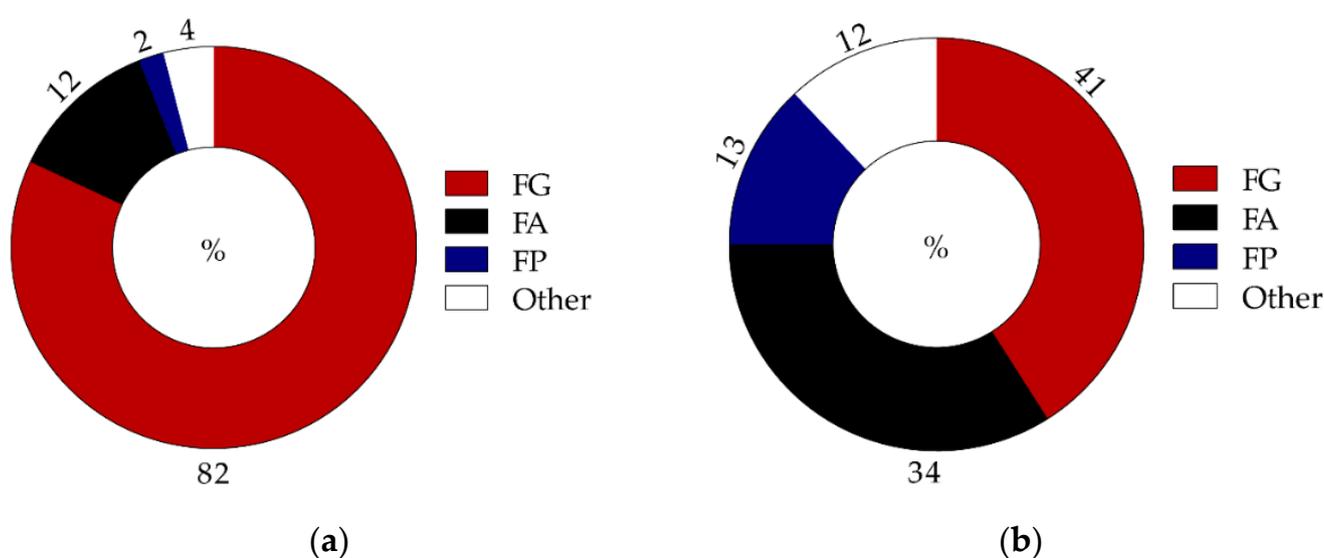
Overall, a higher incidence of FHB causing species in barley grains was observed in 2016 than in 2017 (Table 3). In both years, the predominant *Fusarium* species was *F. graminearum* followed by *F. avenaceum* and *F. poae* (Figure 1). In 2016, species of the non-toxicogenic genus *Microdochium* were present on average in 34% of the grains with 96% being the maximum incidence, whereas in 2017, there was only an average incidence of 2% (Table 3).

The Spearman’s correlation study (Figure 2) for the incidence of *F. graminearum* in grains revealed a strong correlation ( $p < 0.001$ ) with the content of culmorin ( $\rho = 0.750$ ) and deoxynivalenol ( $\rho = 0.711$ ), a moderate correlation ( $p < 0.001$ ) with the content of zearalenone ( $\rho = 0.678$ ), aurofusarin ( $\rho = 0.652$ ), enniatins ( $\rho = 0.590$ ), and equisetin ( $\rho = 0.523$ ) as well as a weak correlation ( $p < 0.001$ ) with moniliformin ( $\rho = 0.478$ ), butenolide ( $\rho = 0.478$ ), and antibiotic Y ( $\rho = 0.349$ ). The incidence of *F. avenaceum* in grains correlated significantly ( $p < 0.001$ ) but weakly with the contents of enniatins ( $\rho = 0.437$ ), moniliformin ( $\rho = 0.375$ ), aurofusarin ( $\rho = 0.371$ ), equisetin ( $\rho = 0.304$ ), and antibiotic Y ( $\rho = 0.296$ ). The incidence of *F. poae* correlated significantly ( $p < 0.001$ ) but weakly with the contents of beauvericin

( $\rho = 0.421$ ), apicidin ( $\rho = 0.321$ ), and nivalenol ( $\rho = 0.304$ ). The amount of *F. graminearum* DNA in grains correlated ( $p < 0.001$ ) with the incidence of *F. graminearum* ( $\rho = 0.752$ ) and the content of culmorin ( $\rho = 0.882$ ), deoxynivalenol ( $\rho = 0.856$ ), zearalenone ( $\rho = 0.756$ ), aurofusarin ( $\rho = 0.630$ ), enniatins ( $\rho = 0.612$ ), moniliformin ( $\rho = 0.511$ ), butenolide ( $\rho = 0.492$ ), and equisetin ( $\rho = 0.442$ ). Furthermore, a strong correlation ( $p < 0.001$ ) was found between the amounts of *F. graminearum* DNA in grains and in straw ( $\rho = 0.717$ ).

**Table 3.** Mean and maximum incidence (%) of Fusarium head blight causing species (*Fusarium graminearum*; *F. avenaceum*; *F. poae*; *Microdochium* spp.) in barley grains from harvest 2016 (n = 123) and harvest 2017 (n = 129).

Fusarium Head Blight Causing Species	2016		2017	
	Mean	Maximum	Mean	Maximum
<i>Fusarium graminearum</i>	15	81	3	31
<i>Fusarium avenaceum</i>	2	15	2	42
<i>Fusarium poae</i>	0.4	8	1	21
<i>Microdochium</i> spp.	34	96	2	22

