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Raised concerns about the safety of barley grains and straw: A Swiss survey reveals a high diversity of mycotoxins and other fungal metabolites

Dimitrios Drakopoulos [a], Michael Sulyok [b], Rudolf Krska [b,c], Antonio F. Logrieco [d], Susanne Vogelgsang [a,*]

[a] Ecological Plant Protection in Arable Crops, Plant Protection, Agroscope, Reckenholzstrasse 191, 8046, Zurich, Switzerland
[b] 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
[c] Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom
[d] Institute of Sciences of Food Production, National Research Council, CNR, Via Amendola 122/O, 70126, Bari, Italy

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ABSTRACT

Barley can be contaminated with a wide range of fungal secondary metabolites, including various mycotoxins that reduce the quality and safety of raw materials as well as cause economic losses. A survey was conducted for the crop seasons 2016 and 2017 to analyse fungal metabolites, including mycotoxins, in grain and straw samples of barley, which originated from fields across Switzerland. In total, 253 grain and 237 straw samples were analysed by LC-MS/MS detecting 87 and 86 fungal metabolites, respectively, which are reported to be produced by *Fusarium*, *Alternaria*, *Claviceps*, *Aspergillus*, *Penicillium* and other genera. None of the grain samples exceeded the permitted limits of mycotoxins set by the European Commission. With regard to straw, three and six samples exceeded the guidance levels set for raw grains for deoxynivalenol and the sum of T-2 and HT-2, respectively. Nevertheless, some samples contained high concentrations of unregulated fungal metabolites, e.g. enniatins, infectopyron, zinniol and rubellin D. This was more frequently observed in straw and, to a lesser extent, in grains, suggesting that the presence of fungal metabolites in straw material should not be neglected. Our study demonstrated that both grain and straw matrices of barley represent large pools of various fungal secondary metabolites, most of them with undetermined toxicity. Hence, future studies should focus on the toxicology of the predominant fungal metabolites that occurred at elevated concentrations as well as the health impact of co-occurrence of toxins primarily with metabolites that revealed strong correlations.

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the most commonly cultivated cereal crops worldwide. In 2018, barley took the fourth position in the global list of cereals with a harvested area of 48 million hectares and a production quantity of 140 million metric tonnes (FAOSTAT, 2020). Barley grains are the main collected material which is utilised for animal feed, brewing, human food and seed production. In addition, barley growers frequently use the straw after grain harvest as bedding material in barns or feed for animals. Numerous fungal organisms are able to infect barley plants during crop cultivation and/or spoil the harvested material during storage. Some of these filamentous fungi, belonging to different genera (e.g. *Fusarium*, *Alternaria*, *Aspergillus* and *Claviceps*), are known to produce hazardous secondary metabolites, called mycotoxins, which not only jeopardise food and feed safety, but also cause economic losses across the entire supply chain (Claeys et al., 2020; Pitt et al., 2012; Pereira, Cunha, & Fernandes, 2019).

To ensure the safety of food and feed products, legal authorities worldwide have regulated several mycotoxins by providing maximum limits and guidance levels depending on the material (e.g. type of cereal, processed or unprocessed products) and the target group (e.g. human or animal consumption). The European Commission (EC) Regulation No 1881/2006 of December 19, 2006 and its amendments set maximum limits for certain mycotoxins in foodstuffs (Anonymous, 2006). Up to date, the maximum levels (in μg kg^{-1}) of the regulated mycotoxins in barley foodstuffs are the following: aflatoxins (B_1: 0.10–2; sum of B_1, B_2, G_1 and G_2: 4), ochratoxin A (unprocessed: 5; processed: 0.5–3), deoxynivalenol (unprocessed: 1,250; processed: 200–750) and

* Corresponding author.
E-mail address: susanne.vogelgsang@agroscope.admin.ch (S. Vogelgsang).

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zearalenone (unprocessed: 100; processed: 20–75). Furthermore, guidance levels (in μg kg^{-1}) have been established for the sum of T-2 and HT-2 toxins regarding barley food (unprocessed including malting barley: 200; processed: 15–100) (Anonymous, 2013). For barley feedstuffs, only guidance levels are provided for certain mycotoxins (e.g. deoxynivalenol and zearalenone at 8,000 and 2,000 μg kg^{-1}, respectively, and the sum of T-2 and HT-2 toxins at 500 μg kg^{-1}). Nevertheless, there is a multitude of other fungal metabolites present in the barley matrix, which can be potentially toxic (Streit et al., 2012). Although several of these metabolites are unregulated and not routinely determined, they frequently occur in agricultural products and have been defined as "emerging mycotoxins" posing a potentially significant threat to food and feed safety (Gruber-Dorninger, Novak, Nagl, & Berthiller, 2017).

Regarding *Fusarium* mycotoxins, deoxynivalenol and zearalenone are two of the most studied metabolites due to their adverse health effects and their frequent occurrence in cereal products (EFSA, 2011, 2013). Deoxynivalenol causes feed refusal, immunosuppression and inhibition of protein synthesis, while zearalenone is mainly associated with estrogenic activity causing reproductive problems in domestic animals (Kuiper-Goodman, Scott, & Watanabe, 1987; Prelusky, Rotter, & Rotter, 1994). Moreover, other *Fusarium* mycotoxins, e.g. beauvericin, butenolide, enniatins and moniliformin, are frequently detected in food and feed products as well (Gruber-Dorninger et al., 2017; Jestoi, 2008). However, mycotoxins from other fungal genera are also of concern. For example, fungi of the genus *Claviceps* produce ergot alkaloids in small-grain cereals including barley. The European Food Safety Authority has recommended to continue collecting analytical data on ergot alkaloids in food and feed commodities due to their potent toxicity (Arcella, Gómez Ruiz, Innocenti, & Roldán, 2017). *Alternaria* mycotoxins, e.g. alternariol and tenuazonic acid, interfere with protein biosynthesis and have shown remarkable cytotoxicity in microbial and mammalian cell cultures (Escrivá, Oueslati, Font, & Manyes, 2017). Moreover, mycotoxins and other fungal metabolites produced by *Penicillium* (e.g. deoxynortryptoquivalin), *Aspergillus* (e.g. gliotoxin) and other genera (e.g. *Ramularia,* producing rubellin D) can be found in barley products jeopardising the safety of food and feed.

The presence of fungal metabolites in straw material has been studied less extensively compared with barley grains (Häggblom & Nordkvist, 2015; Mol, Rijk, Egmond, & Jong, 2014). Nevertheless, animals can consume considerable amounts of barley straw when it is used as bedding material, e.g. up to 14% of the total diet in pigs (Van Barneveld, Edwards, & Choct, 2003). Moreover, Rohweder et al. (2013) investigated the matrix effects of different plant parts of wheat on the bioavailability of deoxynivalenol. The authors found that the bioavailability of deoxynivalenol amounted to 82, 87 and 110% for straw, grain and chaff, respectively, without significant differences between each other. They concluded that the uptake of deoxynivalenol from straw might equally contribute to the overall exposure of animals.

Hence, besides the regulated mycotoxins, a wide range of other fungal metabolites can be expected in the grain and straw matrices of barley, which should not be ignored. Furthermore, it has not been elucidated yet how these metabolites correlate with each other in grains and straw of barley. To fill these knowledge gaps, we carried out a survey on fungal metabolites in grain and straw samples of barley from fields across Switzerland for the crop seasons 2016 and 2017. The main objectives of this study were, first, to elucidate the mycotoxin accumulation and potential exposure by quantifying a broad range of metabolites from different fungal genera and, second, to investigate the correlations between frequently detected metabolites as well as between grain and straw material of these metabolites.

