



An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins

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ARTICLE INFO

Keywords:

Fusarium head blight
Wheat
Deoxynivalenol
Zearalenone
Nivalenol
Cropping factor

ABSTRACT

Over an eight-year period, 686 winter wheat grain samples and information on their cropping history were obtained from Swiss growers. To estimate the risk of *Fusarium* head blight (FHB), grains were examined for *Fusarium* species incidence, mycotoxin content as well as the abundance of *F. graminearum* (FG) and *F. poae* (FP) DNA and three chemotypes, 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON) and nivalenol (NIV). Of all *Fusarium* species, FG and FP were predominant, and the average abundance of the FG DNA was three times higher compared with that of FP. The average detection of the 15ADON chemotype was twice as high as those of 3ADON and NIV, respectively. Deoxynivalenol (DON), zearalenone (ZEA) and nivalenol (NIV) were the most frequently detected toxins. For DON, 11% and for ZEA, 7% of all samples exceeded the European maximum limits for unprocessed cereals intended for human consumption. Furthermore, NIV was most likely produced by four different *Fusarium* species. A multiple correspondence analysis revealed that high levels of FG and DON were mainly observed in grain samples from fields with the previous crop maize, reduced tillage, cultivars with poor FHB resistance and strobilurin-based fungicides. Other previous crops and/or ploughing decreased the DON content by 78 to 95%. ZEA showed a similar pattern. In contrast, high levels of FP and NIV were associated with samples from ploughed fields and the previous crop canola. These findings and the negative correlations between FP DNA and FG incidence, ZEA and DON suggest a different ecological niche for FP or diverging requirements for growth and infection.

1. Introduction

Fusarium head blight (FHB) is one of the world's most noxious cereal diseases affecting wheat, barley and oats (Goswami and Kistler, 2004; McMullen et al., 2012). Based on a polyphasic approach, the genus *Fusarium* comprises about 70 species (Munkvold, 2017) of which 17 are associated with FHB (Parry et al., 1995). In central Europe, the most dominant FHB causing species are *F. graminearum sensu stricto*, *F. poae*, *F. avenaceum*, *F. culmorum*, *F. langsethiae* and *F. cerealis* (syn. *F. crookwellense*) (Bottalico and Perrone, 2002; Edwards et al., 2009; Schöneberg et al., 2018a; Xu et al., 2005). Cereal crops infected by *Fusarium* species suffer substantial yield losses. For instance, as early as 1954, a severe FHB outbreak in Ireland decreased the wheat and oat yield by up to 50% (McKay, 1957). Similarly, a field survey of wheat in 1980 in the Atlantic Provinces of Canada revealed that FHB was

responsible for yield losses between 30 and 70% (Martin and Johnston, 1982). Though, the most important impact is the contamination by various health threatening mycotoxins, particularly trichothecenes and the mycoestrogen zearalenone (Desjardins, 2006).

The overall economic impact is based on the sum of direct and secondary losses, which explains the observed total loss of US \$ 2.7 billion in wheat and barley from North Dakota and Minnesota between 1998 and 2000 (Nganje et al., 2004). More recently, McMullen et al. (2012) reviewed FHB-caused losses in wheat and barley between 1997 and 2011 in several states of the USA. Apart from the high variability between years, geographical regions, crops and grain market classes, the authors reported wheat yield losses between 2 and 54%, and in individual years, total losses of US \$ 14 million (2003: 40 counties across Maryland, North Carolina and Virginia) and \$ 13 million (2009: Kansas) were estimated. In terms of economic impact, *F. graminearum*

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<https://doi.org/10.1016/j.eja.2019.01.002>

Received 27 October 2018; Received in revised form 9 January 2019; Accepted 11 January 2019

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was considered as the fourth most threatening plant-pathogenic fungus in the world (Dean et al., 2012).

Consumption of grains containing trichothecenes may cause intestinal irritation in mammals, feed refusal in livestock, vomiting, skin dermatitis and immunological problems (Pestka, 2010). Trichothecenes have been classified into four groups (Ueno, 1985), but type-A and type-B are the most prevalent trichothecenes occurring widely in cereals (Krska et al., 2001). In Europe, *F. sporotrichioides* and *F. langsethiae* are the main producers of type-A trichothecenes, including T-2 and HT-2 toxins, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS) and neosolaniol (NEO) (Thrane et al., 2004). The type-B trichothecenes, such as deoxynivalenol (DON), the co-contaminants, 3- or 15-acetyl DON (3ADON or 15ADON), and fusarenon-X (FUS-X; synonym 4-acetyl-nivalenol) are produced predominantly by *F. graminearum* and *F. culmorum*, whereas nivalenol (NIV) is a common contaminant in cereals infected with *F. poae* (Schothorst and van Egmond, 2004; Vogelgsang et al., 2008b). Zearalenone (ZEA), frequently produced by *F. graminearum*, is of low cellular toxicity but displays high estrogenic activity, commonly leading to hyperestrogenism and fertility problems (Anonymous, 2004a). In 2006, the European Commission set maximum limits for the *Fusarium* toxins DON and ZEA as well as for fumonisins in cereals, maize and cereal/maize products for human consumption (Anonymous, 2006). For T-2 and HT-2, indicative levels for different cereal types have been established (Anonymous, 2013), whereas for NIV, despite its elevated toxicity (Ueno, 1985), no maximum limits or recommendations exist. The 2017 World Mycotoxin Survey conducted by BIOMIN GmbH revealed for cereals (wheat, barley, oats, triticale, rye, sorghum and millet) contamination rates of DON and ZEA of 37% (mean of positives $468 \mu\text{g kg}^{-1}$) and 15% ($32 \mu\text{g kg}^{-1}$), respectively. Based on the analyses for both small-grain cereals and maize, and on defined corresponding risk levels (thresholds of $150 \mu\text{g kg}^{-1}$ for DON and $50 \mu\text{g kg}^{-1}$ for ZEA), the risks for DON and ZEA contamination in Europe were classified as ‘severe’ (65% of samples above the threshold) and as ‘high’ (44%), respectively (Anonymous, 2017b).

During the last two decades, a number of European cereal grain surveys on FHB causing species and their production of mycotoxins were conducted (e.g. Ios et al., 2004; Müller et al., 2010; Stanciu et al., 2015). Overall, the species and mycotoxin patterns varied tremendously, depending on the geographic area, the host species, cropping factors, soil conditions and weather conditions in the respective sampling years (e.g. Chandelier et al., 2011; Giraud et al., 2010; Hofgaard et al., 2016; Lindblad et al., 2012; Müller et al., 2011). In most countries, *F. graminearum*, *F. culmorum*, *F. langsethiae* and *F. avenaceum*, accompanied by DON, NIV and ZEA, were predominant. In other environments, enniatins (Lindblad et al., 2013; Uhlig et al., 2007), T-2 and HT-2 (Edwards et al., 2009; Fredlund et al., 2013; Hietaniemi et al., 2016) or even fumonisins (Rubert et al., 2013) were the most commonly detected mycotoxins. Some of these studies also evaluated the relationship between cropping factors such as cultivar resistance and the occurrence of various *Fusarium* species or the respective mycotoxins. However, most investigations were conducted during a limited time span and/or focused on a small number of factors (e.g. Bernhofs et al., 2012; Bérubé et al., 2012; Blandino et al., 2012; Fernandez et al., 2005; Wenda-Piesik et al., 2017). In addition, the majority of these surveys as well as a number of reviews (e.g. Blandino et al., 2017; Kazan et al., 2012; Shah et al., 2018) examined the effect of agronomic measures solely on the occurrence of *F. graminearum* and/or DON. Furthermore, it is expected that the FHB species complex and the mycotoxin contamination of cereal grains may change over time and that they are not only dependent on climatic or geographic factors but on a wide array of partially interrelated cropping factors. In addition, it is assumed that the efficacy of a given control measure, including risk reducing cropping factors, depends fundamentally on the composition of the FHB causing species complex (Xu and Nicholson, 2009).

Wheat is the most commonly produced cereal in Switzerland (Anonymous, 2017c) and in many other countries. Between 2007 and

2014, a survey on *Fusarium* mycotoxins in Swiss winter wheat samples was conducted. The results on the effects of the sampling year and the geographic origin on mycotoxin contents were presented previously (Vogelgsang et al., 2017). The main objective of the current study was to elucidate risk-reducing strategies by additionally considering the fungal incidence, the abundance and the cropping history of the respective fields. To better understand the connection between these variables and FHB risk, growers' samples were assembled with information on a large number of cropping factors. Based on the results of fungal incidence, mycotoxin concentrations and DNA quantification of fungal species and genetic chemotypes, we assessed whether and how agronomic measures influenced the fungal attack and the respective toxin contaminations.

2. Materials and methods

2.1. Sampling

From 2007 to 2010, growers from all Swiss wheat cultivation areas were contacted each year and requested to participate in this survey by providing a sample of wheat grains immediately after harvest. Subsequently, between 2011 and 2014, growers of the canton Berne, who participated in a cantonal programme on soil protection (“Kantonales Förderprogramm Boden” / “Programme cantonal de promotion des sols”), provided additional wheat samples (Anonymous, 2017d). Berne is the second largest canton in Switzerland, and together with the canton Vaud, comprises the region with the highest cereal production (Anonymous, 2017c). Thus, it is highly representative for all wheat growing areas north of the Alps. In the written request for a harvest sample, sample collection instructions requested that ten subsamples from different places within the combine harvester (approximately 1 kg each) would be taken and thoroughly mixed. The grower were asked to submit approximately 1 kg of this mixed sample for each field. A questionnaire was included to obtain information on agricultural practices, such as the cropping system, the wheat cultivar, the previous crop, the pre-previous crop, the tillage regime, the use of fungicides, the plot location as well as the sowing, harvest and anthesis start dates. The cropping systems were composed of five different production systems: organic (Bio Suisse), integrated (IP-SUISSE), extenso, ÖLN (‘Ökologischer Leistungs-Nachweis’, meaning ‘ecological proof of performance’) and conventional. For the integrated system, plant protection treatments are carried out only if crop monitoring demonstrates that non-treatment will engender losses of revenue above a given limit (Anonymous, 2018). Extenso production is used only for small-grain cereals and canola, and the difference between this system and integrated production is that the use of synthetic insecticides, fungicides and plant growth regulators is prohibited (Jäggi, 2003). ÖLN requires the allocation of an appropriate ratio of ecological compensation areas, a rational use of fertilisers, crop rotation, soil protection measures, the targeted use of plant protection products and the implementation of animal welfare measures (Anonymous, 2004b). Growers sent their samples in plastic bags overnight along with agronomic data pertaining to each field sample. For all processing steps, raw grain samples were used.

