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Multi-mycotoxin screening of feed and feed raw materials from Africa

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RESEARCH ARTICLE

Abstract

As animal feed is prone to infestation with mycotoxin-producing fungi, mycotoxin contamination of feed should be monitored. Here, we report a multi-mycotoxin survey of feed samples from Africa. We determined the concentrations of aflatoxins, fumonisins, deoxynivalenol, T-2 toxin, zearalenone and ochratoxin A in 1,045 samples of finished feed and feed raw materials (maize, maize silage, other cereals, etc.) from South Africa and 318 samples from Algeria, Tunisia, Morocco, Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya, Tanzania, Zambia and Madagascar. We compared the measured mycotoxin concentrations to regulatory limits or guidance values that are in effect in the European Union and analysed the co-occurrence of these mycotoxins. To determine the occurrence of other fungal secondary metabolites, a subset of the samples was analysed using a multi-analyte liquid chromatography tandem mass spectrometry-based method for the simultaneous detection of over 700 fungal metabolites. We found that 33.3% of maize samples and 54.4% of finished feed samples from Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya and Tanzania exceeded the European regulatory limit of 20 ng/g aflatoxins. The *Fusarium* mycotoxins zearalenone, fumonisins and deoxynivalenol were prevalent in all commodities from all countries, but concentrations were in most cases below European guidance values. Concentrations of deoxynivalenol and zearalenone were correlated. Several other *Fusarium* metabolites occurred frequently (e.g. moniliformin, beauvericin, aurofusarin) or in high concentrations (e.g. aurofusarin, fusaproliferin). Furthermore, high levels of diplodiatoxin were occasionally detected in samples from South Africa and the *Alternaria* metabolite tenuazonic acid was prevalent and reached high concentrations. In conclusion, aflatoxins frequently occurred in African feed samples in potentially unsafe concentrations. While *Fusarium* mycotoxins mostly occurred in concentrations below European guidance values, a correlation between deoxynivalenol and zearalenone concentrations suggests that toxicological interactions of these compounds deserve attention. Several less investigated fungal secondary metabolites occurred frequently or reached high concentrations.

Keywords: South Africa, survey, maize, co-occurrence

1. Introduction

Animal feed is frequently contaminated with mycotoxins such as aflatoxins, fumonisins, trichothecenes, zearalenone (ZEA) and ochratoxin A (OTA) due to fungal growth in the field or during storage. Recent surveys detected mycotoxins in a high proportion (>70%) of analysed feed samples (e.g. Kovalsky *et al.*, 2016; Streit *et al.*, 2013). When mycotoxins occur in sufficiently high concentrations, they cause a variety of adverse health effects in animals, thereby compromising their wellbeing and decreasing their productivity (Bryden, 2012). Moreover, aflatoxins are carried over into milk, thereby posing a health hazard to human consumers (Becker-Algeri *et al.*, 2016).

As different types of mycotoxins may be produced by the same fungal strain or by different strains under similar environmental conditions, mycotoxins frequently co-occur in feed (Rodrigues and Naehrer, 2012; Streit *et al.*, 2012). Consequently, animals may frequently be exposed to multiple mycotoxins at the same time. The effect of a mycotoxin mixture may be equal to ('additive'), greater ('synergistic') or lower ('antagonistic'), compared to the summed effects of the individual mycotoxins. In many cases,

mycotoxin mixtures cause additive or synergistic effects (Alassane-Kpembi *et al.*, 2017; Grenier and Oswald, 2011). Therefore, mycotoxin co-contamination may frequently increase the toxic effect of animal feed.

In recent years, liquid chromatography-mass spectrometry (LC-MS) based methods for the simultaneous analysis of up to several hundreds of fungal metabolites have been developed and are increasingly being used for multi-mycotoxin screening of agricultural commodities. As a result, awareness is increasing that not only major mycotoxins – i.e. fungal metabolites whose toxicity is well documented – frequently occur in agricultural commodities, but also a plethora of other fungal metabolites. For these metabolites, only limited or no toxicological data are available (Fraeyman *et al.*, 2017; Gruber-Dorninger *et al.*, 2017).

Regulatory limits for mycotoxin concentrations in feed have been put in place in many parts of the world, e.g. in the European Union (EU), where maximum levels are enforced for aflatoxin B_1 (AFB_1) and guidance values have been established for fumonisins, deoxynivalenol (DON), ZEA and OTA. For many African countries, no mycotoxin risk assessment is available and regulations or other measures taken against mycotoxin exposure of humans and animals are insufficient (Matumba *et al.*, 2017). One requirement for mycotoxin risk assessment is the availability of occurrence data. Currently, only limited mycotoxin occurrence data from African countries are available (Chilaka *et al.*, 2017; Matumba *et al.*, 2017). Since 2004, BIOMIN has been conducting a survey program to monitor the global occurrence of aflatoxins, fumonisins, trichothecenes, ZEA and OTA in feed and feed raw materials. In total, 1,363 samples from 14 African countries (South Africa, Algeria, Tunisia, Morocco, Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya, Tanzania, Zambia and Madagascar) were collected between March 2006 and November 2017. This dataset was analysed in this study.