2. Materials and methods

2.1. Instruction letters, sample origin and sample size

Instruction letters on the sampling procedure, as described in Schöneberg et al. (2016), were sent to barley growers in 2016 and 2017. In brief, samples of approximately 1,000 g for grains and 150 g for straw were collected directly after the harvest of each field by mixing ten subsamples into one composite sample in plastic bags with vent holes. The samples were subsequently sent to Agroscope (Swiss centre of excellence for agricultural research), Zurich-Reckenholz, Switzerland.

The contact details of barley growers in Switzerland were obtained from the cantonal plant protection offices. Grain and straw samples from 18 Swiss cantons were received and the exact number of samples per canton is provided in Supplementary Table 1. For barley grains, 253 samples (2016: 123; 2017: 130) were received and analysed, from which 218 were produced for animal feed, 20 for human food, nine for seed and six for malting. Regarding barley straw, 237 samples (2016: 115; 2017: 122) were received and analysed, from which 205 were collected from crops that were produced for animal feed, 17 for human food, nine for seed and six for malting.

2.2. Preparation of subsamples

Representative grain subsamples of 150 g each were prepared using a riffle divider (Schieritz & Hauenstein AG, Switzerland) and ground with a sample mill (Cyclotec™ 1093; Foss Tecator, Sweden; 1 mm mesh size). For straw, the material was first cut to approximately 5 cm pieces with a chopper device (Wintersteiger Hege 44, Austria) and then ground with a sample mill (Retsch SM100; Retsch GmbH, Germany; 1 mm mesh size). Afterwards, the ground samples were stored at −20 °C until further processing.

2.3. Analysis of fungal metabolites

Extraction was performed 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 grains and 40 mL per 2.5 g for straw. Raw extracts were diluted 1 + 1 using acetonitrile/water/acetic acid (20/79/1) and 5 μl of the diluted extracts were injected into a LC-MS/MS device without any further pretreatment.

The detection and quantification of fungal metabolites were performed as described in Sulyok, Stadler, Steiner, and Krska (2020) with a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source and a 1290 Series UHPLC System (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150 × 4.6 mm i. d., 5 μm particle size, equipped with a C18 security guard cartridge, 4 × 3 mm i. d. (all from Phenomenex, Torrance, CA, USA). Elution was carried out in a binary gradient mode. Both mobile phases contained 5 mM ammonium acetate and were composed of methanol/water/acetic acid 10:89:1 (v/v/v; eluent A) and 97:2:1 (v/v/v; eluent B). After 2 min at 100% A, the proportion of B was increased linearly to 50% within 3 min. Further linear increase of B to 100% within 9 min was followed by a hold-time of 4 min at 100% B and 2.5 min column re-equilibration at 100% A. The flow rate was 1,000 μL min^{-1}. ESI-MS/MS was performed in the scheduled multiple reaction monitoring (sMRM) mode both in positive and negative polarities in two separate chromatographic runs. The sMRM detection window of each analyte was set to the respective retention time ±27 s and ±42 s in positive and in negative mode, respectively. The target scan time was set to 1 s. Confirmation of positive analyte identification was obtained by the acquisition of two sMRMs per analyte (with the exception of moniliformin and 3-nitropropionic acid that each exhibit only one fragment ion), which yields 4.0 identification points according to commission decision 2002/657/EC (Anonymous,

2002). Analyst® software version 1.5.1 (AB Sciex, Foster City, CA, USA) was used to control the LC-MS/MS instrument, as well as for automatic and manual integration of the peak. Quantitation was performed using external calibration based on serial dilution of a multi-analyte stock solution. Results were corrected for apparent recoveries, which had previously been determined by spiking experiments of both grain and straw samples. Limits of detection (LOD) and limits of quantification (LOQ) were calculated following the EURACHEM guide. The apparent recoveries, LOD and LOQ of each measured analyte are provided in Supplementary File 1. The accuracy of the method is verified on a routine basis by regular participation in interlaboratory testing schemes including a broad variation of matrices of grains, nuts, dried fruits, spices, baby food and animal feed. Satisfactory z-scores, between −2 and 2, were obtained for >94% of the >1,400 results submitted so far.

2.4. Data analysis

Descriptive statistics were performed using the positive samples (x ≥ LOD) for each measured metabolite, including minimum and maximum concentrations, median and mean values. Furthermore, a two-tailed Spearman's correlation study was conducted to investigate the relationships between the concentrations of different fungal metabolites as well as between the concentrations in grain and straw material of the same metabolite. The correlation coefficient (ρ) was calculated with the programme SPSS® Statistics (Version 24; IBM Corporate, USA), while the figures of the correlation heatmaps were prepared with Prism 8 (GraphPad Software Inc., USA). Metabolites with at least 20% positive samples were used for the correlations. The strength of correlation was indicated according to Asuero, Sayago, and González (2006), i.e. "very weak, if any" (ρ = 0–0.29), "weak" (ρ = 0.30–0.49), "moderate" (ρ = 0.50–0.69), "strong" (ρ = 0.70–0.89) and "very strong" (ρ = 0.90–1).

3. Results

3.1. Overview of detected fungal metabolites

In total, 87 and 86 fungal metabolites were measured in grain and straw samples, respectively, belonging to *Fusarium*, *Alternaria*, *Claviceps*, *Aspergillus*, *Penicillium* and other fungal genera. The toxins with the highest incidence (% positive samples) originated from fungi of the genera *Fusarium* and *Alternaria*. Considering the target group (humans or animals), none of the grain samples exceeded the permitted limits of mycotoxins set by the European Commission, while three and six straw samples were above the guidance levels set for unprocessed grains for deoxynivalenol and the sum of T-2 and HT-2, respectively.

3.2. Fusarium metabolites

The percentage of positive samples, the minimum and maximum concentrations, the median and mean values of *Fusarium* metabolites in

Table 1
Analysis of 38 ***Fusarium*** **metabolites in grain samples** (n = 253) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