2.2. Preparation of subsamples and identification of *Fusarium* species

The preparation of subsamples was performed as described in Vogelgsang et al. (2017). In brief, the moisture content of grains was determined, and, if needed, grains were dried to below 15% moisture. Samples were further processed using a grain divider. *Fusarium* species incidence (% infection based on the number of fungal colonies) was determined from 100 grains per sample, using a seed health test as described in Vogelgsang et al. (2008b). The different *Fusarium* species were morphologically identified according to Leslie and Summerell (2006). For DNA extraction and mycotoxin analyses, a sub-sample of

150 g grains was finely ground (mesh size 1 mm) and the resulting flour was stored at -20°C until further analysis.

2.3. Extraction of fungal DNA and quantitative PCR

DNA was extracted as described in Schöneberg et al. (2018a). For samples received between 2007 and 2010, 1 g of the wheat flour subsample was suspended in 15 ml lysis buffer PL1 (part of the DNA extraction kit „NucleoSpin 96 Plant II Kit“, Macherey-Nagel, Düren, Germany) in 45 ml Falcon tubes and vortexed for 30 s. In 2011, a more efficient procedure, requiring only 20 mg wheat flour, was established and used for all subsequent samples (1.2 ml polypropylene tubes [BRAND[®], Sigma-Aldrich, Buchs, Switzerland], one 3 mm Tungsten bead per sample and a TissueLyser II [Qiagen[®], Hombrechtikon, Switzerland], frequency 20 s^{-1} , shaken twice during 15 s). The amount of total DNA was determined by measuring the optical density at 260 nm with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Zurich, Switzerland) and, in parallel, by measuring fluorescence according to a Qubit 2.0 protocol (Invitrogen, USA).

Quantitative PCR (qPCR) was performed to determine the amount of *F. graminearum* and *F. poae* DNA in milled grain samples and to assess chemotype abundance. The primers, PCR protocol specifications and thermocycling parameters for the *F. graminearum* DNA quantification were obtained from Brandfass and Karlovsky (2006) and adapted to the available reaction mixes and laboratory devices. For the samples collected between the years 2007 and 2010, the standard curve for quantitative PCR was prepared with *F. graminearum* strain PH-1 (NRRL 31084) (Fungal Genetics Stock Center, Kansas City, MO, USA) genomic DNA. The standard curve for the samples from the years 2011 to 2014 was prepared with a plasmid as previously described (Brandfass and Karlovsky, 2006). The sequence targeted by the qPCR primers is present as a single copy in the genome; hence, it was possible to determine the copy number based on the known genome size and weight as described in Pasquali et al. (2006) for *F. oxysporum*. The qPCR for *F. poae* DNA was performed as described in Schöneberg et al. (2018b). All standards of the *F. graminearum* and *F. poae* qPCRs were spiked with DNA from healthy wheat (8 to 12 ng total DNA reaction⁻¹ [volume 20 μl] and 20 to 30 ng total DNA reaction⁻¹ [volume 25 μl], respectively), so that the amount of total DNA in the standards was similar to that found in the samples.

2.4. Quantification of genetic chemotypes

For each wheat sample, the genetic chemotypes' abundance (3ADON, 15ADON, NIV) was measured by the qPCR method as described by Kulik et al. (2011) using a TaqMan based approach with minor modifications. The reaction was carried out in a volume of 10 μl using a Takyon low ROX master mix (Eurogentech, Seraing, Belgium). The plate assembly was done using an Ep-Motion Liquid Handler (Eppendorf) in a 384-plate setup. All reactions were triplicated. Values were included when standard deviation between repetitions was below 0.2 Ct. Amplification was carried out using a Via 7 qPCR (Thermo Fischer Scientific, Zurich, Switzerland) in fast mode. Plate normalisation setup was carried out including standard curves in triplicate in each plate.

2.5. Quantification of mycotoxins

Extraction (10 g flour per sample) and quantification of mycotoxins with liquid chromatography tandem mass spectrometry (LC-MS/MS) using two 1200 L systems (Varian Inc., Walnut Creek, CA, USA) was performed as described in Forrer et al. (2014). The analytes were DON, ZEA, NIV, acetyl-deoxynivalenol (ADON: sum of 3ADON and 15ADON), NEO, DAS, HT-2 and T-2 toxin and FUS-X. Each analyte was detected with two transitions (qualifier and quantifier) in multiple reaction monitoring mode (MRM). Analyte identification was confirmed using

chromatographic retention time, correct mass of the parent ion, correct mass of the two daughter ions and agreement of the ratio of qualifier to quantifier with the calibration ($\pm 10\%$). For quantification, the method of matrix matched calibration was implemented to correct for possible ion suppression. Recoveries for low (0.5 mg kg^{-1}) and high (2 mg kg^{-1}) spiked blank samples ($n = 4$) were between 86–126 and 78–107%, respectively. Method precision was in the range of 2 and 12%, whereas instrument precision was between 2 and 10%. The toxin measurements were conducted over a period of several years; hence, due to fluctuations in the sensitivity of the LC-MS/MS instruments over time, detection and quantification limits varied from one sample run to the other. For samples obtained from harvests between 2007 and 2013, the limits of detection (LOD, in $\mu\text{g kg}^{-1}$) for DON ranged from 5 to 26, for ZEA from 1 to 9 and for NIV from 3 to 20. The limits of quantification (LOQ, in $\mu\text{g kg}^{-1}$) for DON ranged from 18 to 32, for ZEA from 5 to 19 and for NIV from 10 to 37. For the samples of 2014, a new LC-MS/MS instrument of the same type with partially higher detection and quantification limits had to be used (DON: LOD 13–20, LOQ 43–65; ZEA: LOD 9–10, LOQ 31–32; NIV: LOD 5–27, LOQ 16–91; all concentrations in $\mu\text{g kg}^{-1}$). For the sporadically detected other analytes (less than 3% of all samples), ADON, NEO, DAS, HT-2 and T-2 toxin and FUS-X, LODs / LOQs (including those from the year 2014) ranged between 3–13 / 9–45, 1–5 / 5–16, 1–2 / 2–8, 6–22 / 20–73, 1–7 / 3–23 and 3–13 / 10–45 $\mu\text{g kg}^{-1}$, respectively. For samples in which no toxin was detected or it was detected but not quantified, half of the LOD or LOQ was used, respectively, to allow for them to be considered in statistical analyses.

2.6. Statistical analyses

All analyses were performed using the statistical software package IBM SPSS Statistics for Windows, Version 24. Graphs with non-transformed data were plotted using the Systat software SigmaPlot, Version 13.0. Pooled across all years, two-tailed Spearman's rank correlations (significance level at 0.01) between fungal incidences (number of fungal colonies expressed in %), mycotoxins ($\mu\text{g kg}^{-1}$), fungal DNA amount (number of genomic copies per ng of genomic DNA) and genetic chemotypes (number of genomic copies per ng of genomic DNA) were computed. Furthermore, a principal component analysis was conducted on the incidences of trichothecene producing *Fusarium* species, the DON, ZEA and NIV contents and the amount of DNA of *F. graminearum* and *F. poae*. The contribution of the two first principal components to the overall variability was calculated. The number of samples exceeding legal limits for food or guidance values for feed was calculated and expressed in percent. Homogeneity of variance and normality of residuals were checked graphically using plots of fitted values versus the root of the standardised residuals and normal Q–Q plot, respectively. To meet the assumptions of homogeneity of variance and normal distribution of residuals, *Fusarium* species incidence, mycotoxin content, fungal DNA and genetic chemotype data were logarithmically transformed (natural logarithm \ln) before further testing. A univariate analysis of variance (ANOVA) was performed to detect differences among the survey years and between the two survey sets 2007 to 2010 and 2011 to 2014.

To elucidate the quantitative impact of cropping factors, samples with DON, ZEA or NIV concentrations below the LOD were removed from the dataset. This way, the following analyses were limited to samples where the environmental conditions were, in principal, suitable for infection and toxin production so that the effects of the cropping factors could be evaluated. To spatially visualise relationships among six predicting variables (previous crop, pre-previous crop, cultivar resistance, fungicide usage, tillage and cropping system) with non-transformed data of fungal and mycotoxin occurrence on dimensional axes, multiple correspondence analyses (MCA) were conducted for *F. graminearum* and DON, for *F. graminearum* and ZEA as well as for *F. poae* and NIV, respectively. Before analysis, the response variables from the

datasets with mycotoxin contents above the LOD were divided into two groups with values either below (designated as “low”) or above (designated as “high”) the calculated median of the respective fungal occurrence and mycotoxin content. As MCAs are not based on assumptions of distributions, they do not offer statistical significance tests. Hence, to determine if there was a significant diversion from the expected 1:1 ratio between predicting and response variables, a contingency table analysis of observed and expected counts was conducted and the Pearson Chi-Square value ($\alpha = 0.05$, Bonferroni corrected p values) was computed (Beasley and Schumacker, 1995). A two-factorial ANOVA for the effects of previous crop and tillage and their effect on *F. graminearum* occurrence and \ln transformed-mycotoxin values was conducted. To estimate the size of the effect (i.e. the contribution of a factor to the overall variability) the partial eta square value η^2 was calculated (Cohen, 1973). When the overall effect of the relevant factor was significant in the ANOVA, an all-pairwise multiple comparison procedure for uneven sample sizes according to Games-Howell (Games et al., 1981) was performed to evaluate differences between means ($\alpha = 0.05$).

3. Results

3.1. Sample size, origin and cropping history

In total, 686 winter wheat grain samples were obtained. In the four years of the Swiss wide survey, the sample numbers were 119 (year 2007), 129 (2008), 59 (2009) and 220 (2010), resulting in a total sample size of 527. In the years of the cantonal survey in Berne (total sample size of 159), the sample numbers were 36 (year 2011), 45 (2012), 45 (2013) and 33 (2014). Between 2007 and 2010, the samples were obtained from nine out of 12 climate regions and from 17 different cantons (Vogelgsang et al., 2017). Wheat cultivars with fewer than 20 samples as well as previous crops and pre-previous crops with fewer than 10 samples were summarised as ‘Other’. Only 26 samples received from growers were indicated as ‘conventional’ for their cropping system, hence, data from ÖLN and conventional systems were

combined and entered as ‘conventional’. The cropping history of the obtained wheat samples varied greatly (Table 1). The most common previous crop and pre-previous crop was maize and cereals, respectively. The grain samples were composed of 44 different wheat cultivars, with 35 bread and eight fodder cultivars as well as one cultivar for biscuit production (Supplementary Table 1). The cultivars with the greatest proportions were Zinal (74 samples) and Arina (71). The cultivar resistances to FHB according to the Swiss recommended cultivar list (based on artificial inoculations with *F. culmorum*) varied as well, and the majority of cultivars belonged to the category of “moderate” resistance. The number of wheat samples from ploughed fields was slightly higher than that from fields with reduced tillage. The majority of growers did not apply fungicides during wheat cultivation, and most wheat samples originated from ÖLN/conventional farms, followed by integrated production, extenso and organic production. Only few growers indicated the period of wheat anthesis; hence, these data were not considered for further analyses.