In this study, we determined the concentrations of aflatoxins, fumonisins, DON, T-2 toxin, ZEA and OTA in 1,045 samples of finished feed and feed raw materials taken from South Africa and 318 samples taken from 13 other African countries during a 10-year period. We compared the concentrations to regulatory limits and guidance values that are in place in the EU. We used European limits for this comparison, as European legislation on mycotoxins in feed is most extensive, whereas many African countries do not have legal limits for mycotoxins or have limits only for some mycotoxins. We furthermore analysed the co-occurrence of these mycotoxins and the year-to-year variation of their concentrations. For a subset of the samples (377 samples from South Africa and 113 samples from the other 13 countries) we analysed fungal secondary metabolite occurrence in more detail by using a liquid chromatography tandem mass spectrometry (LC-MS/MS)-based method for the quantification of over 700 metabolites. Comparison of mycotoxin occurrence data to EU legislation helps to assess feed safety. The collection of multi-mycotoxin datasets and data on mycotoxin co-occurrence can support risk assessment and provide a basis to prioritise research efforts on toxicological effects of less investigated fungal metabolites and on mycotoxin mixtures relevant to animal nutrition in Africa.

2. Materials and methods

For the BIOMIN Mycotoxin survey, a total of 1,363 feed samples were taken from 14 African countries between March 2006 and November 2017. Countries of origin and sample numbers are represented in Figure 1. In total, 1,045 samples of finished feed and feed raw materials were taken from South Africa and 318 samples were taken from the other 13 countries. The dataset comprised samples of finished feed (numbers in South Africa n_{SAf}=311, numbers in the other 13 countries n_{13}=146), samples of maize (n_{SAf}=305, n_{13}=70), samples of maize silage (n_{SAf}=109, n_{13}=6), samples of wheat or wheat bran (n_{SAf}=50, n_{13}=12), and other feed samples (n_{SAf}=270, n_{13}=84).

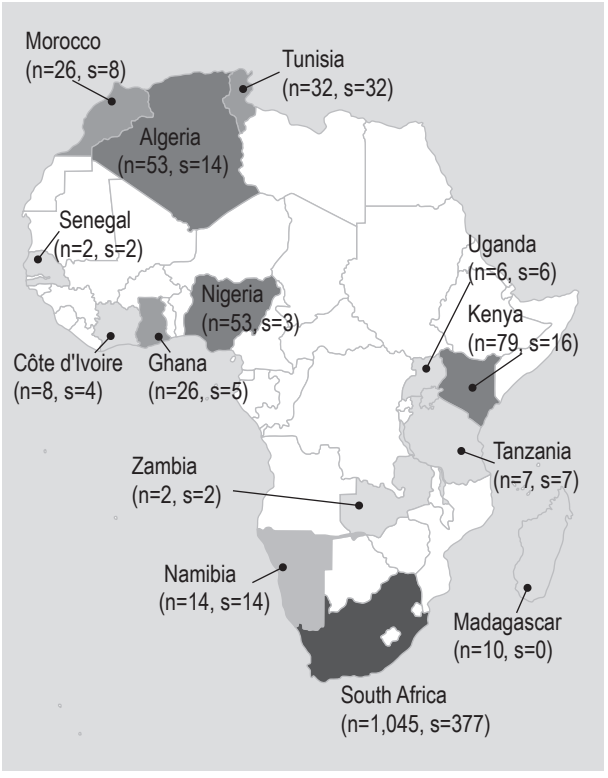

Figure 1. Sample numbers per country of origin. For each country, the number of samples analysed for major mycotoxins ('n') and the number of samples subjected to LC-MS/MS multi-mycotoxin analysis ('s') are given. Countries are coloured in shades of grey according to number of samples analysed for major mycotoxins.