	limit of detection	positive samples	min-max observation	median	mean
	µg kg^{-1}	%	µg kg^{-1}		
Fusarium					
Aminodimethyloctadecanol	16.50	51	27–4,340	27	194
Antibiotic Y	8.70	39	15–8,660	122	458
Apicidin	0.20	29	0.3–256	6.0	24
Aurofusarin	5.00	79	7.5–29,600	200	1,250
Beauvericin	0.01	34	0.1–62	0.6	3.5
Bikaverin	0.46	6	2.2–22	4.4	7.3
Butenolide	3.23	49	5.4–11,300	65	299
Chlamydospordiol	0.09	9	0.4–12	2.2	3.6
Chlamydosporol	0.34	11	6.8–122	15	25
Chrysogin	0.56	80	2.9–291	16	26
Culmorin	1.64	65	18–4,840	141	413
5-Hydroxyculmorin	30.00	33	50–7,470	370	617
15-Hydroxyculmorin	6.20	81	10–9,170	123	428
Deoxynivalenol	1.00	78	3.4–5,940	105	283
Deoxynivalenol-3-glucoside	0.60	59	0.8–343	11	26
3-Acetyldeoxynivalenol	4.80	10	8.0–116	25	27
15-Acetyldeoxynivalenol	9.20	13	15.3–416	73	103
Diacetoxyscirpenol	0.15	5	0.8–8	1.6	2.8
Enniatin A	0.01	91	0.02–90	1.1	3.4
Enniatin A1	0.06	98	0.1–793	19	55
Enniatin B	0.01	100	0.2–1,940	69	138
Enniatin B1	0.03	100	0.3–2,890	67	162
Enniatin B2	0.17	91	0.3–231	3.0	7.0
Enniatin B3	0.001	75	0.002–2	0.03	0.1
Equisetin	0.69	76	1.2–1,470	14	75
Fungerin	0.20	4	2.2–27	5.9	9.9
HT-2	1.49	4	13.8–73	29	31
HT-2 glucoside	1.70	3	2.8–35	17	18
Moniliformin	1.52	75	2.5–577	9.6	23
Monoacetoxyscirpenol	1.57	6	2.6–102	14	23
Neosolaniol	0.87	5	1.5–16	1.5	3.5
Nivalenol	0.77	33	4.9–1,670	23	88
Nivalenol glucoside	1.14	9	3.8–151	16	30
Sambucinol	7.45	0.4	na	na	na
T-2	0.71	8	1.2–39	5.1	8.7
T-2 tetraol	12.00	2	18–163	59	75
W493	0.25	64	1.2–769	9.3	40
Zearalenone	0.19	38	0.3–341	9.9	44

na (not applicable) refers to metabolites with one detected sample (sambucinol: 41 µg kg^{-1}).

Table 2
Analysis of 36 ***Fusarium*** **metabolites in straw samples** (n = 237) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

Fusarium	limit of detection	positive samples	min-max observation	median	mean
	μg kg^{-1}	%	μg kg^{-1}		
Fusarium					
Aminodimethyloctadecanol	66.00	5	78–2,650	420	829
Antibiotic Y	34.90	32	58–12,200	199	683
Apicidin	0.78	55	3.4–1,280	24	89
Aurofusarin	20.00	62	30–13,800	232	939
Beauvericin	0.04	79	0.2–86	2.3	5.8
Bikaverin	1.85	5	3.1–33	14	17
Butenolide	12.90	10	90–1,480	264	420
Chrysogin	2.23	56	9.8–604	59	96
Culmorin	6.58	62	35–6,350	575	866
5-Hydroxyculmorin	120.00	4	498–4,390	1,170	1,830
15-Hydroxyculmorin	24.90	52	42–4,870	207	395
Deoxynivalenol	4.00	69	9.9–43,900	190	909
Deoxynivalenol-3-glucoside	2.40	39	8.3–43,900	39	7,030
3-Acetyldeoxynivalenol	19.20	7	84–311	136	153
15-Acetyldeoxynivalenol	36.70	4	180–884	762	604
Diacetoxyscirpenol	0.59	11	2.7–41	10	12
Enniatin A	0.04	75	0.1–481	0.7	5.3
Enniatin A1	0.24	96	0.3–2,860	13.5	58
Enniatin B	0.05	100	0.5–7,100	81	249
Enniatin B1	0.13	100	0.4–11,100	52	233
Enniatin B2	0.69	81	1.1–807	4.2	15
Enniatin B3	0.005	71	0.01–7.6	0.1	0.19
Equisetin	2.76	68	4.6–7,940	29	342
Fungerin	0.80	9	1.62–93	14	24
HT-2	5.96	12	30–630	120	190
HT-2 glucoside	6.78	4	32–295	64	96
Moniliformin	6.07	65	7–537	10	24
Monoacetoxyscirpenol	6.30	11	26–43,900	50	5,540
Neosolaniol	3.48	7	5.8–88	17	24
Deacetylneosolaniol	6.00	10	10–407	101	138
Nivalenol	3.08	29	12–1,440	109	205
Nivalenol glucoside	4.55	5	35–129	57	60
T-2	2.85	16	8.7–565	73	107
T-2 tetraol	48.00	11	72–941	224	277
W493	1.00	70	3.2–1,770	34	89
Zearalenone	0.76	22	0.8–430	20	60

grains and straws are presented in Tables 1 and 2, respectively.

Overall in grains, we detected 38 *Fusarium* metabolites, from which 16 were present in more than 50% of the samples. These metabolites were the following, in descending order of occurrence: enniatins B, B1, A1, A and B2, 15-hydroxyculmorin, chrysogin, aurofusarin, deoxynivalenol, equisetin, moniliformin, enniatin B3, culmorin, W493, deoxynivalenol-3-glucoside and aminodimethyloctadecanol. Eleven *Fusarium* metabolites had median values above 50 μg kg^{-1}, i.e. in descending order of concentration: 5-hydroxyculmorin, aurofusarin, culmorin, 15-hydroxyculmorin, antibiotic Y, deoxynivalenol, 15-acetyldeoxynivalenol, enniatins B and B1, butenolide and T-2 tetraol. Observations with metabolite concentrations above 1,000 μg kg^{-1} were found for aurofusarin, butenolide, 15-hydroxyculmorin, antibiotic Y, 5-hydroxyculmorin, deoxynivalenol, culmorin, aminodimethyloctadecanol, enniatins B1 and B, nivalenol and equisetin.

Overall in straw, we detected 36 *Fusarium* metabolites, from which 16 were present in more than 50% of the samples. These metabolites were the following, in descending order of occurrence: enniatins B, B1, A1 and B2, beauvericin, enniatins A and B3, W493, deoxynivalenol, equisetin, moniliformin, culmorin, aurofusarin, chrysogin, apicidin and 15-hydroxyculmorin. Twenty *Fusarium* metabolites had median values above 50 μg kg^{-1}, i.e. in descending order of concentration: 5-hydroxyculmorin, 15-acetyldeoxynivalenol, culmorin, aminodimethyloctadecanol, butenolide, aurofusarin, T-2 tetraol, 15-hydroxyculmorin, antibiotic Y, deoxynivalenol, 3-acetyldeoxynivalenol, HT-2, nivalenol, deacetylneosolaniol, enniatin B, T-2, HT-2 glucoside, chrysogin, nivalenol glucoside and enniatin B1. Observations with metabolite concentrations above 1,000 μg kg^{-1} were found for monoacetoxyscirpenol, deoxynivalenol, deoxynivalenol-3-glucoside, aurofusarin, antibiotic Y, enniatin B1, equisetin, enniatin B, culmorin, 15- and 5-hydroxyculmorin, enniatin A1, aminodimethyloctadecanol, W493, butenolide, nivalenol and apicidin.

3.3. *Alternaria* metabolites

The percentage of positive samples, the minimum and maximum concentrations, the median and mean values of *Alternaria* metabolites in grains and straws are presented in Tables 3 and 4, respectively.

Overall in grains, we detected 12 *Alternaria* metabolites, from which only infectopyron was present in more than 50% of the samples. Three *Alternaria* metabolites had median values above 50 μg kg^{-1}, i.e. infectopyron, zinniol and 4-hydroxyalternariol. Observations with metabolite concentrations above 1,000 μg kg^{-1} were found only for infectopyron.