3.2. *Fusarium* species spectrum and fungal incidence

The average incidence of *Fusarium* infected wheat grains throughout all years was 9.1%. There was a significant difference between the Swiss wide (9.4%) and the canton Berne survey (7.8%) ($p = 0.005$). The dominant *Fusarium* species throughout all survey years was *F. graminearum* (62% of all detected *Fusarium* species), followed by *F. poae* (20%) and *F. avenaceum* (11%). Less frequent were *F. cerealis* (1.4%) and *F. culmorum* (1.1%). The year had a strong influence on the average incidences ranging from 1.1 to 8.7% for *F. graminearum* and from 0.2 to 3.6% for *F. poae*. The pattern of the species distribution between the Swiss wide survey (2007–2010) and the survey in the canton Berne (2011–2014) was similar. However, from the Swiss wide to the canton Berne survey, the proportion of *F. avenaceum* (from 10 to 14%; $p > 0.05$) and of *F. poae* (from 19 to 23%; $p > 0.05$) slightly increased, whereas the one of *F. graminearum* significantly decreased (from 64 to 56%; $p = 0.001$) (Fig. 1). The non-toxicogenic FHB causing species *Microdochium majus* / *M. nivale* (species not differentiated in the

Table 1
Cropping history for obtained wheat grain samples ($n = 686$) in descending order of sample frequency (n).

Previous crop ¹	n	Pre-previous crop ²	n	Cultivar resistance ³	n	Tillage ⁴	n	Fungicides ⁵	n	Cropping system ⁶	n
Maize	321	Cereals	312	Moderate	300	Plough	361	None	389	Conventional	270
Sugar beet	111	Pasture	158	Good	118	Reduced	325	Triazoles	148	Integrated	204
Canola	74	Maize	117	Poor	114			Strobilurins & triazoles	138	Extenso	182
Pasture	41	Sugar beet	31	Very good	82			Strobilurins	7	Organic	29
Peas	41	Canola	23	Very poor	59			Unknown	4	Unknown	1
Potatoes	38	Peas	12	Unknown	13						
Sunflower	23	Other	33								
Cereals	17										
Other	20										

¹ “Maize”: silage maize ($n = 223$), grain maize ($n = 89$) and not detailed ($n = 9$), “Other”: soya beans ($n = 9$), various vegetables ($n = 5$), celery ($n = 2$), unknown ($n = 2$), beans/onions ($n = 1/1$);

² “Maize”: grain maize and silage maize, “Other”: various vegetables ($n = 10$), potatoes ($n = 9$), soya beans ($n = 8$), sunflower/unknown ($n = 2/2$), beans/tobacco ($n = 1/1$);

³ Classifications from the Swiss recommended cultivar list. Based on inoculations with *Fusarium culmorum* conidia suspensions;

⁴ “Reduced”: reduced and zero tillage combined. Reduced ($n = 217$), zero tillage ($n = 108$);

⁵ “Strobilurins & triazoles”: growers that used both strobilurins and triazoles;

⁶ “Conventional”: ÖLN (“Proof of ecological performance”) ($n = 244$) and conventional without label ($n = 26$) combined. Details of the different cropping systems are described in the introduction chapter.

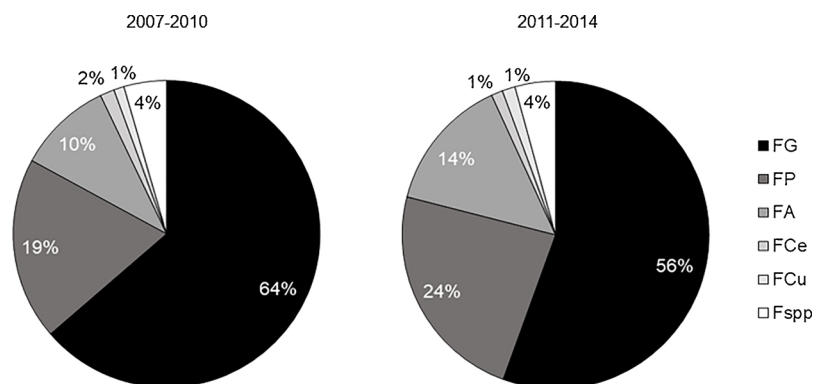


Fig. 1. Ratio (%) of *Fusarium* species identified by a seed health test. 2007-2010: Swiss wide survey (n = 527), 2011–2014: survey in the canton of Berne (n = 159), FG = *F. graminearum*, FP = *F. poae*, FA = *F. avenaceum*, FCe = *F. cerealis*, FCu = *F. culmorum*, F spp = *F. spp* (not identified to the species level).

Table 2

Incidence of *Fusarium* species (%) detected in wheat grain samples between 2007 and 2014 (n = 686). SEM = Standard error of the mean, FG = *F. graminearum*, FP = *F. poae*, FA = *F. avenaceum*, FCe = *F. cerealis*, FCu = *F. culmorum*.

<i>Fusarium</i> species	Mean	SEM	Median	90 th percentile	Maximum incidence
FG	5.6	0.39	2.0	15	73
FP	1.8	0.12	1.0	5	39
FA	1.0	0.08	0.0	3	22
FCe	0.1	0.02	0.0	0	6
FCu	0.1	0.02	0.0	0	5

seed health test) showed an overall average incidence of 12% and was, thus, higher than any of the *Fusarium* species detected (data not shown). The mean, standard error of the mean, median, 90th percentile and the maximum incidence of the respective *Fusarium* species are shown in Table 2.

3.3. Fungal DNA from *Fusarium graminearum* and *Fusarium poae* and genetic chemotypes

Throughout all years, the average number of *F. graminearum* and *F. poae* copies per ng of genomic DNA in wheat grains was 75 and 29, respectively. Similar to the species incidence data, the qPCR results demonstrated a highly significant ($p < 0.001$) influence of the year and the average number of copies per ng of genomic DNA ranged from 26 (year 2010) to 438 (2012) for *F. graminearum* and from 8 (2010 and 2013) to 115 (2009) for *F. poae* (Supplementary Table 2).

The genetic chemotypes 15ADON, 3ADON and NIV were detected in 93, 46 and 42% of all samples, respectively. Across all survey years, the average copy number of 15ADON, 3ADON and NIV genotype was 1484, 81 and 76 per ng of genomic DNA, respectively. Likewise, the year showed a highly significant ($p < 0.001$) effect on the amount of genetic chemotypes, and for 15ADON a wide range of yearly average concentrations per ng of genomic DNA, from 694 (year 2013) to 3890 (2008) (Supplementary Table 2).

3.4. Detected mycotoxins

Of the nine mycotoxins analysed, the most frequently detected toxins were DON, ZEA and NIV at average levels of 592, 39 and 15 $\mu\text{g kg}^{-1}$, respectively. Only few samples were contaminated with 3- or 15ADON (20 samples), T-2 (11 samples), HT-2 (8 samples) or with NEO, DAS and FUS-X (1 sample each), and hence, no statistics were performed with these toxin data. Similar to the detected fungal species and the measured DNA, the year showed a highly significant ($p < 0.001$) effect on the mycotoxin occurrence: between 2007 and

2014, the ratio of samples where DON was detected ranged between close to half (52%) to almost all (98%) samples. ZEA and NIV were detected in fewer samples, with yearly average values ranging between 9 and 43% and between 0 and 49%, respectively. Details on the yearly averages as well as the percentiles, median and maximum concentrations are described in Vogelgsang et al. (2017). Despite the heterogeneous climatic conditions within Switzerland, sample origin showed only minor effects on the occurrence of *Fusarium* mycotoxins (Vogelgsang et al., 2017).

3.5. Correlations between fungal incidences, mycotoxins, fungal DNA and genetic chemotypes

A great variability of non-significant as well as significant positive and negative correlations was observed (Supplementary Table 3). Despite the significant relationships, most correlation coefficients were rather low. The highest positive correlations were detected between the DON content and the *F. graminearum* DNA ($r = 0.718$), between DON and the *F. graminearum* incidence ($r = 0.695$) and between *F. graminearum* incidence and *F. graminearum* DNA ($r = 0.678$). The positive correlations between *F. poae* incidence and NIV content as well as between *F. poae* incidence and *F. poae* DNA were also highly significant but with substantially lower correlation coefficients ($r = 0.188$, $r = 0.379$, respectively). The ZEA content showed the highest positive and highly significant correlations with *F. graminearum* DNA ($r = 0.425$), followed by the DON content ($r = 0.388$) and the *F. graminearum* incidence ($r = 0.370$). The *F. graminearum* incidence showed also highly significant positive correlations with the 15ADON ($r = 0.569$), the 3ADON ($r = 0.375$) and the NIV ($r = 0.375$) genetic chemotype. The *F. cerealis* incidence demonstrated highly significant positive correlations with all genetic chemotypes (15ADON genotype: $r = 0.228$; 3ADON: $r = 0.160$; NIV: $r = 0.164$) while the *F. culmorum* incidence showed only a positive significant correlation with the 3ADON chemotype ($r = 0.115$). The correlations between the *F. graminearum* incidence and that of other FHB causing species incidences ranged between non-significant (*F. poae*: $r = -0.018$) and significant (*F. avenaceum*: $r = 0.252$, *F. cerealis*: $r = 0.245$, *F. culmorum*: $r = 0.126$). Few significant negative but weak correlations were detected, such as that between the *F. poae* DNA and the three genetic chemotypes (r values between -0.121 and -0.185), between *F. poae* DNA and *F. graminearum* incidence ($r = -0.148$) and that between the *F. poae* DNA and the DON and ZEA contents ($r = -0.183$, $r = -0.122$, respectively). With respect to the incidence of the non-toxigenic species *M. majus* / *M. nivale*, weak positive but significant correlations were found with the incidences of *F. graminearum*, *F. avenaceum*, *F. cerealis*, the DON content, the *F. graminearum* DNA as well as with the genetic chemotypes 15ADON and 3ADON (r values between 0.101 and 0.235). In contrast, weak negative but significant correlations were detected between *M.*