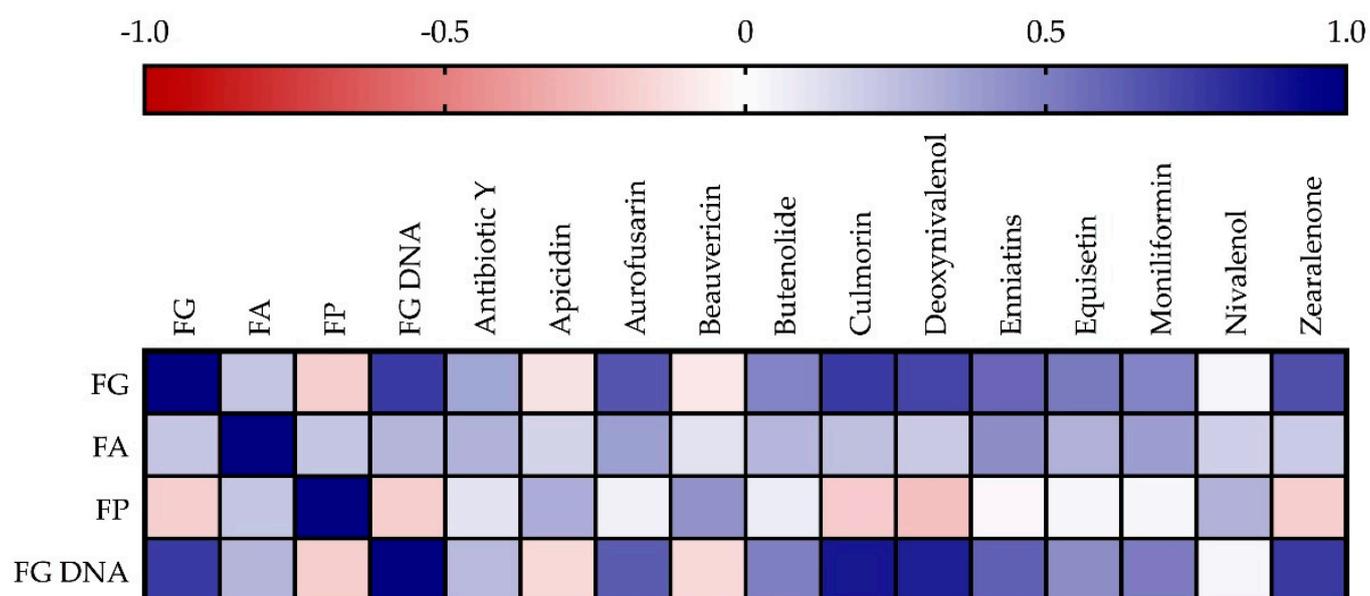


**Figure 1.** Incidence (%) of *Fusarium* species (FG: *Fusarium graminearum*; FA: *F. avenaceum*; FP: *F. poae*; Other: *F. culmorin*, *F. cerealis*, *F. equiseti*, other *Fusarium* spp.) in *Fusarium*-infected barley grains in harvest 2016 (a; n = 123) and harvest 2017 (b; n = 129). Average values across the entire dataset of each harvest year are presented.

### 3.2. Influence of Agricultural Practices on *Fusarium* Head Blight Species Incidence and *Fusarium graminearum* DNA Amount in Grains

The significant associations between the explanatory variables (i.e., agricultural practices and harvest year) and the response variables (i.e., incidence of FHB causing species and amount of *F. graminearum* DNA in grains) are provided in Table 4. The standardized residuals from the cross tabulation analysis are reported in Table 5.

The incidence of *F. graminearum* in grains was significantly associated with tillage and harvest year. An increased frequency of samples with higher incidence of *F. graminearum* (i.e., above the median) was observed under reduced tillage, while the opposite occurred under conventional tillage.



**Figure 2.** Heatmap presenting the Spearman's coefficient ( $\rho$ ) of the correlations between the incidence (%) of the three most prevalent *Fusarium* species (FG: *F. graminearum*; FA: *F. avenaceum*; FP: *F. poae*), the amount of FG DNA (copies of FG DNA per ng total DNA) and *Fusarium* mycotoxins content ( $\mu\text{g kg}^{-1}$ ) in barley grains.

The incidence of *F. avenaceum* in grains was significantly associated with the sowing season. An increased frequency of samples with higher incidence of *F. avenaceum* was observed with spring barley.

The incidence of *F. poae* in grains was significantly associated with production system, sowing season, previous crop, pre-previous crop, tillage, plant height at harvest and fertilizer type. An increased frequency of samples with higher incidence of *F. poae* was observed: in organic production systems, with spring barley, when pasture was the previous and pre-previous crop, under conventional tillage, with shorter barley plants and when manure was applied.

The incidence of *Microdochium* species in grains was significantly associated with sowing season, plant height at harvest, grain yield, and harvest year. An increased frequency of samples with higher incidence of *Microdochium* species was observed: with winter barley, taller plants, lower grain yields, and in 2016.

The amount of *F. graminearum* DNA in grains was significantly associated with sowing season, previous crop, fertilizer type, and harvest year. An increased frequency of samples with higher amount of *F. graminearum* DNA was observed: when maize was the previous crop (Figure 3a) and in 2016. Contrarily, an increased frequency of samples with lower amount of *F. graminearum* DNA (i.e., below or equal to the median) was observed with spring barley and when manure was applied.

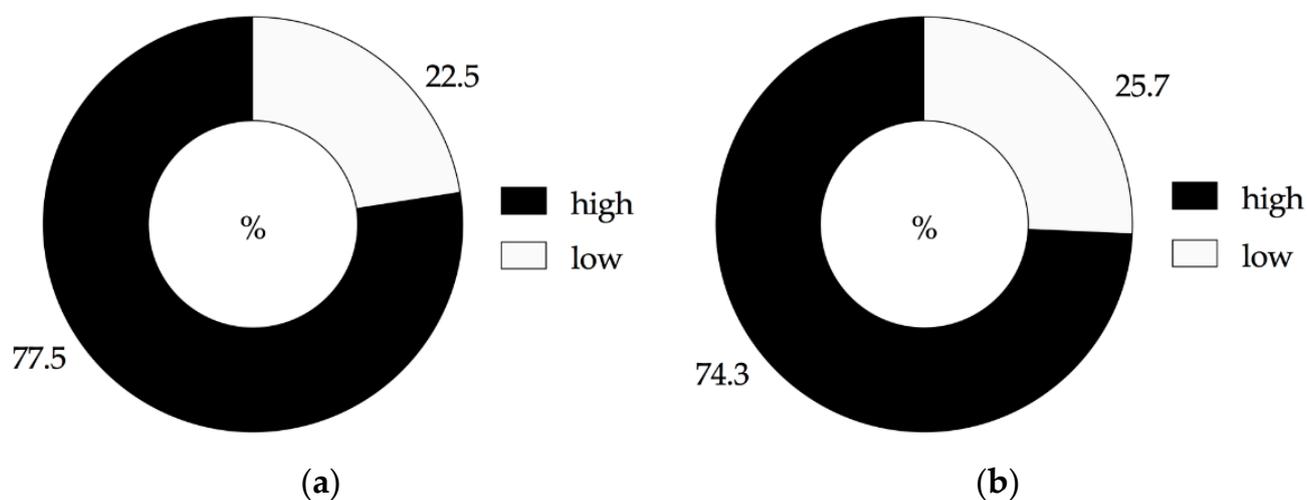
### 3.3. Influence of Agricultural Practices on *Fusarium* mycotoxins Content in Grains

The significant associations between the explanatory variables (i.e., agricultural practices and harvest year) and the response variable (i.e., *Fusarium* mycotoxins in grains) are provided in Table 6. The standardized residuals from the cross tabulation analysis are reported in Table 5.