Overall in straw, we detected 12 *Alternaria* metabolites, from which four were present in more than 50% of the samples, i.e. infectopyron, zinndiol, zinniol and alternariol. Eight *Alternaria* metabolites had median values above 50 μg kg^{-1}, i.e. in descending order of concentration: infectopyron, zinniol, tenuazonic acid, zinndiol, 4-hydroxyalternariol, porritoxinol, alternariol and altertoxin-I. Observations with metabolite concentrations above 1,000 μg kg^{-1} were found for infectopyron, zinniol, alternariol and zinndiol.

Table 3
Analysis of 12 ***Alternaria*** **and** 16 ***Claviceps*** **metabolites in grain samples** (n = 253) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

	limit of detection	positive samples	min-max observation	median	mean
	μg kg^{-1}	%	μg kg^{-1}		
Alternaria					
Alternariol	0.10	37	0.2–50	3.7	7.5
Alternariolmethylether	0.15	33	0.3–4.8	0.6	0.9
4-Hydroxyalternariol	2.87	1	37–69	53	53
Altersetin	1.08	45	1.8–440	10	34
Altertoxin-I	1.46	28	2.4–40	5.4	8.1
Infectopyron	13.38	63	22–1,880	122	183
Porritoxinol	1.23	2	2.1–34	5.4	11
Tentoxin	0.11	50	0.2–8.4	0.7	0.9
Tenuazonic acid	10.00	40	15–922	38	87
Zinndiol	2.64	23	4.4–48	4.4	7.9
Zinniamide	1.11	1	1.9–1.9	1.9	1.9
Zinniol	5.20	7	8.7–454	92	132
Claviceps					
Ergine	0.07	3	0.1–3.3	0.4	1.1
Ergocornine	0.36	9	0.6–250	27	47
Ergocorninin	0.33	9	0.6–229	16	34
Ergocristine	0.37	10	1.8–745	27	119
Ergocristinine	0.27	10	1.5–461	20	77
Ergocryptine	0.42	12	0.7–183	20	42
Ergocryptinine	0.34	9	1.5–98	12	25
Ergometrine	1.08	8	1.8–254	6.3	41
Ergometrinine	0.03	16	0.1–175	1.1	13
Ergosine	0.42	17	0.7–2,150	16	202
Ergosinin	0.08	17	0.1–549	4.7	51
Ergotamine	0.92	8	4.0–4,980	50	554
Ergotaminine	0.40	8	0.7–445	9.0	71
Secalonic acid B	0.86	4	27–1,650	152	497
Secalonic acid D	0.86	11	3–1,700	49	183
Secalonic acid F	0.86	10	11–103,200	2,500	15,100

3.4. *Claviceps* metabolites

The percentage of positive samples, the minimum and maximum concentrations, the median and mean values of *Claviceps* metabolites in grains and straw are presented in Tables 3 and 4, respectively.

Sixteen *Claviceps* metabolites were detected in grains and the percentage of positive samples ranged from 3 to 17% depending on the metabolite. Two *Claviceps* metabolites had median values above 50 μg kg^{-1} (i.e. secalonic acids F and B). Observations with metabolite concentrations above 1,000 μg kg^{-1} were found for five *Claviceps* metabolites, i.e. secalonic acid F, ergotamine, ergosine, secalonic acids D and B.

Fifteen *Claviceps* metabolites were detected in straw and the percentage of positive samples ranged from 1 to 4% depending on the metabolite. Nine *Claviceps* metabolites had median values above 50 μg kg^{-1}, i.e. in descending order of concentration: secalonic acids F, D and B, ergocornine, ergocryptine, ergocorninin, ergosine, ergocryptinine and ergotamine. Observations with metabolite concentrations over 1,000 μg kg^{-1} were found only for secalonic acid F.

3.5. *Aspergillus, Penicillium and other metabolites*

The percentage of positive samples, the minimum and maximum concentrations, the median and mean values of metabolites from *Aspergillus*, *Penicillium* and other fungal genera in grains and straw are presented in Tables 5 and 6, respectively.

In grains, we detected three *Aspergillus*, ten *Penicillium* and eight metabolites from other fungal genera. From these metabolites, only rubellin D (genus *Ramularia*) and monocerin (genus *Exserohilum*) were present in more than 50% of the samples. The median values (μg kg^{-1}) ranged from 6.4 to 64 for *Aspergillus* metabolites, from 0.1 to 8.5 for *Penicillium* metabolites and from 0.3 to 67 for metabolites from other fungal genera. Observations with metabolite concentrations above 100 μg kg^{-1} were found for rubellin D, ilicicolin H (*Cylindrocladium*) and 3-nitropropionic acid.

In straw, we detected six *Aspergillus*, eight *Penicillium* and nine metabolites from other fungal genera. From these metabolites, only rubellin D, monocerin and antibiotic PF 1052 (genus *Phoma*) were present in over 50% of the samples. The median values (μg kg^{-1}) ranged from 0.7 to 51 for *Aspergillus* metabolites, from 0.1 to 85 for *Penicillium* metabolites and from 1.4 to 1,980 for metabolites from other fungal genera. Observations with metabolite concentrations above 100 μg kg^{-1} were found for antibiotic PF 1052, rubellin D, pyrenocin A, destruxin B (genus *Metarhizium*), curvularin, ilicicolin H, bis(methylthio)gliotoxin, 3-nitropropionic acid, monocerin, gliotoxin, deoxynortryptoquivalin and barceloneic acid.

3.6. *Correlations*

3.6.1. *Metabolites in grains*

In grains, we observed several strong to very strong ($p \leq 0.001$) positive correlations among *Fusarium* metabolites (Fig. 1): aurofusarin with all enniatins ($\rho = 0.71$–0.80) and equisetin (0.71); chrysogin with 15-hydroxyculmorin (0.79), culmorin (0.77) and enniatins A1, B, B1, B2 and B3 (0.71–0.78); deoxynivalenol with 15-hydroxyculmorin (0.94), culmorin (0.89), deoxynivalenol-3-glucoside (0.85), chrysogin (0.76), 5-hydroxyculmorin (0.76) and zearalenone (0.72); deoxynivalenol-3-glucoside with culmorin (0.81) and 15-hydroxyculmorin (0.81); culmorin with 15-hydroxyculmorin (0.90) and 5-hydroxyculmorin (0.77); 5-hydroxyculmorin with 15-hydroxyculmorin (0.77); all enniatins with each other (0.75–0.98); enniatins B and B1 with equisetin (0.70–0.71); moniliformin with enniatins A1, B, B1, B2 and B3 (0.76–0.87); zearalenone with culmorin (0.76), 15-hydroxyculmorin (0.74) and 5-hydroxyculmorin (0.71).