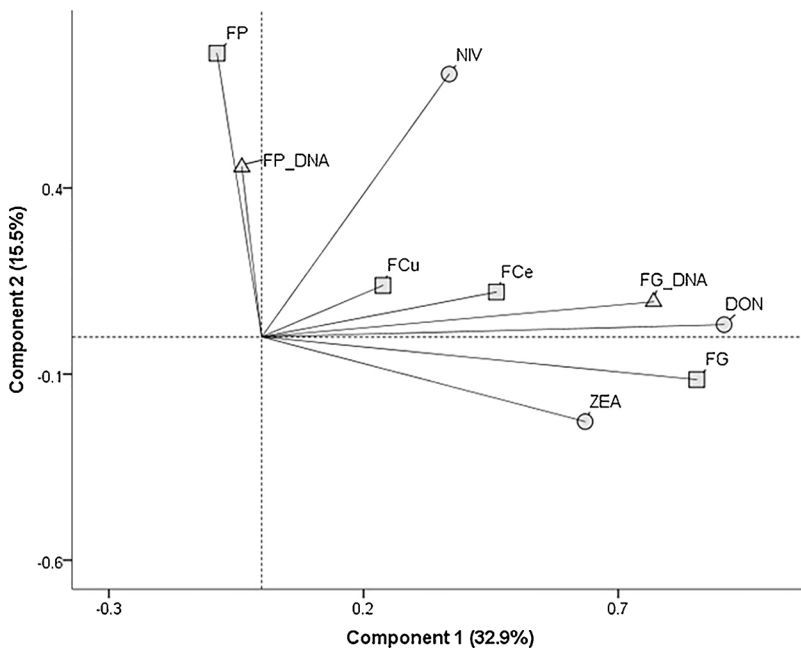


Fig. 2. Component plot in rotated space based on a principal component analysis (rotation method Varimax with Kaiser normalisation) on the response variables *Fusarium* species incidence (%), mycotoxins ($\mu\text{g kg}^{-1}$) and fungal DNA (number of genomic copies per ng of genomic DNA). Dotted lines represent the origin at zero for each component. Percentages in parentheses indicate the contribution to the overall variance. FG = *F. graminearum*, FP = *F. poae*, FCe = *F. cerealis*, FCu = *F. culmorum*, DON = deoxynivalenol, ZEA = zearalenone, NIV = nivalenol, FG_DNA = *F. graminearum* DNA, FP_DNA = *F. poae* DNA, n = 686.

majus / *M. nivale* and the incidence of *F. poae* ($r = -0.147$) (Supplementary Table 3).

The principal component analysis (PCA) on the response variables incidence of trichothecene producing *Fusarium* species, mycotoxins and fungal DNA revealed a contribution to the overall variance of 33% (component 1) and 16% (component 2) (Fig. 2). The PCA demonstrated a strong association of DON content, ZEA content, *F. graminearum* incidence and *F. graminearum* DNA and to a certain extent to *F. cerealis* and *F. culmorum* incidence along the first component. Furthermore, a strong association between *F. poae* incidence and *F. poae* DNA along the second component axis was detected. In contrast, the NIV content showed a similar level of association to both, *F. poae* on the one hand and *F. culmorum*, *F. cerealis* and *F. graminearum* on the other hand (Fig. 2).

3.6. Effect of cropping factors on mycotoxins and fungal occurrence

3.6.1. Associations between six cropping factors on toxin accumulation and fungal occurrence

The multiple correspondence analysis (MCA) for DON, *F. graminearum* incidence and *F. graminearum* DNA (n = 549) revealed an accumulated contribution of 51% to the overall variability (Fig. 3). The analysis showed that the two response variable groups, based on values above or below the median, were far away from the origin and discriminated on the horizontal axis, and hence, predicting variables close to either of these groups were influential. Higher levels of DON, *F. graminearum* DNA and *F. graminearum* incidence were mainly observed in wheat grain samples from fields where the previous crop was maize (Pearson chi square value of $p < 0.0001$ for all three response

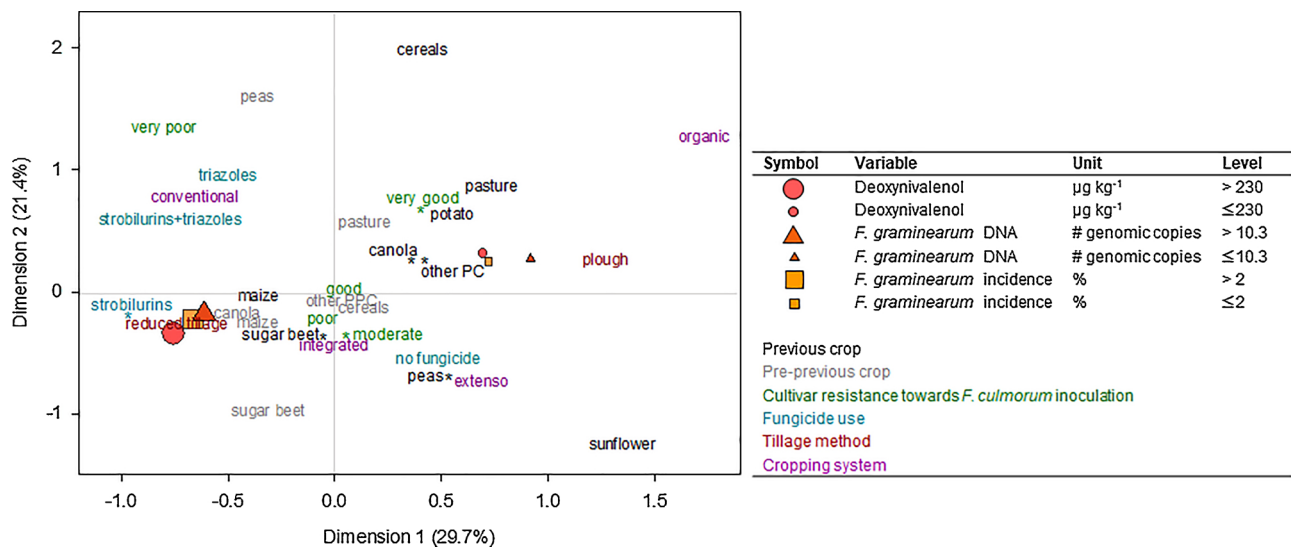


Fig. 3. Graphical representation of a multiple correspondence analysis between deoxynivalenol (DON) concentrations in winter wheat grains, *Fusarium graminearum* disease severity parameters (number of genomic copies per ng of genomic DNA and % incidence) and cropping factors. For analysis, a reduced dataset including only samples with a DON concentration above the limit of detection was used (n = 549). Response variables were grouped each into two classes based on the median of DON or *F. graminearum* disease severity, respectively. Objects that are close to each other were often observed together while objects that are distant were rarely observed together. 'other PC/PPC': other previous/pre-previous crops, respectively. Overlapping cropping factors were separated by placing asterisks on the respective positions. Grey lines represent the origin at zero for each component.

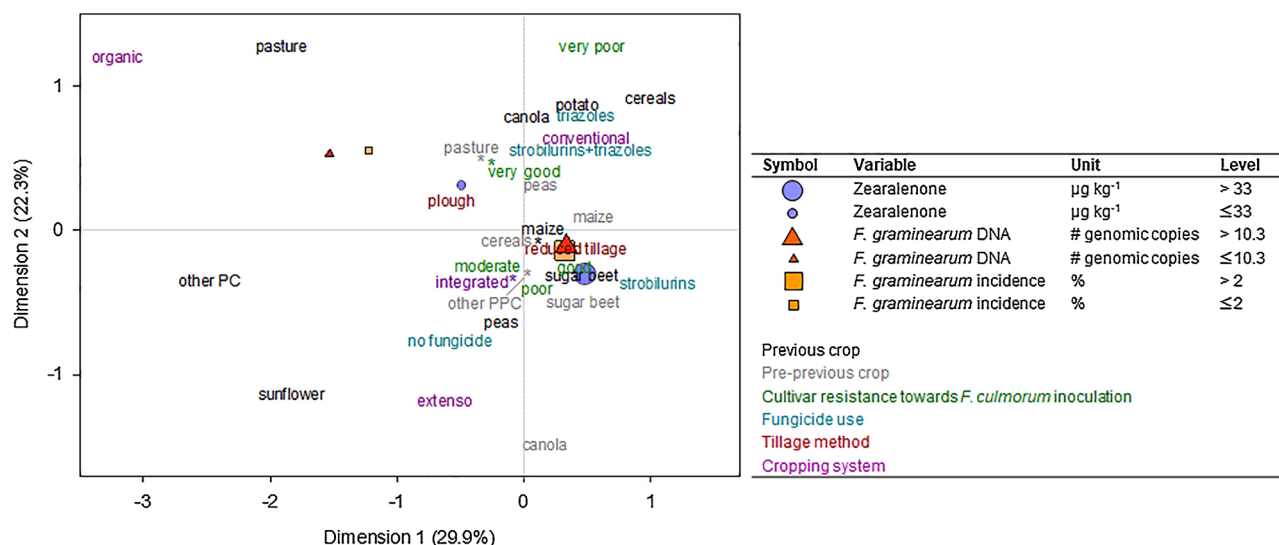


Fig. 4. Graphical representation of a multiple correspondence analysis between **zearalenone** (ZEA) concentrations in winter wheat grains, *Fusarium graminearum* disease severity parameters (number of genomic copies per ng of genomic DNA and % incidence) and cropping factors. For analysis, a reduced dataset including only samples with a ZEA concentration above the limit of detection was used (n = 216). Response variables were grouped each into two classes based on the median of ZEA or *F. graminearum* disease severity, respectively. Objects that are close to each other were often observed together while objects that are distant were rarely observed together. ‘other PC/PPC’: other previous/pre-previous crops, respectively. Overlapping cropping factors were separated by placing asterisks on the respective positions. Grey lines represent the origin at zero for each component.

variables) and where reduced tillage was employed ($p < 0.0001$ for DON and *F. graminearum* DNA, $p = 0.0006$ for *F. graminearum* incidence). In contrast, low levels of DON and *F. graminearum* occurrence were closely associated with previous crops potato, canola, pasture, ‘other’ previous crops (containing mostly soya bean) and ploughed fields. High levels of DON and *F. graminearum* occurrence were also clearly linked to samples with the pre-previous crops maize and canola ($p = 0.007$ for DON and maize) and low levels were closely associated with the pre-previous crop pasture ($p = 0.007$). Grain samples from wheat cultivars considered to be highly resistant against FHB (“very good”) were mainly found in samples with low levels of DON ($p = 0.002$), however, there were no further contrasts between the

other cultivar resistance categories. The use of fungicides was a highly discriminating variable as samples with high levels of DON and fungal occurrence were mainly found in samples from fields where strobilurins were applied compared with those where no fungicides were applied (DON: $p = 0.007$, *F. graminearum* incidence: $p = 0.0003$, *F. graminearum* DNA: $p < 0.0001$). The cropping systems also showed a highly discriminating attribute on the horizontal axis, and grain samples from organically managed fields showed a closer association to samples with low DON contents compared with all three other cropping systems ($p < 0.0001$). In contrast, high *F. graminearum* incidences were mostly observed in samples from conventional and integrated farms compared with organic or extenso farms ($p < 0.0001$).