**Table 4. Grains:** Associations between the explanatory variables (agricultural practices and harvest year) and the response variables (incidence (%) of *Fusarium* head blight causing species and amount of *Fusarium graminearum* (FG) DNA (copies of FG DNA per ng total DNA)) in grains according to Pearson’s chi-square test (\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ) of the cross tabulation analysis. FG: *F. graminearum*; FA: *F. avenaceum*; FP: *F. poae*; M: *Microdochium* spp. n = number of positive samples.

Response Variable	Incidence of FG (n = 207)	Incidence of FA (n = 143)	Incidence of FP (n = 64)	Incidence of M (n = 200)	Amount of FG DNA (n = 227)
<b>Explanatory variable <sup>1</sup></b>					
Production system			*		
Sowing season		*	***	**	*
Barley variety					
Previous crop			*		***
Pre-previous crop			**		
Tillage	**		**		
Plant height at harvest			*	*	
Grain yield				*	
Fungicide type					
Growth regulator application					
Fertilizer type			***		*
Harvest year	***			***	***

<sup>1</sup> The levels of the explanatory variables are provided in Table 1.



**Figure 3.** Observed frequencies (%) of grain (a) and straw (b) samples of barley with “high” or “low” amounts of *Fusarium graminearum* (FG) DNA (copies of FG DNA per ng total DNA) collected from fields where maize (grain or silage) was the previous crop. “High” refers to samples with FG DNA amounts above the median value, while “low” refers to samples with FG DNA amounts below or equal to the median value. The median values of *Fusarium graminearum* DNA amount in grain and straw samples were 5.1 and 26 copies of DNA per ng total DNA, respectively. Average values across the entire dataset from both harvest years (2016 and 2017) are presented.

**Table 5. Grains:** Cross tabulation analysis and Pearson's chi-square test (\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ) to determine the strength of associations between the explanatory variables (agricultural practices and harvest year) and the response variables (incidence (%) of *Fusarium* head blight causing species, amount of *Fusarium graminearum* (FG) DNA (copies of FG DNA per ng total DNA) and *Fusarium* mycotoxins content ( $\mu\text{g kg}^{-1}$ ) in grains). Only significant explanatory variables are reported along with the standardized residuals in brackets (plus sign: increased frequency of samples with values above the median observed than expected; minus sign: increased frequency of samples with values below or equal to the median observed than expected). n = number of positive samples.

<b>Incidence of <i>Fusarium graminearum</i>, n = 207, median: 6%</b>	
Tillage **	conventional tillage (−1.6), reduced tillage (+1.6)
Harvest year ***	2017 (−4.5), 2016 (+3.8)
<b>Incidence of <i>Fusarium avenaceum</i>, n = 143, median: 2%</b>	
Sowing season *	autumn (−0.6), spring (+1.7)
<b>Incidence of <i>Fusarium poae</i>, n = 64, median: 2%</b>	
Production system *	ÖLN (−1.2), extenso (−0.1), organic (+2.0)
Sowing season ***	autumn (−1.8), spring (+3.2)
Previous crop species *	small-grain cereals (−0.9), maize (−0.3), canola (−0.3), other (+0.2), pasture (+2.4)
Pre–previous crop species **	canola (−1.4), other (−1.0), maize (−0.9), small-grain cereals (+0.2), pasture (+2.5)
Tillage **	reduced tillage (−1.7), conventional tillage (+1.3)
Plant height at harvest *	tall (−1.5), short (+1.2)
Fertilizer type ***	mineral + manure (−1.8), mineral (−0.6), manure (+2.4)
<b>Incidence of <i>Microdochium</i> species, n = 200, median: 14.5%</b>	
Sowing season **	spring (−2.2), autumn (+0.6)
Plant height at harvest *	short (−1.0), tall (+1.1)
Grain yield *	high (−1.2), low (+1.1)
Harvest year ***	2017 (−5.9), 2016 (+4.7)
<b>Amount of <i>Fusarium graminearum</i> DNA, n = 227, median: 5.1 DNA copies per ng total DNA</b>	
Sowing season *	spring (−1.4), autumn (+0.3)
Previous crop species ***	other (−1.4), pasture (−0.9), small-grain cereals (−0.7), canola (+0.3), maize (+2.5)
Fertilizer type *	manure (−1.7), mineral + manure (+0.4), mineral (+0.5)
Harvest year ***	2017 (−3.6), 2016 (+3.5)
<b>Enniatins content, n = 253, median: 157 <math>\mu\text{g kg}^{-1}</math></b>	
Production system *	extenso (−1.6), ÖLN (+0.9), organic (+1.1)
Sowing season **	autumn (−0.6), spring (+2.0)
Barley variety *	Semper (−1.9), Cassia (−0.6), Caravan (−0.5), Hobbit (−0.3), other (−0.2), Wootan (−0.2), Meridian (+0.5), Tonic (+0.8), Etincel (+1.0), Eunova (+1.9)
Previous crop species *	other (−0.7), small-grain cereals (−0.5), canola (−0.3), maize (+0.9), pasture (+2.0)
Harvest year ***	2017 (−4.6), 2016 (+4.7)

Table 5. Cont.