Regarding *Alternaria* metabolites, a strong positive correlation was

Table 4
Analysis of 12 ***Alternaria*** **and** 15 ***Claviceps*** **metabolites in straw samples** (n = 237) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

	limit of detection	positive samples	min-max observation	median	mean
	µg kg^{-1}	%	µg kg^{-1}		
Alternaria					
Alternariol	0.39	59	1.1–2,990	65	286
Alternariolmethylether	0.60	27	0.6–61	4.1	6.6
4-Hydroxyalternariol	11.50	6	19–617	154	184
Altersetin	4.34	10	6.5–394	29	51
Altertoxin-I	5.83	1	55–70	63	63
Infectopyron	53.50	100	279–53,600	10,600	12,700
Porritoxinol	4.92	27	17–296	72	89
Tentoxin	0.40	2	0.4–4	2.0	2.0
Tenuazonic acid	40.00	4	60–875	200	265
Zinndiol	10.57	64	15–2,340	157	363
Zinniamide	4.44	21	7.4–93	7.4	16
Zinniol	20.79	62	35–21,400	1,040	2,670
Claviceps					
Ergocornine	1.44	1	116–432	210	253
Ergocorninin	1.34	1	56–208	105	123
Ergocristine	1.49	1	36–55	46	46
Ergocristinine	1.08	1	21–36	29	29
Ergocryptine	1.68	1	68–226	117	37
Ergocryptinine	1.35	2	2.3–147	85	80
Ergometrine	4.30	2	7.2–129	28	46
Ergometrinine	0.12	3	0.2–53	21	22
Ergosine	1.67	4	2.8–848	95	220
Ergosinin	0.32	4	1.6–333	50	98
Ergotamine	3.70	1	57–68	63	63
Ergotaminine	1.60	1	15–22	19	19
Secalonic acid B	3.43	2	5.7–358	274	228
Secalonic acid D	3.43	1	238–651	342	410
Secalonic acid F	3.43	1	664–1,160	1,060	962

observed between alternariol and alternariolmethylether ($p \leq 0.001$; 0.77), whereas the remaining metabolites were not or only weakly correlated with each other.

Regarding metabolites from other fungal genera, monocerin was moderately correlated ($p \leq 0.001$) with the following metabolites: culmorin ($\rho = 0.58$), 15-hydroxyculmorin (0.58), deoxynivalenol (0.55), zearalenone (0.55), altersetin (0.54), chrysogin (0.53) and all enniatins (0.53–0.56). Moreover, rubellin D was moderately correlated ($p \leq 0.001$) with altersetin (0.56) and deoxynortryptoquivalin (0.58), while zinndiol was correlated ($p \leq 0.001$) with equisetin (0.53).

3.6.2. Metabolites in straw

Compared with grains, a lower number of strong to very strong ($p \leq 0.001$) positive correlations were found in straw (Fig. 2).

Regarding *Fusarium* metabolites, strong to very strong correlations ($p \leq 0.001$) were found between 15-hydroxyculmorin and chrysogin ($\rho = 0.71$), between beauvericin and W493 (0.79) as well as among all enniatins (0.67–0.98).

Regarding *Alternaria* metabolites, alternariol was correlated strongly ($p \leq 0.001$) with porritoxinol (0.71), zinniol (0.85) and zinndiol (0.85); additionally, for porritoxinol, strong correlations ($p \leq 0.001$) were found with zinniamide (0.78), zinndiol (0.75) and zinniol (0.73), while the last two were correlated very strongly with each other (0.91, $p \leq 0.001$).

Regarding metabolites from other fungal genera, ilicicolin H was moderately correlated ($p \leq 0.001$) with enniatins A, A1, B and B1 (0.52–0.56) and W493 (0.54). Deoxynortryptoquivalin was moderately correlated ($p \leq 0.001$) with rubellin D (0.56).

3.6.3. Between grains and straw for each detected metabolite

Regarding *Fusarium* metabolites, moderate correlations ($p \leq 0.001$) between grains and straw were found for the following metabolites: 15-hydroxyculmorin ($\rho = 0.60$), culmorin (0.59), aurofusarin (0.59), chrysogin (0.56), zearalenone (0.55), antibiotic Y (0.53), enniatins B (0.51) and B1 (0.51). Moreover, a moderate correlation ($p \leq 0.001$) between grains and straw was found with respect to rubellin D (0.67). For the remaining metabolites, no or weak correlations between grains and straw were observed.

4. Discussion

Barley is one of the most widely cultivated cereal crops worldwide and hence, it is important to ensure high levels of food and feed safety by reducing the risks of mycotoxin accumulation in the harvested products. In the current study, we detected and quantified regulated and unregulated mycotoxins as well as other fungal metabolites in grain and straw samples of barley, which originated from fields across Switzerland. We provide a thorough record of fungal metabolites with measures of central tendency and spread. In addition, we explored the relationships between the concentrations of different fungal metabolites as well as between the concentrations of the same metabolite in grain and straw material. In grains, the main detected metabolites originated from the genera *Fusarium* (38), *Alternaria* (12), *Claviceps* (16) and *Penicillium* (10). A similar pattern was observed for straw, i.e. *Fusarium*: 36, *Alternaria*: 12, *Claviceps*: 15, *Penicillium*: 8.

Barley, after wheat and maize, is the third most studied crop regarding individual mycotoxins in cereal-based food and feed products showing high mean concentrations for several classes of mycotoxins, e.g. deoxynivalenol, deoxynivalenol-3-glucoside, zearalenone, T-2 and HT-2 toxins (Palumbo et al., 2020). Gruber-Dorninger, Jenkins, and Schatzmayr (2019) conducted a ten-year global mycotoxin survey on aflatoxin B1, zearalenone, fumonisins, ochratoxin A, deoxynivalenol and T-2 toxin in feed lots and in feed raw material. The authors reported that mycotoxins were almost ubiquitously present in feed, and with respect to barley, deoxynivalenol was the main contaminant and to a lesser extent, T-2 and zearalenone. Běláková, Benešová, Čáslavský, Svoboda,

Table 5
Analysis of 3 ***Aspergillus*****,** 10 ***Penicillium*** **and** 8 of **other fungal genera metabolites in grain samples** (n = 253) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

	limit of detection	positive samples	min-max observation	median	mean
	μg kg^{-1}	%	μg kg^{-1}		
Aspergillus					
Bis(methylthio)gliotoxin	1.04	3.6	1.7–15	6.4	7.5
Sterigmatocystin	0.08	0.4	na	na	na
3-Nitropropionic acid	0.74	1	4.7–122	64	63
Penicillium					
Agroclavine	0.10	4	0.2–1.8	1.0	0.9
Andrastin A	0.25	0.4	na	na	na
Chanoclavin	0.18	9	0.3–91	1.8	8.0
Citreohybridinol	0.09	1	0.8–12	7.4	6.8
Curvularin	0.61	3	1.0–11	2.4	3.5
Deoxynortryptoquivalin	0.62	47	1.0–37	8.5	11
Mycophenolic acid	1.10	2	1.8–64	5.8	16
Mycophenolic acid IV	0.20	0.4	na	na	na
Pyrenocin A	1.56	8	2.6–60	7.0	13
Quinolactacin A	0.01	12	0.02–1.2	0.1	0.2
Other fungal genera					
Ascochlorin	0.07	4	0.1–2.1	0.3	0.5
Calphostin	5.91	2	–	–	–
Cercosporamide	0.09	4	0.1–1.5	0.4	0.5
Destruxin B	0.34	2	0.6–18	6.7	8.2
Ilicicolin H	2.00	19	2.4–210	12	24
Monocerin	0.06	78	0.2–21	2.5	3.9
Phomalone	0.06	6	0.1–2.2	0.5	0.9
Rubellin D	0.09	96	0.7–1,010	67	139

na (not applicable) refers to metabolites with one detected sample (sterigmatocystin: 1.1 μg kg^{-1}; andastrin A: 3.9 μg kg^{-1}; mycophenolic acid IV: 1.5 μg kg^{-1}). For calphostin, all values were below the limit of quantification.