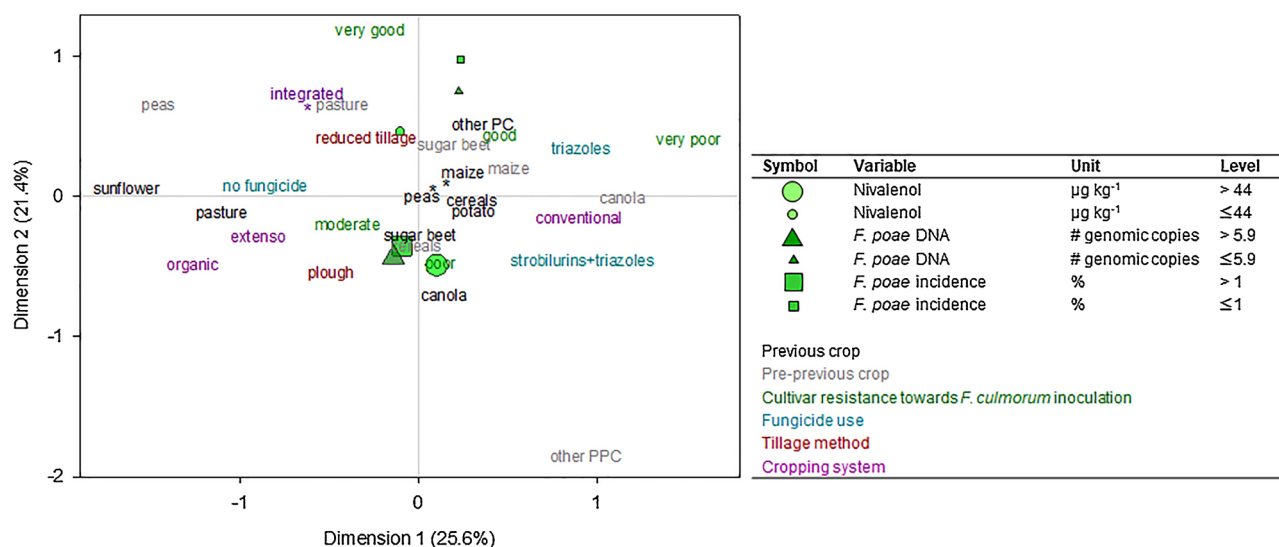


Fig. 5. Graphical representation of a multiple correspondence analysis between **nivalenol** (NIV) concentrations in winter wheat grains, *Fusarium poae* disease severity parameters (number of genomic copies per ng of genomic DNA and % incidence) and cropping factors. For analysis, a reduced dataset including only samples with a NIV concentration above the limit of detection was used (n = 138). Response variables were grouped each into two classes based on the median of NIV or *F. poae* disease severity, respectively. Objects that are close to each other were often observed together while objects that are distant were rarely observed together. ‘other PC/PPC’: other previous/pre-previous crops, respectively. Overlapping cropping factors were separated by placing asterisks on the respective positions. Grey lines represent the origin at zero for each component.

Table 3

Results of a two-factorial analysis of variance for the cropping factors previous crop (PC), tillage (T) and the interaction (PC x T) based on *ln* transformed data on mycotoxin content ($\mu\text{g kg}^{-1}$), *F. graminearum* incidence (%) and DNA (number of genomic copies per ng of genomic DNA). DON = deoxynivalenol, ZEA = zearalenone, FG incidence = *F. graminearum* incidence, FP incidence = *F. poae* incidence, FG DNA = *F. graminearum* DNA, FP DNA = *F. poae* DNA. df = degrees of freedom. Unless stated otherwise, numbers in parentheses indicate the eta square value η^2 .

Cropping factor (df) ¹	DON ²	ZEA ²	FG incidence	FG DNA
PC (8)	** (13.5)	* (11.3)	** (17.6)	** (17.2)
T (1)	** (3.0)	–	* (2.1)	** (3.7)
PC x T (1)	* (3.1)	–	** (4.7)	* (2.9)

¹ Details of the cropping factors are described in Table 1.

² For mycotoxins, only samples above the respective limit of detection were used for the analysis. DON: n = 549, ZEA: n = 216, ** = significant at $p < 0.01$, * = significant at $p < 0.05$; – = not significant.

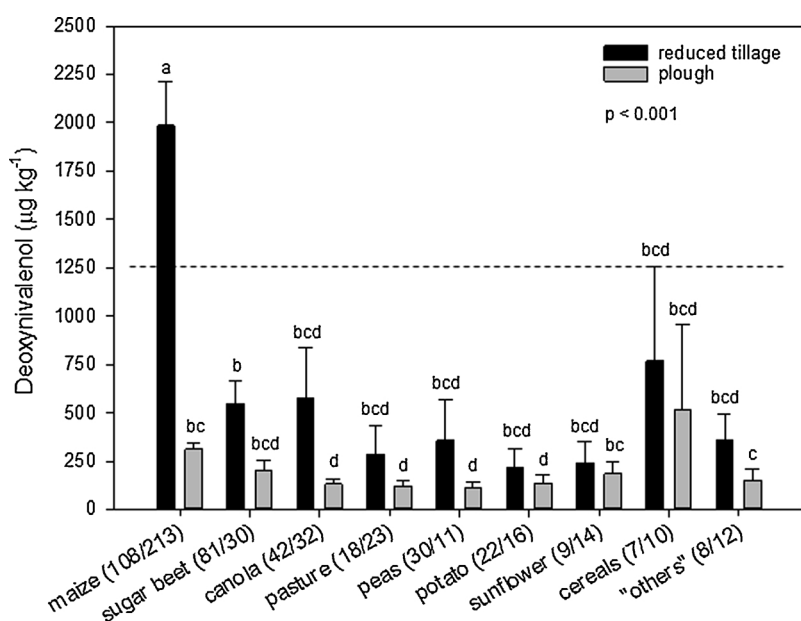


Fig. 6. Deoxynivalenol (DON) concentrations in wheat grains obtained between 2007 and 2014 depending on the previous crop and tillage method. Previous crops “others” are indicated in Table 1. Numbers in parentheses represent the sample sizes from fields with reduced tillage or plough, respectively. The dashed line indicates the European maximum limit for DON in unprocessed cereals intended for human consumption ($1250 \mu\text{g kg}^{-1}$). Statistical analyses were based on *ln* transformed data from samples containing DON above the detection limit. Error bars represent the standard error of the means. Means with the same letters are not significantly different according to a Games-Howell post-hoc multiple comparison method (unequal variances and unequal sample sizes) ($\alpha = 0.05$).

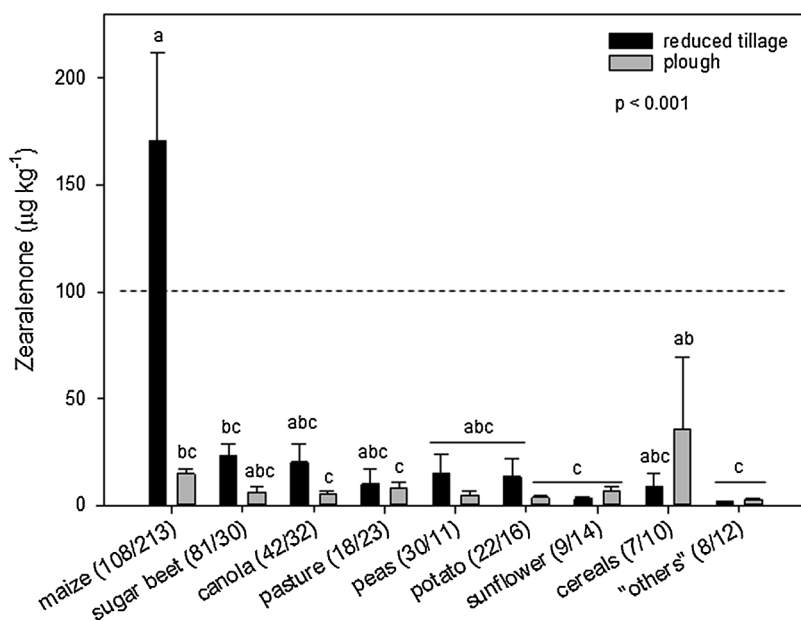


Fig. 7. Zearalenone (ZEA) concentrations in wheat grains obtained between 2007 and 2014 depending on the previous crop and tillage method. Previous crops “others” are indicated in Table 1. Numbers in parentheses represent the sample sizes from fields with reduced tillage or plough, respectively. The dashed line indicates the European maximum limit for ZEA in unprocessed cereals intended for human consumption ($100 \mu\text{g kg}^{-1}$). Statistical analyses were based on *ln* transformed data from samples containing ZEA above the detection limit. Error bars represent the standard error of the means. Means with the same letters are not significantly different according to a Games-Howell post-hoc multiple comparison method (unequal variances and unequal sample sizes) ($\alpha = 0.05$).

more often found together with samples that had lower fungal occurrence (*F. graminearum* incidence: $p < 0.0001$, *F. graminearum* DNA: $p = 0.002$). For ZEA, fungicides did not discriminate.

With the MCA on NIV, *F. poae* incidence and *F. poae* DNA ($n = 138$), the accumulated contribution to the overall variability (47%) was somewhat lower than those for the MCAs on DON and ZEA (Fig. 5). The response variables of each level were closely linked to each other, and the higher versus the lower levels were mainly separated on the second dimension. Higher levels of NIV, *F. poae* DNA and *F. poae* incidence were mainly observed in grain samples from fields with the previous crop canola or sugar beet and where the pre-previous crop was cereals, even though the contingency table did not reveal a significant effect. In contrast to the MCAs on DON and ZEA, lower NIV and *F. poae* fungal occurrence levels were closer to ‘other’ previous crops, maize and peas. Furthermore, while reduced tillage was highly linked with samples containing high DON, ZEA and *F. graminearum* levels, the opposite was the case for NIV and *F. poae*, where samples with low levels were mainly observed in samples from fields where reduced tillage was employed (significant for both *F. poae* incidence and *F. poae* DNA: $p = 0.002$). In addition, higher levels of NIV were closer with applications of strobilurins compared with no applications or triazoles alone ($p = 0.032$). Neither the choice of wheat cultivar nor the cropping system did discriminate with respect to NIV content or *F. poae* occurrence.