<b>Aurofusarin content</b> , n = 200, median: 200 $\mu\text{g kg}^{-1}$	
Sowing season ***	autumn (−0.8), spring (+2.7)
Barley variety *	Semper (−1.6), Hobbit (−0.9), Tonic (−0.4), Etincel (−0.4), Cassia (−0.1), Caravan (+0.3), Wootan (+0.3), Meridian (+0.4), other (+1.0), Eunova (+2.3)
Previous crop species *	other (−0.7), canola (−0.4), small-grain cereals (−0.1), maize (0), pasture (+2.0)
Harvest year ***	2017 (−3.8), 2016 (+3.2)
<b>Deoxynivalenol content</b> , n = 197, median: 105 $\mu\text{g kg}^{-1}$	
Previous crop species **	canola (−1.0), pasture (−0.7), other (−0.6), small-grain cereals (−0.5), maize (+2.3)
Tillage **	conventional tillage (−1.5), reduced tillage (+1.4)
Fungicide type *	triazole (−0.6), triazole + strobilurin (0), strobilurin (+1.5)
Harvest year ***	2017 (−3.0), 2016 (+2.8)
<b>Equisetin content</b> , n = 192, median: 14 $\mu\text{g kg}^{-1}$	
Growth regulator *	yes (−1.2), no (+1.3)
Harvest year ***	2017 (−1.9), 2016 (+1.4)
<b>Moniliformin content</b> , n = 191, median: 9.6 $\mu\text{g kg}^{-1}$	
Sowing season **	autumn (−0.6), spring (+1.9)
Growth regulator *	no (−1.0), yes (+0.9)
Harvest year ***	2017 (−2.3), 2016 (+1.9)
<b>Culmorin content</b> , n = 158, median: 141 $\mu\text{g kg}^{-1}$	
Production system *	organic (−1.6), extenso (−0.9), ÖLN (+1.1)
Sowing season *	spring (−1.7), autumn (+0.3)
Growth regulator *	no (−1.3), yes (+1.0)
Fertilizer type **	manure (−1.9), mineral (−0.3), mineral + manure (+1.2)
Harvest year ***	2017 (−2.9), 2016 (+2.2)
<b>Butenolide content</b> , n = 124, median: 65 $\mu\text{g kg}^{-1}$	
Production system **	extenso (−1.7), ÖLN (+0.9), organic (+1.1)
Sowing season ***	autumn (−1.0), spring (+2.3)
Barley variety ***	Wootan (−1.7), Semper (−1.6), Caravan (−1.4), Etincel (−0.9), Hobbit (−0.4), other (0), Cassia (+0.2), Meridian (+0.3), Tonic (+1.8), Eunova (+2.3)
Growth regulator *	no (−1.2), yes (+1.2)
<b>Antibiotic Y content</b> , n = 98, median: 122 $\mu\text{g kg}^{-1}$	
Sowing season **	autumn (−0.8), spring (+1.8)
Grain yield *	high (−1.2), low (+1.0)
<b>Zearalenone content</b> , n = 95, median: 9.9 $\mu\text{g kg}^{-1}$	
Plant height at harvest *	short (−1.0), tall (+1.0)
Harvest year ***	2017 (−2.2), 2016 (+1.0)

Table 5. Cont.

<b>Beauvericin content</b> , n = 85, median: 0.6 µg kg <sup>-1</sup>	
Production system ***	ÖLN (−2.1), extenso (+1.0), organic (+2.3)
Sowing season ***	autumn (−1.6), spring (+3.1)
Barley variety ***	Tonic (−1.7), Meridian (−1.4), Wootan (−0.7), Hobbit (−0.2), Cassia (0), Etincel (0), Semper (+0.4), other (+1.2), Eunova (+2.4)
Previous crop species *	canola (−1.7), other (−0.2), maize (0), small-grain cereals (+0.1), pasture (+1.5)
Pre-previous crop species ***	other (−1.5), maize (−1.3), canola (−0.2), small-grain cereals (+0.3), pasture (+2.6)
Plant height at harvest ***	tall (−2.0), short (+1.3)
Grain yield **	high (−1.4), low (+1.3)
Fungicide type *	triazole (−1.2), triazole + strobilurin (+0.1), strobilurin (+1.9)
Growth regulator ***	yes (−2.2), no (+2.1)
Fertilizer type ***	mineral + manure (−1.3), mineral (−1.1), manure (+3.0)
<b>Nivalenol content</b> , n = 83, median: 23 µg kg <sup>-1</sup>	
Production system ***	ÖLN (−2.0), extenso (+0.8), organic (+2.1)
Sowing season ***	autumn (−1.5), spring (+3.0)
Barley variety **	Meridian (−1.3), Caravan (−1.0), Tonic (−0.9), Semper (−0.8), Cassia (−0.4), Hobbit (0), Etincel (+0.4), Wootan (+0.4), other (+1.4), Eunova (+2.4)
Pre-previous crop species *	maize (−1.6), small-grain cereals (0), canola (+0.3), other (+0.3), pasture (+1.6)
Plant height at harvest ***	tall (−2.2), short (+1.5)
Growth regulator **	yes (−1.7), no (+1.4)
Fertilizer type ***	mineral + manure (−1.7), mineral (−0.5), manure (+2.5)
<b>Apicidin content</b> , n = 73, median: 6 µg kg <sup>-1</sup>	
Sowing season ***	autumn (−1.0), spring (+2.1)
Barley variety **	Hobbit (−2.1), Etincel (−1.0), Semper (−0.7), Meridian (−0.5), Cassia (−0.2), Wootan (0), Caravan (+0.7), other (+0.7), Tonic (+1.4), Eunova (+1.5)
Previous crop species *	canola (−1.2), other (−1.1), maize (−0.9), small-grain cereals (+0.7), pasture (+1.6)
Fertilizer type *	mineral + manure (−1.0), mineral (−0.3), manure (+1.6)

The content of enniatins (sum of enniatin A, A1, B, B1, B2, and B3) in grains was significantly associated with production system, sowing season, barley variety, previous crop, and harvest year. An increased frequency of samples with higher enniatins content (i.e., above the median) was observed: under ÖLN and organic production systems, with spring barley and the variety Eunova, when maize and pasture were the previous crops and in 2016.

The aurofusarin content in grains was significantly associated with sowing season, barley variety, previous crop, and harvest year. An increased frequency of samples with higher aurofusarin content was observed: with spring barley and the variety Eunova, when pasture was the previous crop and in 2016.

The deoxynivalenol content in grains was significantly associated with previous crop, tillage, fungicide type and harvest year. An increased frequency of samples with higher deoxynivalenol content was observed: when the previous crop was maize, under reduced tillage, when strobilurin-based fungicides were applied and in 2016.

**Table 6. Grains:** Associations between the explanatory variables (agricultural practices and harvest year) and the response variables (*Fusarium* mycotoxins content ( $\mu\text{g kg}^{-1}$ )) in grains according to Pearson's chi-square test (\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ) of the cross tabulation analysis. n = number of positive samples.

Response Variable	Enniatins <sup>2</sup> (n = 253)	Aurofusarin (n = 200)	Deoxynivalenol (n = 197)	Equisetin (n = 192)	Moniliformin (n = 191)	Culmorin (n = 158)	Butenolide (n = 124)	Antibiotic Y (n = 98)	Zearalenone (n = 95)	Beauvericin (n = 85)	Nivalenol (n = 83)	Apicidin (n = 73)
<b>Explanatory variable<sup>1</sup></b>												
Production system	*					*	**			***	***	
Sowing season	**	***			**	*	***	**		***	***	***
Barley variety	*	*					***			***	**	**
Previous crop	*	*	**							*		*
Pre-previous crop										***	*	
Tillage			**									
Plant height at harvest									*	***	***	
Grain yield								*		**		
Fungicide type			*							*		
Growth regulator application				*	*	*	*			***	**	
Fertilizer type						**				***	***	*
Harvest year	***	***	***	***	***	***			***			

<sup>1</sup> The levels of the explanatory variables are provided in Table 1. <sup>2</sup> Enniatins: sum of enniatin A, A1, B, B1, B2, and B3.