Table 6
Analysis of 6 ***Aspergillus*****,** 8 ***Penicillium*** **and** 9 of **other fungal genera metabolites in straw samples** (n = 237) of barley with the respective limit of detection (LOD), percentage of positive samples (x ≥ LOD), minimum to maximum (min-max) concentrations, median and mean values.

	limit of detection	positive samples	min-max observation	median	mean
	μg kg^{-1}	%	μg kg^{-1}		
Aspergillus					
Averantin	0.16	0.4	na	1.5	1.5
Averufin	0.08	7	0.1–20.3	0.7	2.7
Gliotoxin	3.60	12	14.9–464	51	100
Bis(methylthio)gliotoxin	4.17	30	5.59–831	43	88
Sterigmatocystin	0.30	9	1.0–48	3.3	7.9
3-Nitropropionic acid	2.96	0.4	na	na	na
Penicillium					
Andrastin A	0.99	1	1.7–6.1	3.9	3.9
Barceloneic acid	4.00	17	7.8–148	49	54
Chanoclavin	0.73	4	1.2–13	4.7	5.9
Curvularin	2.46	6	2.8–1,100	29	175
Deoxynortryptoquivalin	2.49	45	9.7–428	67	79
Mycophenolic acid	4.39	0.4	na	na	na
Pyrenocin A	6.25	39	10–1,540	85	144
Quinolactacin A	0.05	3	0.1–0.6	0.1	0.2
Other fungal genera					
Antibiotic PF 1052	10.00	67	238–118,000	1,980	8,150
Ascochlorin	0.29	13	0.5–65	1.4	7.3
Ascofuranone	0.36	6	0.6–28	2.1	6.5
Cercosporamide	0.35	0.4	na	4.3	4.3
Destruxin B	1.36	7	2.3–1,150	6.6	89
Ilicicolin B	2.40	4	3.8–19	3.8	5.8
Ilicicolin H	8.00	27	9.1–959	41	76
Monocerin	0.24	77	0.4–494	6.9	22
Rubellin D	0.37	99	5.7–5,920	674	989

na (not applicable) refers to metabolites with one detected sample (3-nitropropionic acid: 597 μg kg^{-1}; mycophenolic acid: 23 μg kg^{-1}).

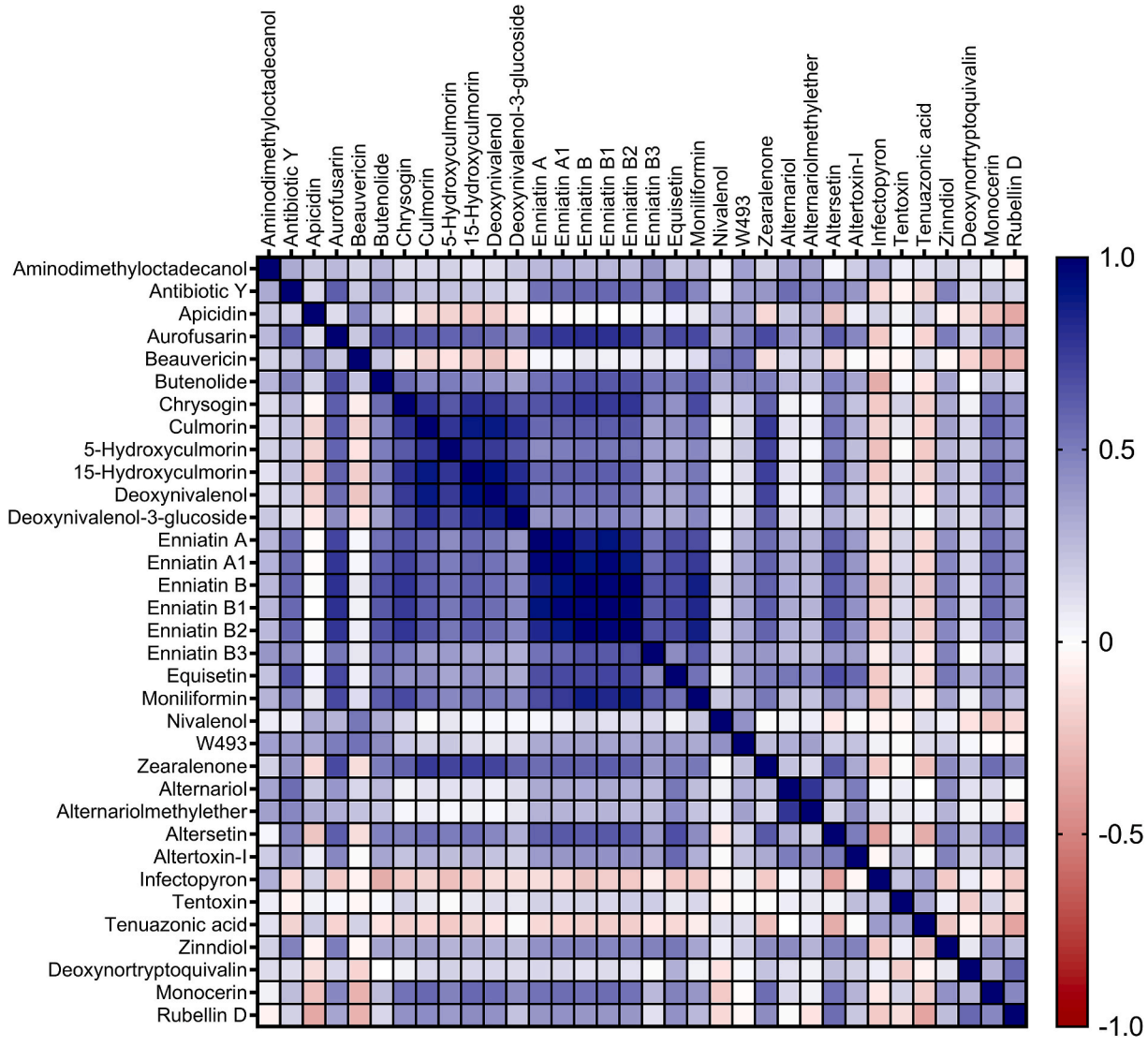

Fig. 1. Correlation matrix heatmap of fungal metabolites in **grains** with the respective Spearman's coefficient (ρ). The concentrations of metabolites that were detected in at least 20% of samples were included in the correlation study.

and Mikulíková (2014) studied the occurrence of *Fusarium* mycotoxins (i.e. deoxynivalenol, zearalenone, T-2 and HT-2) in 325 malting barley samples and the most frequently detected mycotoxin was again deoxynivalenol. However, in another study on grains of malting barley in Italy, the most frequently detected mycotoxin was HT-2 followed by enniatins B and B1, T-2 toxin and nivalenol (Beccari, Caproni, Tini, Uhlig, & Covarelli, 2016). In our study, we found that deoxynivalenol, zearalenone, nivalenol, T-2 and HT-2 were present in 78, 38, 33, 8 and 4% of grain samples and in 69, 22, 29, 16 and 12% of straw samples, respectively. Moreover, we showed that enniatins were present in 75–100% of grain samples and in 71–100% of straw samples, depending on the enniatin type. Likewise, Bolechová et al. (2015) measured a wide range of mycotoxins including enniatins (A, A1, B and B1) in malting barley and malt in Czech Republic and found that enniatins were detected in all samples. In the same study, none of the samples exceeded the maximum limits set by the European Commission. Likewise, Ibáñez-Vea, Lizarraga, González-Peñas, and López de Cerain (2012) analysed 123 barley samples for type-A and type-B trichothecenes and all samples were below the maximum limits. In the current study, taking into account the target group (humans or animals), none of the analysed grain samples exceeded the permitted limits for unprocessed cereals with respect to deoxynivalenol and zearalenone as well as the guidance levels for the sum of T-2 and HT-2 toxins. In contrast, regarding the analysed straw samples (target group: animals), we found three and six samples, respectively, that exceeded the guidance levels for deoxynivalenol (8,000 μg kg^{-1}) and the sum of T-2 and HT-2 toxins (500 μg kg^{-1}).