3.6.2. Effect of previous crop, tillage and their interaction on *Fusarium graminearum* occurrence, DON, ZEA and the exceedance of maximum limits

Throughout all years, 11 or 7% of the samples contained DON or ZEA concentrations above the European maximum limit for unprocessed cereals intended for human consumption, 1250 and 100 $\mu\text{g kg}^{-1}$, respectively. Levels per year varied between 0 and 27% for DON and between 0 and 12% for ZEA. With respect to guidance values for complementary and complete feeding stuffs for pigs, 14 or 3% samples contained a DON or ZEA content above the value of 900 $\mu\text{g kg}^{-1}$ (DON for pigs) and 250 $\mu\text{g kg}^{-1}$ (ZEA for sows and fattening pigs), respectively. Levels per year varied between 0 and 29% for DON and between 0 and 5% for ZEA. The two-factorial ANOVA on the effects of previous crop and tillage revealed significant effects of these factors and the respective interaction on the DON content, the *F. graminearum* incidence and the *F. graminearum* DNA. The contribution to the overall variability represented by the eta square value η^2 was substantially higher for the factor previous crop compared with the factor tillage and the interaction (Table 3). For ZEA, a significant effect was only observed for the factor previous crop.

Based on the entire dataset, wheat samples from fields with the previous crop maize and reduced tillage contained on average 1990 $\mu\text{g kg}^{-1}$ DON and, thus, exceeded the maximum limit of 1250 $\mu\text{g kg}^{-1}$. These samples had an average DON amount that was six times higher than that in wheat samples from fields where the previous maize crop residues were ploughed under. Additionally, the wheat samples from fields with maize and reduced tillage had DON levels that were 18 times higher than those from fields with peas as the previous crop and ploughing before wheat sowing. These modified cropping factors were associated with a reduction of the DON content by 84 or 95% reduction, respectively (Fig. 6). When pooled over both tillage types, the reduction of DON from previous crop maize to previous crop pasture was 78%. Similarly, the highest average ZEA content (170 $\mu\text{g kg}^{-1}$), above the maximum limit of 100 $\mu\text{g kg}^{-1}$, was found in wheat samples from fields with the previous crops maize and reduced tillage. The ZEA contents were reduced by 91 and 99% when maize residues were ploughed under or when wheat samples came from a field with other previous crops (including soya beans and various vegetables), respectively (Fig. 7). Pooled over both tillage types, the reduction of ZEA from previous crop maize to previous crop ‘others’ (including mostly soya bean) was 96%.

4. Discussion

In the current study, a dataset of 686 grain samples collected during eight years and throughout 17 Swiss cantons allowed to reveal the risk of *Fusarium* infection and mycotoxin occurrence in commercially grown wheat under natural fungal inoculum conditions. Moreover, the cropping factors that have a considerable effect on FHB infection were elucidated by evaluating the abundance of different FHB causing species and their main mycotoxins in combination with the respective cropping history.

4.1. Cropping history

By far, the most common previous crop was maize, comprising almost three times as many samples as those where sugar beet was the previous crop. This finding was expected since maize is a crop that is harvested rather late while winter wheat can be sown as late as November. Therefore, in Switzerland, the harvest of maize and sowing of wheat is commonly done on the same day. Most growers used wheat cultivars with a moderate resistance to FHB, and comparably few growers used cultivars with very good or very poor resistance. Certainly, the choice of cultivar depends on various other factors such as yield potential, baking quality, protein content as well as resistance to lodging and to other wheat diseases. In fact, some of the Swiss cultivars with a very good resistance to FHB, such as Arina and Titlis, are in turn not well performing with respect to yield, which represents a challenge for growers. Samples from fields that were ploughed or where reduced tillage (including zero tillage) was employed were almost equally distributed. Direct payments to growers in several cantons can explain the high number of the latter tillage regime. The relatively widespread usage of reduced tillage is also due to the awareness of maintaining soil fertility and a reduced risk of erosion associated with this practice and because ploughing is a costly measure in terms of time and fuel. It was surprising that the majority of samples were obtained from fields where no fungicides were applied (close to 400) compared with close to 300 fields where triazoles, strobilurins or a combination of both were used. The choice of the fungicidal active ingredient represents a challenge as strobilurins have a high efficacy against several leaf diseases, while some strobilurins perform poorly towards certain *Fusarium* species (Dubos et al., 2013, 2011) or might even increase DON contamination by 50 to 95% as it was shown for *F. culmorum* (Forrer et al., 2000). Swiss cropping systems are highly diverse, comprising five different systems, i.e. organic production, integrated production, extenso, ÖLN (“proof of ecological performance”) and conventional. Although comparatively few samples were obtained from organic cropping systems, the sum of samples from extenso and integrated farming represented more than half of all samples, which in turn, indicates the absence or reduced applications of both fungicides and growth regulators.

4.2. *Fusarium* species spectrum, fungal incidence, DNA and genetic chemotypes

As expected, the dominant FHB causing species in the wheat samples investigated was *F. graminearum*. This finding is in line with its worldwide occurrence (cited in Backhouse, 2014) and parallels results from other European surveys, such as those in Belgium (Hellin et al., 2016), Luxembourg (Beyer et al., 2014), France (Boutigny et al., 2014), Germany (Talas et al., 2011), Sweden (Karlsson et al., 2017) and Italy (Covarelli et al., 2015). Nevertheless, *F. poae* and *F. avenaceum* were detected in all years, with alarmingly high levels in individual samples that reached maxima of 39 and 22% incidence, respectively. The year had a considerable effect on the fungal incidence, and *F. graminearum* and *F. poae* average incidences were 8 and 18 times greater, respectively, in the year with the highest incidences compared to the year with the lowest incidences. This observation is most probably due to

different weather conditions being favourable or unfavourable for the infection by a given FHB causing species and subsequent mycotoxin accumulation (Vogelgsang et al., 2017; Xu et al., 2008). The fact that the proportions of *F. poae* and *F. avenaceum* during the survey of the canton Berne between 2011 and 2014 were higher compared with those from the Swiss wide survey between 2007 and 2010 could be due to a shift within the FHB species community. Certainly, the sample sizes between 2011 and 2014 were considerably smaller. Still, potential species shifts towards an increase of *F. poae* in wheat due to climate change and its direct (warmer, drier conditions) and indirect effects (modified cropping systems) were suggested in previous reports from the Czech Republic, Poland, Sweden and Finland (Chrpová et al., 2016; Kulik and Jestoi, 2009; Lindblad et al., 2013; Parikka et al., 2012, respectively). Similarly, field sampling carried out in Hungary, Ireland, Italy and the United Kingdom showed that *F. poae* was associated with relatively drier and warmer conditions, whereas *F. graminearum* was associated with warmer but humid conditions (Xu et al., 2008). Kliks et al. (2008) found a significant negative correlation between the DON content and the percentage of samples infected with *F. poae*, suggesting that DON producers and *F. poae* rarely occur at the same time in the same place. Hence, different environmental conditions may differentially affect the infection and colonisation processes of individual FHB causing species (Xu and Nicholson, 2009).

Similar to the fungal incidence, the average number of *Fusarium* species and genetic chemotype copies varied considerably throughout the survey years. The highest yearly average amount of *F. graminearum* and *F. poae* DNA, the sum of 15- and 3ADON and NIV were 17, 14, 6 and 18-fold higher, respectively, compared with the lowest yearly average. For the *F. graminearum* DNA, these differences were even greater than those for the *F. graminearum* incidences. This result could be due to detrimental conditions for the living fungal material of some grain samples between harvest and arrival or due to triazole-based fungicides, which would not affect DNA measurements but might have partially destroyed mycelium, thus reducing the number of outgrowing colonies. These cases could have contributed to an underestimated incidence of infected grains and, thus, decreasing some of the year effects.

From all chemotypes, the average amount of 15ADON was clearly the highest. This result underlines the dominance of 15ADON in Europe and is in accordance with a comprehensive report on *F. graminearum* and *F. culmorum* isolates across 17 countries (Pasquali et al., 2016).

4.3. Detected mycotoxins

Based on the finding that *F. graminearum* and *F. poae* were the two most dominant species, it was expected that DON, ZEA and NIV were the most frequent toxins detected. Depending on the year, DON was detected in close to half to almost all samples. Less pronounced but similar trends were observed for ZEA and NIV. As presented in Vogelgsang et al. (2017), the highest annual average DON, ZEA and NIV contents were about 19, 11 and 5 times higher than those of the year with the lowest content. However, based on the observation that the variability even within a survey year was substantial, it was assumed that other factors such as agronomic measures were partially overriding the year effects. It was remarkable that only 20 samples contained 15ADON and 3ADON despite the fact that the respective genetic chemotypes were detected in a large fraction of wheat grains. This finding confirms previous observations that highlight the *Fusarium* species' co-production of DON and its acetylated forms in different concentrations, which can lead to minor concentrations in field samples (Pasquali et al., 2010). Furthermore, this result is also likely due to the fact that the plant quickly metabolises 3ADON to DON (Schmeitzl et al., 2015).

In previous investigations (Liu et al., 1998; Thrane et al., 2004; Vogelgsang et al., 2008a, 2008b), it was shown that *F. poae* frequently produces MAS as well as DAS, NEO and FUS-X in addition to the previously discussed metabolites. Yet in the present study, some of these metabolites were not at all or only sporadically detected. However,

most of the aforementioned studies focused on *in vitro* trials with a limited number of isolates. In turn, mycotoxin production depends not only on the fungal genotype and the geographic origin (Toth et al., 2004; Vesonder et al., 1991) but also on environmental conditions such as substrate, water availability and temperature (Magan et al., 2002; Thrane et al., 2004; Vogelgsang et al., 2008a). Thus, it was anticipated that not all of these toxins would be detected in the collected wheat grain samples. Furthermore, agricultural practices also have a strong influence on mycotoxin accumulation (discussed below in chapter 4.5).

4.4. Correlations between fungal incidences, mycotoxins, fungal DNA and genetic chemotypes

The majority of the detected correlation coefficient values were rather low and the highest value of 0.72 was found for the association between the DON content and the *F. graminearum* DNA. The overall low values are most probably due to the fact that the wheat samples were not obtained from an experiment with defined parameters but from a long-term survey using wheat grains from growers across an entire country.

Except for the *F. poae* incidence, positive and highly significant correlations were observed between all investigated *Fusarium* species incidences. This was not surprising and confirms a previous study investigating the occurrence of FHB in Hungary, Ireland, Italy and the United Kingdom, where up to six species were found at one site (Xu et al., 2008).

The fact that in the current study, *F. poae* incidence was not correlated with those of other *Fusarium* species could be an indication for a separate ecological niche and/or for different climatic requirements for growth and infection. The observed significant negative correlations between *F. poae* DNA and *F. graminearum* incidence, DON content and ZEA content could further strengthen the different ecological niche hypothesis. If the *F. poae* incidence is extrapolated onto NIV as its main associated mycotoxin, the hypothesis is in parallel with results from monitoring data in Finland, Sweden, Norway and the Netherlands over a 20 year period: the occurrence of NIV was negatively associated with higher rainfall and relative humidity whereas the opposite was the case for DON and ZEA (Van der Fels-Klerx et al., 2012). Hence, the authors suggested that climatic conditions that are conducive for a given toxin might have a decreasing effect on other toxins.