The equisetin content in grains was significantly associated with the application of plant growth regulators and harvest year. An increased frequency of samples with higher equisetin content was observed when plant growth regulators were not applied and in 2016.

The moniliformin content in grains was significantly associated with sowing season, the application of plant growth regulators and harvest year. An increased frequency of samples with higher moniliformin content was observed: with spring barley, when plant growth regulator was applied and in 2016.

The culmorin content in grains was significantly associated with production system, sowing season, the application of plant growth regulator, fertilizer type, and harvest year. An increased frequency of samples with higher culmorin content was observed: in ÖLN production system, when plant growth regulators were applied and in 2016. Contrarily, an increased frequency of samples with lower culmorin content was observed with spring barley and when manure was applied.

The butenolide content in grains was significantly associated with production system, sowing season, barley variety and the application of plant growth regulators. An increased frequency of samples with higher butenolide content was observed: in ÖLN and organic production systems, with spring barley and the varieties Tonic and Eunova, and when plant growth regulators were applied.

The content of antibiotic Y in grains was significantly associated with sowing season and grain yield. An increased frequency of samples with higher antibiotic Y content was observed with spring barley and lower grain yields.

The zearalenone content in grains was significantly associated with plant height at harvest and harvest year. An increased frequency of samples with higher zearalenone content was observed with taller barley plants and in 2016.

The beauvericin content in grains was significantly associated with all the examined explanatory variables except for harvest year and tillage. An increased frequency of samples with higher beauvericin content was observed: in extenso and organic production systems, with spring barley and the variety Eunova, when pasture was the previous and the pre-previous crop, with shorter barley plants and lower grain yields, when plant growth regulator was not applied, with the use of strobilurin-based fungicides and when manure was applied.

The nivalenol content in grains was significantly associated with production system, sowing season, barley variety, pre-previous crop, plant height at harvest, the application of plant growth regulators and fertilizer type. An increased frequency of samples with higher nivalenol content was observed: in extenso and organic production systems, with spring barley and the variety Eunova, when the pre-previous crop was pasture, with shorter barley plants, when plant growth regulator was not applied and when manure was applied.

The apicidin content in grains was significantly associated with sowing season, barley variety, previous crop and fertilizer type. An increased frequency of samples with higher apicidin content was observed: with spring barley, with the varieties Tonic and Eunova, when pasture was the previous crop and when manure was applied.

#### 3.4. Influence of Agricultural Practices on *Fusarium graminearum* DNA Amount and *Fusarium* mycotoxins Content in Straw

The significant associations between the explanatory variables (i.e., agricultural practices and harvest year) and the response variables (i.e., *F. graminearum* DNA amount and *Fusarium* mycotoxins in straw) are provided in Table 7. The standardized residuals from the cross tabulation analysis are reported in Table 8.

**Table 7. Straw:** Associations between the explanatory variables (agricultural practices and harvest year) and the response variables (amount of *Fusarium graminearum* (FG) DNA (copies of FG DNA per ng total DNA) and *Fusarium* mycotoxins content ( $\mu\text{g kg}^{-1}$ )) in straw according to Pearson's chi-square test (\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ) of the cross tabulation analysis. n = number of positive samples.

Response Variable	Amount of FG DNA (n = 207)	Enniatins <sup>2</sup> (n = 237)	Beauvericin (n = 187)	Deoxynivalenol (n = 163)	Equisetin (n = 161)	Moniliformin (n = 154)	Aurofusarin (n = 146)	Culmorin (n = 141)	Apicidin (n = 131)	Antibiotic Y (n = 77)	Nivalenol (n = 68)	Zearalenone (n = 53)	Butenolide (n = 23)
<b>Explanatory variable<sup>1</sup></b>													
Production system			*	***	***			*					
Sowing season	**	**	***	*	***	***							
Barley variety													
Previous crop	***					*							
Pre-previous crop			*		**			*					
Tillage				*									
Plant height at harvest	*			*									
Fungicide type													
Growth regulator application				***	***								
Fertilizer type	**		***	**	***			***					
Harvest year	***	***	***				*	*	***		**		

<sup>1</sup> The levels of the explanatory variables are provided in Table 1. <sup>2</sup> Enniatins: sum of enniatin A, A1, B, B1, B2 and B3.

**Table 8. Straw:** Cross tabulation analysis and Pearson's chi-square test (\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ) to determine the strength of associations between the explanatory variables (agricultural practices and harvest year) and the response variables (amount of *Fusarium graminearum* (FG) DNA (copies of FG DNA per ng total DNA) and *Fusarium* mycotoxins content ( $\mu\text{g kg}^{-1}$ ) in straw). Only significant explanatory variables are reported along with the standardized residuals in brackets (plus sign: increased frequency of samples with values above the median observed than expected; minus sign: increased frequency of samples with values below or equal to the median observed than expected). n = number of positive samples.