For commonly studied mycotoxins in barley, we found elevated mean concentrations in grain and straw samples, i.e. (in μg kg^{-1}, respectively) deoxynivalenol: 283 and 909, zearalenone: 44 and 60, HT-2: 31 and 190, T-2: 9 and 107. For the same mycotoxins, the maximum observed concentration was very high in some cases, especially in straw material (e.g. 43,900 and 630 μg kg^{-1} for deoxynivalenol and HT-2). More importantly though, in some samples, we detected exceedingly high concentrations of other fungal metabolites for which neither legislated limits nor guidance levels exist. With respect to grains (maximum concentration in μg kg^{-1}), some of these metabolites were aurofusarin (29,600), butenolide (11,300) and antibiotic Y (8,660). With respect to straw (maximum concentration in μg kg^{-1}), some of these metabolites were antibiotic PF 1052 (118,000), infectopyron (53,600), monoacetoxyscirpenol (43,900), zinniol (21,400), aurofusarin (13,800), antibiotic Y (12,200) and enniatin B1 (11,100). Khoshal et al., (2019) found that some less studied mycotoxins, which are frequently present in pig feed, e.g. apicidin and enniatin A1, showed higher toxicity

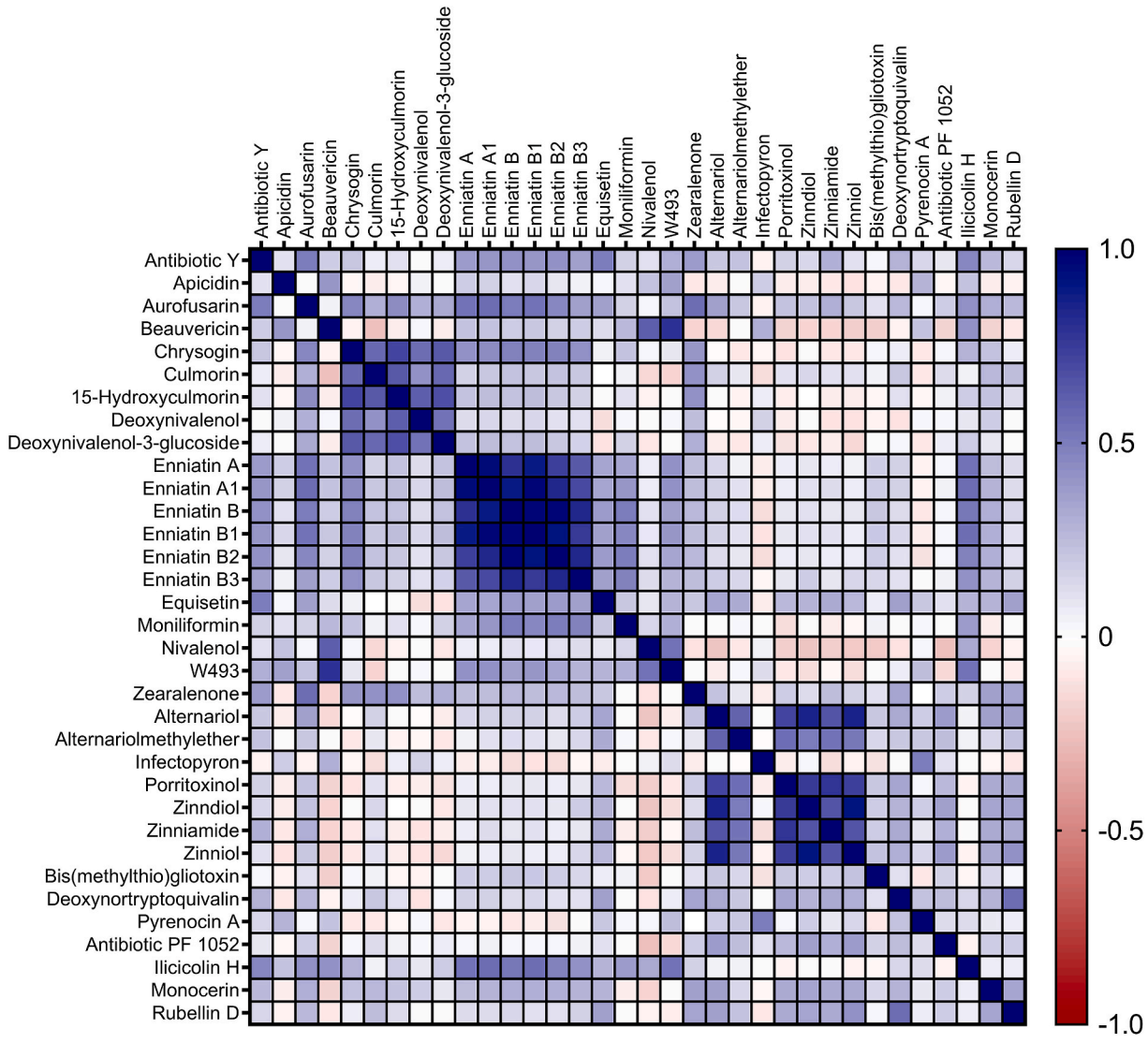

Fig. 2. Correlation matrix heatmap of fungal metabolites in **straw** with the respective Spearman's coefficient (ρ). The concentrations of metabolites that were detected in at least 20% of samples were included in the correlation study.

in intestinal cells than deoxynivalenol. As the contamination of harvested products by unregulated mycotoxins represents a problem of global concern (Spanic et al., 2020), further research on the occurrence and toxicological studies are needed.

Alternaria is another major toxigenic fungal genus including several species which produce metabolites that can cause mutagenicity, carcinogenicity and induction of DNA strand break (Escrivá et al., 2017). An Argentinian study on the natural occurrence of *Alternaria* mycotoxins in barley grains showed that alternariol, tenuazonic acid and alternariolmethylether occurred in 64, 37 and 8% of all samples, respectively, with mean concentrations ranging from 712 to 2,201 μg kg^{-1} (Castañares et al., 2020). Likewise, we found that 40, 37 and 33% of grain samples contained detectable levels of tenuazonic acid, alternariol and alternariolmethylether, respectively, but in lower mean concentrations (i.e. 0.9–87 μg kg^{-1}). Besides, we detected additional *Alternaria* toxins in the grain and straw matrices. For example, infectopyron, altersetin and zinndiol were present in 63, 45 and 23% of grain samples as well as in 100, 10 and 64% of straw samples, respectively. Interacting abiotic factors, i.e. water activity, temperature and pH, can influence the growth of *Alternaria* species in the lab (Lee, Patriarca, & Magan, 2015). Moreover, in a 10-year field survey in the Northeast of Germany, Müller and Korn (2013) investigated cropping factors that affect the accumulation of tenuazonic acid. The authors found increased contamination with tenuazonic acid in wheat samples when maize and winter wheat were the previous crops as well as under reduced tillage practices. Considering the potent toxicity of *Alternaria* toxins in both humans and animals, further investigations should focus on the influencing factors, including agronomic and environmental effects, which have an impact on the toxin accumulation in barley under field conditions.