In the present study, a positive and highly significant correlation was detected between the *F. poae* incidence, the *F. poae* DNA and the NIV content, which confirms the role of *F. poae* as one of the major NIV producing species. However, the *F. graminearum* incidence was also positively and highly significantly associated with the NIV content as well as the NIV chemotype concentration. These observations explain the result obtained with the principal component analyses where the NIV content was plotted in-between several trichothecene producing species, suggesting that NIV detected in this survey originated from several species and not exclusively from *F. poae* and *F. graminearum*. Furthermore, *F. graminearum*, *F. cerealis* and *F. culmorum* could not be distinguished based on this plot. This result parallels findings from a Belgian study by Hellin et al. (2016) where NIV was also found to be produced by several *Fusarium* species. Moreover, a cereal monitoring in England and Wales demonstrated the presence of both DON and NIV producing chemotypes of *F. graminearum* in wheat (Jennings et al., 2004) and a literature review by Pasquali and Migheli (2014) confirmed the occurrence of both *F. graminearum* DON and *F. graminearum* NIV chemotypes in nearly all wheat-growing areas. The same holds true for *F. cerealis* as it also showed significant correlations with DON and ZEA as well as with the NIV content. In fact, this species is considered to be primarily a NIV producer (Amarasinghe et al., 2015; Chandler et al., 2003) and it might be argued that the correlation with DON and ZEA is mainly due to its close association with *F. graminearum*, possibly using the same ecological niche. However, it has been reported that some isolates showed also the ability to produce ZEA (e.g. López et al., 1997).

For *F. culmorum*, positive and highly significant correlations were found with the DON content, the *F. graminearum* DNA, the 3ADON genetic chemotype but not with the 15ADON or the NIV chemotype. The latter result is agreeing with findings from the above mentioned database study where the majority of the European *F. culmorum* isolates belonged to the 3ADON chemotype (Pasquali et al., 2016). Apart from the *F. poae* and *F. culmorum* incidences, the 15ADON and the NIV genetic chemotypes were both positively correlated with the incidences of other *Fusarium* species, all three toxins and the *F. graminearum* DNA. This finding suggests multiple toxigenic abilities of various FHB causing species and closely linked ecological niches for infection and toxin production.

As expected, there was a positive and highly significant association between the NIV content and the NIV genetic chemotype. However, the *F. poae* DNA showed negative significant correlations to all three chemotypes including that of NIV, although *F. poae* is able to produce NIV. In fact, the qPCR primers for the different chemotypes were developed for the *F. graminearum* species complex (Kulik et al., 2011), hence, these results represent a further confirmation of the lack of association between the *F. poae* and *F. graminearum* species complex.

4.5. Effect of cropping factors on mycotoxins and fungal occurrence

With respect to *F. graminearum* and DON in wheat, it has been well documented that the risk is increased with maize as the previous crop, especially if combined with reduced tillage (Dill-Macky, 2008; Edwards and Jennings, 2019; Gourdain et al., 2011; Vogelgsang et al., 2011), leaving *F. graminearum* infested crop residues on the soil surface. In the current study, one of the aims was to verify whether the effect of this cropping factor combination holds true for growers' wheat samples collected over an extended time span and whether this pattern could be extrapolated onto other toxins and other FHB causing species. Furthermore, another objective was to verify the effect of other agricultural measures that could be taken into account for reducing the risk of FHB in wheat. For the multiple correspondence analyses (MCA), samples with toxin contents below the respective LODs were removed from the dataset, therefore, the results of the analyses discussed in the following paragraphs should not be inferred to situations where environmental conditions might suppress fungal growth and toxin production.

4.5.1. *Fusarium graminearum* and DON

The MCA on *F. graminearum* and DON showed that higher disease and toxin levels were closely associated with the previous crop maize, pre-previous crop maize and reduced tillage, confirming results from earlier studies in Europe (Blandino et al., 2012; Edwards, 2004) and North-America (Dill-Macky and Jones, 2000; Schaafsma et al., 2005). It should be noted though that the presence of residues rather than the tillage itself has an effect on the disease pressure (Maiorano et al., 2008). The pre-previous crop canola was also closely linked to samples with high DON content. This finding could be an illustration of the wide host range of *F. graminearum* as shown in a study by Chongo et al. (2001) where infected wheat florets were used as inoculum for seed-borne diseases in a large number of arable crops. Another reason could be the fact that 83% of the cases with pre-previous crop canola came from samples with maize as the previous crop. Interestingly, low disease levels and toxin contents were not only associated with samples from ploughed fields but with the previous crops potato, canola and pasture. Therefore, these crops may provide an alternative previous crop, especially for growers who employ reduced or zero tillage. Low DON levels were also closely linked to 'other' previous crops, which contained mostly soya beans. In fact, Dill-Macky and Jones (2000) demonstrated in a three-year field experiment 25 and 50% less FHB infection when the previous crop was soya beans compared to when it was wheat or maize, respectively. As the cultivation of soya beans in Switzerland is experiencing increased interest, it would be worthwhile

to further investigate this option. Certainly, the choice of a less susceptible wheat cultivar would be a simple and sustainable approach to help growers to reduce the risk of FHB in cereals. The Swiss recommended cultivar list classifies the FHB resistance based on inoculations with *F. culmorum* conidia and visual observation of symptoms. Hence, it is not clear whether a breeding programme relying on a less frequent FHB causing species and lacking toxin measurements is sufficient for truly estimating the susceptibility. Nevertheless, in the current study, samples from the cultivars with the least susceptible cultivars, Arina and Titlis, were mostly observed in samples with the lowest DON content. However, both Arina and Titlis are not performing well with respect to yield and are susceptible to brown rust (*Puccinia triticina* (Eriks.)). Furthermore, Arina is a tall cultivar, which *per se* represents an advantage with respect to FHB resistance (Gervais et al., 2003), but without growth regulators, this cultivar is prone to lodging which could further increase the risk for late FHB infections during the season (Langseth and Stabbetorp, 1996).

With respect to fungicides, samples with high levels of DON and fungal occurrence were mainly found together with samples from fields where strobilurin-based fungicides were applied. This finding is in line with results from inoculations with *F. culmorum* in Switzerland (Forrer et al., 2000), from naturally and artificially inoculated field trials in the United Kingdom (Simpson et al., 2001) and from inoculated paddy fields in Japan (Nakajima, 2010) where azoxystrobin applications led to increased DON concentrations. Forrer et al. (2000) and Simpson et al. (2001) observed that this fungicide selectively controlled *Microdochium nivale* and, thus, proposed that the elimination of this species allowed greater colonisation by toxigenic *Fusarium* species. Likewise, naturally-infected field experiments with wheat and barley in France reported similar findings on the selective control of *M. nivale* with strobilurins but significantly reduced FHB disease levels after applications of triazole-based fungicides (Ioos et al., 2005). Moreover, *in vitro* studies by Müllenborn et al. (2008) indicated differences in fungicide sensitivity among different *Fusarium* species and saprophytic wheat head-colonising fungi antagonistic to *Fusarium* species. The authors suggested that fungicides could modify the balance within the fungal flora of wheat heads, which in turn, might affect the subsequent mycotoxin contamination. Ideally, fungicides to control *F. graminearum* should be applied at early or mid-anthesis, hence, the timing of fungicide application is highly critical as observed by Giraud et al. (2011) and reviewed in Beres et al. (2018). Within the current study, very few growers indicated the anthesis period and thus it was not possible to know at which exact growth stage the fungicides were applied. On the horizontal axis, the cropping system was highly discriminating and samples from organic growers were more closely associated with samples containing low amounts of DON, whereas samples with higher DON contents and higher disease severity were mostly observed in samples from integrated or conventional farms. Even though the number of samples from organic farms was rather low, they were present in years with both high and low disease pressure. Therefore, we can rule out that the better results of samples from organic cropping systems were induced by a preferential collection of samples from organic farms in years with low disease pressure. This pattern of lower mycotoxin levels in wheat samples from organic farms compared with samples from integrated or conventional farms has already been reported in earlier multi-year studies for DON (Birzele et al., 2002; Munger et al., 2014), DON and ZEA (Meister, 2009), DON and moniliformin (Bernhoft et al., 2010) and for T-2/HT-2 (Edwards, 2009b). Hence, it is unlikely that organic cereal production would increase the risk of FHB epidemics and DON. It is assumed, however, that various agronomic practices associated with organic farming are responsible for lower levels of mycotoxins, such as reduced nitrogen applications, more ploughing, fewer cereal intense rotations (Bernhoft et al., 2010; Edwards, 2009a, 2009b) and earlier harvest dates (Edwards and Jennings, 2019), compared with conventional farming systems. These

associated but varying factors might also explain reports from various countries with contradictory results, demonstrating either no differences in toxin contamination between cropping systems versus higher or lower contaminations as reviewed in Brodal et al. (2016).

4.5.2. *Fusarium graminearum* and ZEA

The two groups of response variables with high versus low levels of *F. graminearum* abundance and ZEA contents were not as closely associated as those with DON. The decreased association among these variables may be due to the fact that the accumulation of ZEA depends not only on *F. graminearum* infection during or shortly after anthesis but is also highly influenced by the weather conditions before harvest. In fact, an analysis of mycotoxin data over ten years in the UK showed that the ZEA levels were particularly high in years with delayed wet harvests (Edwards, 2011) and, thus, can be highly variable from one year to the next. As observed for DON, high ZEA levels were mainly observed in wheat samples from fields with reduced tillage and where the previous crop or the pre-previous crop was maize. In addition, the previous and pre-previous crop sugar beet was highly linked to samples with high ZEA concentrations. It has been shown that sugar beets can be infected by a wide array of species from the genus *Fusarium*, including the ZEA producing species *F. culmorum* and *F. graminearum* (e.g. Bosch and Mirocha, 1992; Nitschke et al., 2009), resulting in damping off and root rot. Furthermore, in the complete dataset of the current study, sugar beet was the previous crop with the second highest frequency, suggesting that inoculum from soil-borne *Fusarium* diseases in sugar beet could also serve as an important infection source for the following wheat crop. On the other hand, samples with low disease levels and low ZEA concentrations were mostly observed together with samples where the previous crop was pasture. This finding could be surprising as several grasses can be infected by *F. graminearum* (e.g. Inch and Gilbert, 2003). However, in Switzerland, pastures are sown mostly in mixtures that typically also include several legume species such as different clover types (*Medicago*, *Trifolium* and *Lotus* species) and sainfoin (*Onobrychis viciifolia*) (Azuhwi et al., 2011; Nyfeler et al., 2011). Hence, this richness suggests that mixed species pastures could suppress *F. graminearum* development and subsequent toxin production. As for DON, samples from fields with strobilurin-based fungicide applications were associated with high ZEA contents whereas samples from organic farms were associated with lower ZEA contents, but these effects were not as pronounced as for DON. This could be due to the strong influence of weather conditions before harvest on ZEA accumulation and also because of the overall higher variations of ZEA contents observed in various monitoring studies (reviewed in Brodal et al., 2016).