<b>Amount of <i>Fusarium graminearum</i> DNA, n = 207, median: 26 DNA copies per ng total DNA</b>	
Sowing season **	spring (−1.8), autumn (+0.4)
Previous crop species ***	other (−1.7), pasture (−1.1), small-grain cereals (−0.5), canola (+0.6), maize (+2.1)
Plant height at harvest *	short (−1.0), tall (+1.1)
Fertilizer type **	manure (−1.6), mineral (−0.5), mineral + manure (+1.5)
Harvest year ***	2017 (−5.1), 2016 (+4.7)
<b>Enniatins content, n = 237, median: 148 <math>\mu\text{g kg}^{-1}</math></b>	
Sowing season **	autumn (−0.6), spring (+2.1)
Harvest year ***	2017 (−2.5), 2016 (+2.6)
<b>Beauvericin content, n = 187, median: 2.3 <math>\mu\text{g kg}^{-1}</math></b>	
Production system *	extenso (−0.7), ÖLN (−0.2), organic (+1.8)
Sowing season ***	autumn (−0.9), spring (+2.9)
Pre-previous crop species *	canola (−1.1), other (−0.7), small-grain cereals (−0.6), maize (+0.1), pasture (+2.0)
Fertilizer type ***	mineral (−1.3), mineral + manure (−0.2), manure (+2.8)
Harvest year ***	2016 (−4.0), 2017 (+3.7)
<b>Deoxynivalenol content, n = 163, median: 188 <math>\mu\text{g kg}^{-1}</math></b>	
Production system ***	extenso (−1.7), organic (−1.6), ÖLN (+1.8)
Sowing season *	spring (−1.5), autumn (+0.3)
Tillage *	conventional tillage (−1.0), reduced tillage (+1.0)
Plant height at harvest *	short (−1.1), tall (+1.1)
Growth regulator ***	no (−2.0), yes (+1.8)
Fertilizer type **	manure (−2.1), mineral (+0.1), mineral + manure (+1.0)
<b>Equisetin content, n = 161, median: 29 <math>\mu\text{g kg}^{-1}</math></b>	
Production system ***	ÖLN (−2.5), extenso (+1.1), organic (+2.7)
Sowing season ***	autumn(−0.7), spring (+2.1)
Pre-previous crop species **	other (−1.1), canola (−0.7), maize (−0.6), small-grain cereals (0), pasture (+2.3)
Growth regulator ***	yes (−2.7), no (+2.2)
Fertilizer type ***	mineral (−1.5), mineral + manure (−0.3), manure (+3.0)
<b>Moniliformin content, n = 154, median: 10 <math>\mu\text{g kg}^{-1}</math></b>	
Sowing season ***	autumn (−1.1), spring (+3.3)
Previous crop species *	canola (−0.9), small-grain cereals (−0.4), other (−0.2), maize (+0.1), pasture (+2.5)
<b>Aurofusarin content, n = 146, median: 232 <math>\mu\text{g kg}^{-1}</math></b>	
Harvest year *	2017 (−1.3), 2016 (+1.1)
<b>Culmorin content, n = 141, median: 575 <math>\mu\text{g kg}^{-1}</math></b>	

Table 8. Cont.

Production system *	organic (−1.9), extenso (0), ÖLN (+0.5)
Pre-previous crop species *	other (−1.6), small-grain cereals (−1.1), maize (+0.3), pasture (+0.3), canola (+1.6)
Fertilizer type ***	manure (−2.2), mineral + manure (−0.5), mineral (+1.3)
Harvest year *	2017 (−1.4), 2016 (+1.1)
<b>Apicidin content</b> , n = 131, median: 24 µg kg <sup>−1</sup>	
Harvest year ***	2016 (−1.7), 2017 (+1.6)
<b>Antibiotic Y content</b> , n = 77, median: 199 µg kg <sup>−1</sup>	
No significant associations with explanatory variables	
<b>Nivalenol content</b> , n = 68, median: 109 µg kg <sup>−1</sup>	
Harvest year**	2017 (−1.1), 2016 (+1.8)
<b>Zearalenone content</b> , n = 53, median: 20 µg kg <sup>−1</sup>	
No significant associations with explanatory variables	
<b>Butenolide content</b> , n = 23, median: 264 µg kg <sup>−1</sup>	
No significant associations with explanatory variables	

The amount of *F. graminearum* DNA in straw was significantly associated with sowing season, previous crop, plant height at harvest, fertilizer type, and harvest year. An increased frequency of samples with higher amount of *F. graminearum* DNA (i.e., above the median) was observed: when the previous crop was maize (Figure 3b), with taller barley plants and in 2016. In contrast, an increased frequency of samples with lower amount of *F. graminearum* DNA (below or equal to the median) was observed with spring barley and when manure was applied.

The content of enniatins (sum of enniatin A, A1, B, B1, B2, and B3) in straw was significantly associated with sowing season and harvest year. An increased frequency of samples with higher content of enniatins was observed with spring barley and in 2016.

The beauvericin content in straw was significantly associated with production system, sowing season, pre-previous crop, fertilizer type and harvest year. An increased frequency of samples with higher beauvericin content was observed: in organic production system, with spring barley, with pasture as pre-previous crop, when manure was applied and in 2017.

The deoxynivalenol content in straw was significantly associated with the production system, sowing season, tillage, plant height at harvest, plant growth regulator application and fertilizer type. An increased frequency of samples with higher deoxynivalenol content was observed: in ÖLN, under reduced tillage, with taller plants, and when plant growth regulators were applied. In contrast, an increased frequency of samples with lower deoxynivalenol content was observed with spring barley and when manure was applied.

The equisetin content in straw was significantly associated with production system, sowing season, pre-previous crop, the application of plant growth regulator and fertilizer type. An increased frequency of samples with higher equisetin content was observed: in extenso and organic production systems, with spring barley, when pasture was the pre-previous crop, when plant growth regulators were not applied and when manure was applied.

The moniliformin content in straw was significantly associated with sowing season and previous crop. An increased frequency of samples with higher moniliformin content was observed with spring barley and when pasture was the previous crop.

The aurofusarin content in straw was significantly associated with harvest year. An increased frequency of samples with higher aurofusarin content was observed than expected in 2016.

The culmorin content in straw was significantly associated with production system, pre-previous crop, fertilizer type, and harvest year. An increased frequency of samples with higher culmorin content was observed when the pre-previous crop was canola and in 2016. In contrast, an increased frequency of samples with lower culmorin content was observed in organic production system and when manure was applied.

The apicidin content in straw was significantly associated with harvest year. An increased frequency of samples with high apicidin content was observed in 2017.

The nivalenol content in straw was significantly associated with the harvest year. An increased frequency of samples with higher nivalenol content was observed in 2016.

No significant associations were observed between the explanatory variables and the contents of antibiotic Y, zearalenone and butenolide in straw.

## 4. Discussion

### 4.1. Incidence of *Fusarium* Head Blight Causing Species in Grains and Correlations with Mycotoxins

Although several *Fusarium* species can cause FHB in small-grain cereals, *F. graminearum* is the predominant species across Switzerland and in most parts of the world as shown in nationwide multiyear surveys in barley [10] and wheat [12] as well as in global epidemiological studies [26]. For example, Schöneberg, et al. [10] found that, on average in harvests of 2013 and 2014, *F. graminearum* was isolated from 58% *Fusarium*-infected barley grains followed by *F. avenaceum* (30%) and *F. poae* (7%). These findings are in line with the results of the current study where *F. graminearum* was the predominant species followed by *F. avenaceum* and *F. poae* in both 2016 and 2017. In oats, however, *F. poae* was by far the predominant *Fusarium* species in a Swiss survey between 2013 and 2015 [27], indicating that the FHB species complex is greatly dependent on host plant characteristics. Despite the fact that fungal species belonging to the *Microdochium* genus are non-toxigenic, they are able to cause FHB in small-grain cereals resulting in yield losses [28]. In our study, *Microdochium* species were isolated on average from 34% of grains in 2016, indicating a high presence in Swiss barley cropping systems.