Ergot alkaloids are produced by fungal species of the genus *Claviceps* and comprise, among others, ergocristine, ergosine, ergotamine, ergometrine, ergocornine and ergocryptine, which are frequently detected in food and feed matrices (Di Mavungu, Malysheva, Sanders, Larionova, Robbens & Dubruel, 2012). For example, in western Canada, 49 out of 67 barley grain samples were positive for ergot alkaloids and the mean concentrations ranged from 121 to 555 μg kg^{-1} for ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine (Shi, Schwab, Liu, & Yu, 2019). In the current study, although the mean concentrations of the same ergot alkaloids in grains were on a similar range (41–554 μg kg^{-1}), the percentage of positive samples was much lower (8–17%) depending on the metabolite. The occurrence of ergot alkaloids in straw samples was also very low (1–4%). The geographic region and weather conditions are two major factors that influence the

contamination of crops with ergot alkaloids. Extended periods of increased moisture and cold weather during flowering promote ergot development in cereal crops (Coufal-Majewski et al., 2016). Despite the lower incidences found in our study, some samples contained alarmingly high concentrations of *Claviceps* metabolites, e.g. ergosine and ergotamine in grains. Severe ergot toxicity in mammals causes writhing, tremors and hallucinations and can even lead to death (Coufal-Majewski et al., 2016).

Another important finding of this study is that the mean concentrations of 65 fungal metabolites were higher in straw compared with grain samples, i.e. *Fusarium*: 34, *Alternaria*: 12, *Claviceps*: 8, *Penicillium*: 3, *Aspergillus*: 2, other fungal genera: 6. The opposite was found for 11 fungal metabolites, i.e. *Claviceps*: 7, *Fusarium*: 2, *Aspergillus*: 1, *Penicillium*: 1. There is a limited number of surveys focusing on neglected harvested material, but Mol et al. (2014) investigated the occurrence of mycotoxins in straw and hay in the Netherlands. Besides the mycotoxins for which legal maximum limits or guidance levels were established (e.g. deoxynivalenol, zearalenone, T-2 and HT-2), other mycotoxins were also detected in straw (e.g. enniatins and *Alternaria* toxins) as well as sterigmatocystin and enniatins in hay. In addition, Häggblom and Nordkvist (2015) studied the contamination of straw material with deoxynivalenol and zearalenone at various pig farms in Sweden and found that all barley samples were positive for both mycotoxins concluding that the mycotoxin exposure from straw can be significant and should not be neglected.

Commonly, natural contaminants occur simultaneously in plant products (Palumbo et al., 2020; Kovalsky, Kos, Nährer, Schwab, Jenkins & Schatzmayr, 2016). The health risks of human and animal co-exposure to multiple mycotoxins has raised an increasing public concern (Assunção, Silva, & Alvito, 2016; Grenier & Oswald, 2011). In a mycotoxin survey of barley in the northern region of Spain, 77% of the samples were contaminated with two or more toxins, belonging to type-A and type-B trichothecenes (Ibáñez-Vea et al., 2012). Likewise, Gruber-Dorninger et al. (2019) showed that $\geq$ two mycotoxins were detected in 64% of 74,821 feed samples (e.g. maize, wheat, soybean) collected from 100 countries when tested for $\geq$ three mycotoxins, indicating that co-occurrence is the rule rather than the exception. Moreover, the interactions of co-occurring mycotoxins could act synergistically or additively leading to a higher risk of adverse health effects (Speijers & Speijers, 2004). Therefore, it is also important to investigate the correlations among the concentrations of fungal metabolites that are present in food and feed matrices. We observed moderate to very strong positive correlations among the concentrations of several fungal metabolites in grain and straw material of barley. With respect to grains, several strong positive correlations were expected and observed between the concentrations of *Fusarium* metabolites, e.g. aurofusarin and enniatins, deoxynivalenol and culmorin, enniatins and moniliformin as well as zearalenone and culmorin. This could be explained by the presence of the same or related species in the genus *Fusarium* infecting barley crops. Perkowski, Kiecana, & Kaczmarek (2003) found significant relationships between type-B trichothecenes (deoxynivalenol, 15-acetyldeoxynivalenol and nivalenol) as well as between type-A trichothecenes (T-2, T-2 tetraol and HT-2) in naturally contaminated barley grains. However, the authors showed that deoxynivalenol, produced by the dominating species *F. graminearum* and *F. culmorum*, was not correlated with the type-A trichothecenes, produced by *F. sporotrichioides* and *F. poae*, indicating that stronger correlations occur between toxins that are formed by the same *Fusarium* species. We found weak or no correlations in grains between *Alternaria* metabolites possibly due to the relatively lower occurrence compared with *Fusarium* metabolites. Moderate correlations of some less frequently studied metabolites were observed, i.e. monocerin with some *Fusarium* and *Alternaria* metabolites, and rubellin D with altersetin and deoxynortryptoquivalin. With respect to straw, the number of strong correlations was substantially lower compared with that of grains. For example, strong correlations were found between the concentrations of beauvericin and W493 (genus *Fusarium*) as well as between alternariol and other *Alternaria* metabolites (e.g. zinniol and zinndiol).

Moreover, we demonstrated that the concentrations of certain fungal metabolites in grains correlate well with the ones in the straw material. The strongest correlations between grains and straw occurred, in decreasing order, for rubellin D, culmorin, aurofusarin, chrysogin, zearalenone, antibiotic Y as well as enniatins B and B1.

These findings, combined with the investigation of the influencing cropping factors and climatic conditions in the field, can improve our understanding regarding the expected levels and relationships of common natural contaminants, but also of less frequently studied metabolites in barley raw materials.

5. Conclusions

Our study demonstrated that both grain and straw matrices of barley are large pools of fungal secondary metabolites. Thus, a high diversity of regulated and unregulated ("emerging") mycotoxins as well as other fungal metabolites was detected in samples from fields across Switzerland. In total, 87 and 86 metabolites were detected in grain and straw samples, respectively, mainly produced by the fungal genera *Fusarium*, *Alternaria*, *Claviceps*, *Aspergillus* and *Penicillium*. Some samples were heavily contaminated, which was more frequently observed in straw compared with grains. Therefore, despite the additional costs, barley straw should also be tested for a broad range of mycotoxins prior to use as animal feed and bedding material to prevent health problems in livestock. From a perspective of food and feed safety, it is evident that more research is necessary to elucidate the toxicity of the detected fungal metabolites, alone or in co-occurrence, especially for the most prevalent ones. Additionally, future toxicological studies should examine combined effects of mycotoxin mixtures with strong correlations in barley products, e.g. deoxynivalenol with culmorin and zearalenone in grains.

CRediT author contribution statement

Dimitrios Drakopoulos: Conceptualization, Methodology, Data curation, Writing - original draft. **Michael Sulyok:** Methodology, Writing - review & editing. **Rudolf Krska:** Writing - review & editing. **Antonio F. Logrieco:** Writing - review & editing. **Susanne Vogelgsang:** Conceptualization, Methodology, Writing - review & editing.

Declaration of competing interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.107919.

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