4.5.3. *Fusarium poae* and NIV

In contrast to DON and ZEA, the previous crop maize was not associated to either of the two *F. poae* occurrence and NIV concentration groups. This suggests that if *F. poae* were to become a more prominent species within the FHB complex, maize as a previous crop might not increase the risk of infection. High levels of *F. poae* occurrence and NIV were associated with samples where the previous crop was canola or sugar beet. To our knowledge, there has been no report on *F. poae* isolated from canola, however, *F. poae* has, apart from wheat, a wide host range, including barley and oats (e.g. Schöneberg et al., 2018a, 2016), rice, alfalfa, soya bean, sunflower (Booth, 1971), several grasses (Nedělník et al., 2015), various broad-leaved weed species (Jenkinson and Parry, 1994), tomato (Stenglein et al., 2009) and sugar beet (Bosch and Mirocha, 1992). The high NIV contents could be partially due to the presence of *F. graminearum* NIV chemotypes. Additionally, the hosts canola and sugar beet might provide a trigger in the fungus to express genes for *FgTri13*, which lead to NIV instead of DON (Kimura et al., 2007). It was also suggested that drought and increased cropping of maize might select for *F. culmorum* NIV chemotypes (Beyer et al., 2014; Pasquali et al., 2010). This hypothesis has to be tested further and

should also include *F. graminearum*. The cultivar testing in Switzerland is based on artificial inoculations with *F. culmorum*, hence, it was not surprising that the association between low *F. poae* occurrence and low NIV levels with wheat cultivars of the resistance categories 'very good' and 'good' was rather weak. In the reduced dataset, where samples with toxin contents below the LOD were removed, there was no sample from a field where solely strobilurin-based fungicides were applied. Associations with toxin levels and fungicide use were weak and the closest one was observed between samples with higher NIV content and those where both strobilurins and triazoles were applied compared with those where no fungicides were used. While the poor efficacy of strobilurins towards some *Fusarium* species has been documented (Dubos et al., 2013, 2011, Forrer et al., 2000; Müllenborn et al., 2008), the unclear association between triazoles and high NIV levels raises the question whether triazole is sufficiently effective against the NIV producers that occurred in the present study. Furthermore, an *in vitro* study by Müllenborn et al. (2008) investigated the sensitivity of various FHB causing species and several saprophytes to triazoles and strobilurins, which demonstrated that the sensitivity to triazoles differs between compounds and, as expected, also between *Fusarium* species. For example, the experiment with the triazole prothioconazole showed that, compared with *F. poae*, the species *F. cerealis*, *F. graminearum* and *F. avenaceum* were 36, four and three times less sensitive, respectively. Compared with the MCAs for DON and ZEA, the cropping system was not a discriminating factor, which also reflects the unclear associations of the *F. poae* and NIV groups with fungicides and previous crops. It was striking that lower NIV levels were clearly associated with reduced tillage whereas higher NIV levels and higher *F. poae* occurrences were closely linked with samples from ploughed fields. This finding is in sharp contrast to the results for DON and ZEA and suggests that ploughed fields promote *F. poae* infection and NIV production while suppressing *F. graminearum* inoculum. The reason for the obtained results are not clear but underline that *F. poae* possesses most probably not only a different life cycle but also other means of survival and primary infection routes compared with those of *F. graminearum*.

4.5.4. Effect of previous crop and tillage on *Fusarium graminearum* occurrence, DON, ZEA and the exceedance of maximum limits

Certainly, the effect of the year on the ratio of samples exceeding the maximum limits for DON and ZEA by 0 to 27% and 0 to 12%, respectively, illustrates the importance of the weather conditions during and after wheat anthesis (Vogelgsang et al., 2017). Similar effects were observed in a wheat monitoring project in the UK between 2006 and 2013, where the ratio of samples exceeding the maximum limits ranged between 0 to 13% for DON and between 0 and 29% for ZEA (Edwards and Jennings, 2019). Nevertheless, the MCAs and the two-factorial ANOVA demonstrated that both previous crop and tillage had significant effects on DON and the occurrence of *F. graminearum*, while for ZEA, the previous crop showed a significant effect. Although the high DON content in samples from fields with previous crop maize and reduced tillage was expected, it was nevertheless remarkable that the average DON content was decreased by 84% when maize residues were ploughed under and even by 95% when fields with the previous crop peas were ploughed. This eminent reduction of mycotoxin contents from commercial wheat samples underlines that the growers can strongly reduce the risk by the choice of agricultural measures, regardless of year effects. During the years with the highest average toxin concentrations, 29 and 5% of the samples exceeded the DON and ZEA guidance values for pig feeding stuffs, respectively. The toxicological relevance of this material is not clear. In general, the ratio of wheat in pig feed can comprise up to 40% (personal communication Claude Chaubert, Agroscope). This might lead to a considerable 'dilution' effect, however, maize grains are also commonly used as an important feed component, and it is well known that maize grains can contain even higher amounts of mycotoxins (e.g. Dorn et al., 2011, 2009).

The fact that ploughing, as opposed to reduced or zero tillage, decreases the risk for *F. graminearum* infection represents a dilemma for sustainable wheat production. Several Swiss cantons support reduced tillage through direct payments to growers and currently, reduced and zero tillage are employed on approximately 4 and 22% of the arable land (without pastures), respectively (Anonymous, 2017a). Indeed, there are many positive effects of reduced tillage, such as decreased soil loss through erosion, less fuel, machine and labour costs, reduced CO₂ emissions and an increase in beneficial soil organisms (cited in Holland, 2004). Furthermore, while maize crop residues might increase the risk of *F. graminearum* infections, they might in turn decrease stem-based diseases in wheat (Bateman et al., 2007). Fine mulching of crop residues can greatly accelerate the decomposition and, thus, reduce the survival of *F. graminearum*, however, in certain years with high disease pressure, the DON content in harvested grains might still be above the maximum limits (Vogelgsang et al., 2011).

4.5.5. Effects of cropping factors on *Fusarium* communities and other cereal associated species

As of now, it is unclear which agricultural factors might affect entire *Fusarium* communities. For example, Klix et al. (2008) conducted a 3-year wheat monitoring in Northern Germany and suggested that tillage and previous crops did not show an effect on the FHB species composition in wheat heads. In contrast, a structured sampling of wheat from southern Sweden demonstrated that the agricultural intensity, including application of fertilisers, pesticides and tillage, did have an effect on the abundance of certain *Fusarium* species in wheat grains (Karlsson et al., 2017). Moreover, efficacies of individual control measures, including cropping factors, may critically depend on the exact composition of the FHB complex in a given area (Xu and Nicholson, 2009), rendering the choice of the appropriate measure a challenge. Furthermore, management strategies, including different tillage regimes and cropping systems could influence the wheat microbiome and thus, by favouring antagonistic endophytes, could suppress disease pressure by FHB causing pathogens such as *F. graminearum* (Gdanetz and Trail, 2017). Xu and Nicholson (2009) suggested that agricultural measures which target certain individual species within the FHB complex might create niche vacancies that will be filled by other species within the complex. This might well be the case for *F. poae* considering the opposing effects of reduced tillage on *F. graminearum* with DON and ZEA versus *F. poae* and NIV or NIV chemotypes.

4.6. Remaining questions with respect to *Fusarium poae*

Fusarium poae does not lead to typical head blight symptoms but rather to small, distinct necroses on glumes, hence, infections can be easily overseen. In addition, Vanheule et al. (2017) discussed not only the ability of *F. poae* to produce highly toxic type A and type B trichothecenes but also its ability to undergo both asexual and sexual reproduction, further enhancing its genetic diversity for successful spread and adaptation. Apart from cropping factors, climate change will have direct (weather variables) and indirect (changed cultivation practices) effects on mycotoxin contamination of small-grain cereals (West et al., 2012). Several surveys from geographic areas already reported an increase or even dominance of *F. poae* (Chrpová et al., 2016; Lindblad et al., 2013; Parikka et al., 2012). Roháček and Hudec (2005) reported that the highest incidence of *F. poae* corresponded to its abundance in warm places and suggested that *F. poae* is more adaptable to agro-environmental conditions than *F. graminearum*. Likewise, Xu et al. (2008) associated *F. poae* with relatively drier and warmer conditions, whereas *F. graminearum* was associated with warmer/humid conditions. Although it remains to be seen which FHB causing species will predominate in the future and, thus, which control measures should become more prominent, further research on the life cycle and the epidemiology of *F. poae* is warranted to develop risk reducing strategies against this highly toxigenic species.

5. Conclusions

This is the first long-term survey on FHB in wheat, considering four different response variables, including fungal incidence, fungal DNA, genetic chemotypes and mycotoxins, and studying them in association with six different cropping factors.

For DON and ZEA, the risk of exceeding maximum limits can be greatly reduced by avoiding the combination of maize as a previous crop with reduced tillage. Soya bean as a previous crop might potentially contribute to a reduced risk of infection by *F. graminearum* and *F. poae*. The data suggest that NIV is produced by several species, including *F. poae*, *F. cerealis*, *F. culmorum* and *F. graminearum*. Low NIV contents and low occurrences of *F. poae* were associated with reduced tillage as opposed to low DON and ZEA contents, which were associated with samples from ploughed fields. The discrepancy between the occurrences of *F. poae* and those of *F. graminearum* indicates different ecological niches for infection.

In the current study, *F. poae* was the second most dominant species and several other reports indicate an increase or even dominance within the FHB species complex. Since NIV is several times more toxic than DON, this finding prompts future monitoring for the presence of this toxin in food and feed products.

Funding

SV acknowledges the support from the Swiss federal research institution Agroscope for the project 'Fusaria and mycotoxins in maize and cereals'. MB acknowledges the Administration des Services Techniques de l'Agriculture, Luxembourg, for supporting the project 'Sentinelle'.

Acknowledgements

We thank Irene Bänziger, Andreas Kägi and various interns for excellent technical assistance. We are also grateful to cantonal plant protection officers who provided addresses of wheat growers and helped in distributing annual results. Finally, we would like to thank all participating growers for sending samples and filling in the questionnaires.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eja.2019.01.002>.

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