As it was expected and has already been shown in previous studies [29–31], we found positive correlations between the incidence of *F. graminearum* in grains and several *Fusarium* mycotoxins, e.g., deoxynivalenol, culmorin, zearalenone, and aurofusarin. However, the observed positive correlations between the incidence of *F. graminearum* and the contents of equisetin and moniliformin in grains could be due to possible microscopic misclassifications in the seed health tests. Specifically, *F. incarnatum* (syn. *F. semitectum*) can be misclassified as *F. graminearum* due to similarities in the shape and size of macroconidia. The former species has been isolated from barley grains in the past [32] and is able to produce equisetin and moniliformin [21]. In addition, we cannot exclude the possibility that *F. graminearum* and *F. avenaceum* (prominent producer of moniliformin) share a similar ecological niche in cereal-based cropping systems.

### 4.2. Influence of Agricultural Practices Performed before and during Barley Production

#### 4.2.1. Before Barley Production

Agricultural practices prior to crop production, such as crop rotation and tillage, can greatly affect the severity of FHB and subsequent contamination with *Fusarium* mycotoxins in small-grain cereals [33].

The implementation of crop rotations with a high frequency of non-host plant species is considered one of the most effective strategies to prevent FHB epidemics in cereals [34,35]. Several studies have shown that growing maize increases the risk of FHB and mycotoxin contamination in subsequent wheat and barley crops compared with other previous crops. For example, Dill-Macky and Jones [36] found that the level of deoxynivalenol in wheat following maize was 25% higher than in wheat following wheat and 50% higher than in wheat following soybean. Another study on *Fusarium* mycotoxins in Switzerland also showed that growing maize prior to barley resulted in higher *F. graminearum* incidence

and deoxynivalenol content in barley grains compared with other preceding crops, such as canola and small-grain cereals [10]. Likewise, we showed that the previous crop species was associated with the amount of *F. graminearum* DNA in both barley grains and straw as well as with the content of five *Fusarium* mycotoxins in grain and one in straw samples. In particular, growing maize before barley was associated with increased amount of *F. graminearum* DNA in grains and straw as well as with elevated concentrations of deoxynivalenol and to a lesser extent of enniatins in grains. Interestingly, when pasture was cultivated prior to barley, an increased frequency of grain samples with elevated concentrations of enniatins, aurofusarin, beauvericin and apicidin was observed. Moreover, pasture as pre-previous crop was associated with increased incidence of *F. poae* and elevated concentrations of beauvericin and nivalenol in grains and beauvericin and equisetin in straw. These findings support the epidemiological theory that agricultural practices targeting individual *Fusarium* species might create vacancies on ecological niches that could be filled by other FHB causing species within the disease complex [12,13]. Nichea, et al. [37] studied the presence of multiple mycotoxins in natural grasses intended for grazing cattle and detected several unregulated toxins in the samples, including beauvericin, equisetin, aurofusarin, and enniatin B. Twaruzek, et al. [38] analyzed samples from 26 plant species (e.g., Poaceae, Fabaceae, and Plantaginaceae families) in pastures and demonstrated that plant material was contaminated by toxigenic species from several fungal genera, e.g., *Alternaria* and *Fusarium*. Thus, a judicious selection of crop species in a crop rotation should also account for potential phytopathological risks caused by emerging *Fusarium* species and other mycotoxigenic fungi, which are producing secondary metabolites with undetermined toxicity.

The tillage practice prior to cereal production for weed control, seedbed preparation and management of crop residues is another crucial factor influencing FHB development and contamination with *Fusarium* mycotoxins. Reduced tillage leads to increased amounts of crop residues on the soil surface, resulting in elevated *F. graminearum* inoculum levels and subsequently high risks of mycotoxin contamination in cereals [33]. Steinkellner and Langer [39] investigated the influence of long-term conventional and conservation tillage treatments on the incidence and diversity of *Fusarium* species. The authors reported that conventional tillage (moldboard plough) resulted in lower diversity of *Fusarium* species than reduced tillage (chisel plough or rotary tiller). We demonstrated that, in the short-term, tillage practice was associated with the incidence of *F. graminearum* and *F. poae* in grains as well as with the deoxynivalenol content in grains and straw. In particular, reduced tillage was linked to increased incidence of *F. graminearum* and deoxynivalenol content in grains and straw, while the opposite was found with the use of conventional tillage. Moreover, use of conventional tillage was associated with a higher incidence of *F. poae* in grains, which is in line with the findings of another study in wheat where higher *F. poae* occurrence was closely linked to samples from ploughed fields [12]. From all analyzed *Fusarium* mycotoxins, tillage was only associated with deoxynivalenol, indicating a strong association of this agronomic practice with the presence of *F. graminearum*, which is one of the main producers of this toxin [40].

#### 4.2.2. During Barley Production

Agricultural practices during crop production have a great impact on FHB and mycotoxin accumulation in small-grain cereals [41,42]. We found strong associations between several explanatory variables (e.g., plant variety, application of plant growth regulators, and fungicide type) and *Fusarium* response variables on barley.

In Switzerland, a list of recommended crop varieties with resistance against FHB exists for wheat but not for barley. Therefore, barley varieties could not be grouped according to their resistance level against FHB. Nevertheless, we found strong associations between barley variety and six *Fusarium* mycotoxins in grains. In particular, the spring variety Eunova was linked to increased concentrations of enniatins, aurofusarin, butenolide, beauvericin, nivalenol, and apicidin in grains. In parallel, we observed that the sowing

season (winter versus spring barley) was linked to several FHB variables. More specifically, spring barley was linked to increased concentrations of eight *Fusarium* mycotoxins in grains (i.e., enniatins, aurofusarin, moniliformin, butenolide, antibiotic Y, beauvericin, nivalenol, and apicidin) and four in straw (i.e., enniatins, beauvericin, equisetin, and moniliformin). Moreover, spring barley was associated with increased incidence of *F. avenaceum* and *F. poae* in grains. Here, it is important to point out that the majority of spring barley fields were ploughed prior to crop production, which could explain the increased incidence of *F. poae*. On the other hand, spring barley was linked to lower incidence of *Microdochium* species in grains and decreased amount of *F. graminearum* in grains and straw. Schöneberg, et al. [10] showed that the use of winter barley resulted in higher incidence of *F. graminearum* and deoxynivalenol content in grains compared with spring barley. Linkmeyer, et al. [43] found that *F. graminearum* had a dominant role in the FHB complex on winter barley, while on spring barley, *F. graminearum*, *F. culmorum*, and *F. langsethiae* were regularly present. The authors suggested that the incidence of FHB causing species might be influenced by the flowering period of barley, which occurs earlier for winter varieties, and the prevailing weather conditions.

Previous studies have shown that triazole-based fungicides can be effective against type B trichothecene-producing *Fusarium* species (e.g., *F. graminearum* and *F. culmorum*) leading to reduced deoxynivalenol contamination and increased grain yield in wheat systems [44,45]. However, studies on fungicide efficacy for reducing FHB and mycotoxins in barley are scarce and the findings vary. For example, Cowger, et al. [46] found modest efficacy of a triazole-based fungicide (prothioconazole and tebuconazole) against *F. graminearum* in barley, which could decrease deoxynivalenol up to 75% when combined with a moderately resistant variety. Furthermore, Schöneberg, et al. [10] reported that the combination of fungicides belonging to the group of triazoles and strobilurins led to the highest mean *F. graminearum* incidence and deoxynivalenol content in barley grains. In the current study, we showed that application of strobilurin-based fungicides was associated with increased beauvericin and deoxynivalenol concentrations in grains. The application of strobilurin-based fungicides is able to control *Microdochium nivale* [47], but often not the deoxynivalenol-producing *Fusarium* species. In fact, several studies on wheat have demonstrated that strobilurins even increase the amount of deoxynivalenol in grains [48–50].

The effect of plant height on FHB and accumulation of *Fusarium* mycotoxins in grains has been partially elucidated in the past [34]. When the fungal inoculum is splash-dispersed from the soil or stem base [51], shorter varieties are at higher risk of infection. Mesterházy [52] studied the types and components of resistance to FHB, caused by *F. graminearum* and *F. culmorum*, in wheat and reported that dwarf genotypes were more severely infected than taller genotypes under natural conditions. In our study, use of shorter plants correlated with increased incidence of *F. poae*, whose life cycle has not been elucidated yet, and its associated mycotoxins (i.e., beauvericin and nivalenol) in grains. Moreover, the application of plant growth regulators was associated with increased concentrations of moniliformin, culmorin and butenolide in grains and deoxynivalenol in straw. On the other hand, absence of growth regulators application was linked to increased concentrations of equisetin, beauvericin and nivalenol in grains and equisetin in straw. Fauzi and Paulitz [53] suggested that plant growth regulators do not change the inherent susceptibility of wheat heads to *F. graminearum*, but shortened plants may be subject to increased inoculum loads since they are closer to the soil surface and crop residues.

The production system was associated with the incidence of *F. poae* in grains as well as with five *Fusarium* mycotoxins in grains and four in straw. Specifically, ÖLN was linked to increased contents of enniatins, culmorin, and butenolide in grains as well as deoxynivalenol in straw. Moreover, we found that the organic production system was linked to increased incidence of *F. poae* in grains and elevated concentrations of enniatins, butenolide, beauvericin, and nivalenol in grains as well as beauvericin and equisetin in straw. Nevertheless, in our study, no clear differences between the production systems

*per se* were observed in terms of overall grain contamination with *Fusarium* mycotoxins, because several agricultural practices (e.g., previous crop and tillage regime) that are components of all these systems have differential effects on the FHB species complex as shown above. For example, organic growers are mainly using moldboard plough to control weeds and bury crop residues, therefore reducing the inoculum potential of FHB species that overwinter on crop residues (e.g., *F. graminearum*) and the associated mycotoxins (e.g., deoxynivalenol) in grains. On the other hand, we showed that *F. poae* was strongly linked to ploughed fields. Thus, a particular production system that reduces the risk of *F. graminearum* and deoxynivalenol might not be suitable when considering multiple FHB causing species with different life cycles and ecological niches.

Cereal growers produce their crops with the necessary nutrients by applying mineral fertilizers and/or organic amendments, such as manure. We showed that manure application was linked to increased incidence of *F. poae* and elevated concentrations of beauvericin, nivalenol and apicidin in grains as well as beauvericin and equisetin in straw. On the other hand, when manure was applied, an increased frequency of samples with decreased amount of *F. graminearum* DNA in grains and straw as well as lower concentrations of culmorin and deoxynivalenol was found. Organic growers are not allowed to use mineral fertilizers, and therefore usually apply manure. Additionally, organic growers are usually applying conventional tillage, and hence these findings are in line with the effects of the above explanatory variables (e.g., tillage regime).

In wheat crops, yield loss is frequently associated with *F. graminearum* infection in grains [26,54]. However, in barley, we found that yield loss was only related to increased incidence of *Microdochium* species in grains, pointing out the importance of this fungal species in the disease complex despite the fact that it is non-toxicogenic.

The severity of FHB epidemics and mycotoxin contamination may be subject to large seasonal variations due to the prevailing climatic conditions [33,34]. Thus, as expected, we found that the harvest year had a strong effect on seven *Fusarium* mycotoxins in grains and six in straw as well as on the incidence of *F. graminearum* and *Microdochium* spp. in grains.

## 5. Conclusions

In the current study, we investigated whether certain agricultural practices before or during barley production have an impact on the incidence of FHB causing species in grains and the mycotoxin accumulation in grains and straw of barley. It is evident that selecting individual agricultural practices can suppress the development of some *Fusarium* species and reduce the associated mycotoxins in barley systems, but can promote others. This might be a consequence of opening ecological niches for otherwise less prevalent toxigenic species with diverse life cycles. Thus, integrated control strategies that combine several prevention (e.g., crop rotation, tillage, less susceptible barley varieties) and intervention (e.g., rational use of effective plant protection products) measures are needed to sustainably manage mycotoxins in barley. Further research should focus on elucidating the life cycle of neglected *Fusarium* species and the triggers for production of their associated mycotoxins. Additionally, monitoring the dynamics of FHB causing species under changing climate and varying crop rotations is crucial in order to choose the most suitable strategy to reduce the risk of health threatening mycotoxins.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11040801/s1>, Table S1: Total number of grain and straw samples received per canton of Switzerland in 2016 and 2017. Table S2: Limit of detection (LOD,  $\mu\text{g kg}^{-1}$ ) and number of positive samples ( $\geq\text{LOD}$ ) for each analyzed *Fusarium* mycotoxin in grain (n = 253) and straw (n = 237) samples of barley.

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