Sampling as well as milling and homogenisation of samples was performed as described by Kovalsky *et al.* (2016). For sampling, paper bags or bags with ventilation were used to prevent humidity build up, drying was applied if the samples had a high moisture content and samples were immediately sent to the laboratory for analysis. In total, 377 samples from South Africa and 113 samples from the other 13 countries were analysed using a LC-MS/MS multi-mycotoxin analysis method for the simultaneous detection of over 700 fungal metabolites at the Department of Agrobiotechnology (IFA-Tulln) at the University of Natural Resources and Life Sciences Vienna (BOKU) in Tulln, Austria. Analysis and quality control was carried out as described previously (Kovalsky *et al.*, 2016). In total, 471 samples from South Africa and 8 samples from the other 13 countries were analysed by Romer Labs GmbH (Tulln, Austria) using LC-MS/MS. Limits of detection (LODs) were 0.2 ng/g for AFB_1, 0.2 ng/g for aflatoxin B_2 (AFB_2), 0.2 ng/g for aflatoxin G_1 (AFG_1), 0.2 ng/g for aflatoxin G_2 (AFG_2), 4 ng/g for ZEA, 20 ng/g for DON, 20 ng/g for fumonisin B_1 (FB_1), 20 ng/g for fumonisin B_2 (FB_2), 0.2 ng/g for OTA and 2 ng/g for T-2 toxin. A further 10 samples from the other 13 countries were analysed using the same method at Romer Labs in Singapore (Singapore). In total, 156 samples from South Africa and 181 samples from the other 13 countries were analysed by Romer Labs GmbH (Tulln, Austria) using high performance liquid chromatography (HPLC). LODs were 0.3 ng/g for AFB_1, 0.1 ng/g for AFB_2, 0.1 ng/g for AFG_1, 0.1 ng/g for AFG_2, 10 ng/g for ZEA, 50 ng/g for DON, 25 ng/g for T-2 toxin, 25 ng/g for FB_1, 25 ng/g for FB_2 and 0.2 ng/g for OTA. In total, 5 samples from South Africa and 6 samples from the other 13 countries were analysed by Romer Labs GmbH in Tulln using enzyme-linked immunosorbent assay (ELISA). LODs were 1 ng/g for AFB_1, 1 ng/g for AFB_2, 1 ng/g for AFG_1, 1 ng/g for AFG_2, 20 ng/g for ZEA, 200 ng/g for DON, 10 ng/g for T-2 toxin, 1.9 ng/g for OTA, 200 ng/g for FB_1 and 200 ng/g for FB_2. In total, 36 samples from South Africa were analysed at the Southern African Grain Laboratory (SAGL, The Willows, South Africa) using LC-MS/MS. LODs were 2.5 ng/g for AFB_1, 2.5 ng/g for AFB_2, 2.5 ng/g for AFG_1, 2.5 ng/g for AFG_2, 10 ng/g for ZEA, 50 ng/g for DON, 10 ng/g for T-2 toxin, 10 ng/g for FB_1, 10 ng/g for FB_2 and 2.5 ng/g for OTA.

For all analysed compounds except aflatoxins, the threshold of relevant concentrations was defined as >1.0 ng/g or >LOD, whichever was higher. For the sum of AFB_1, AFB_2, AFG_1 and AFG_2, the threshold of relevant concentration was defined as >0.5 ng/g. Correlations between mycotoxin types was analysed with the ggpairs in the ggally package (Schloerke *et al.*, 2017) using R software version 3.3.0 (R Core Team, 2017). Results below the LOD were treated as zero values in the correlation analysis.

3. Results

Occurrence of major mycotoxins in different commodities

Aflatoxins were frequently detected in samples from Algeria, Tunisia, Morocco, Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya, Tanzania, Zambia and Madagascar (Table 1). In total, 49.4% of maize samples, 64.1% of finished feed samples and 28.6% of other cereal samples were contaminated with aflatoxins. Total aflatoxin concentrations (sum of AFB_1, AFB_2, AFG_1 and AFG_2) that exceeded 20 ng/g, the highest EU limit for aflatoxin B_1 (AFB_1), were detected in samples from Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya and Tanzania. In total 33.3% of maize samples and 54.4% of finished feed samples from these countries contained more than 20 ng/g aflatoxins. Aflatoxins were less prevalent (≤9.6% positive samples) in maize, maize silage, other cereal and finished feed samples from South Africa and merely ≤3.2% and ≤1.6% of samples exceeded the lowest and highest EU limit, respectively (Table 2).

ZEA was most frequently detected in finished feed (57.5% positive samples) from South Africa and in other cereals (75.0% positive samples) and finished feed (64.4% positive samples) from the other 13 countries (Tables 1 and 2). High levels of ZEA were occasionally detected in maize and maize silage from South Africa (Table 2). In total, 8.4 and 17.7% of samples exceeded the lowest EU guidance value, but only 0.3 and 1.0% of samples exceeded the highest EU guidance value in case of South African maize and maize silage, respectively. For all other commodities, all samples complied with the highest EU guidance value.

DON was prevalent in all commodities from South Africa (67.2-80.6% positive samples) and in finished feed (62.3% positive samples) and other cereals (81.0% positive samples) from the other 13 countries (Tables 1 and 2). High levels of DON were occasionally detected in all commodities. In case of other cereals from South Africa, 15.9% of samples exceeded the lowest EU guidance value for DON and still 3.2% of samples exceeded the highest EU guidance value. In case of other cereal samples from the other 13 countries, 19.1% exceeded the lowest EU guidance value for DON, but all samples complied with the highest EU guidance value. T-2 toxin showed a low prevalence (≤10.3% positive samples) and low concentrations (≤80 ng/g) in all commodities (Tables 1 and 2).

Fumonisins were most prevalent in finished feed (83.3% positive samples) and maize (80.1% positive samples) from South Africa and in finished feed (93.7% positive samples) and maize (92.4% positive samples) from the other 13 countries (Tables 1 and 2). High fumonisin levels were occasionally observed and a minor fraction (≤5.3%) of maize and finished feed samples from South Africa and

Table 1. Occurrence of main mycotoxins in different commodities from 13 African countries.

Mycotoxin	n[1]	Positive samples[2]		Median concentration (ng/g)	75th percentile (ng/g)	90th percentile (ng/g)	Maximum concentration (ng/g)	Samples above lowest EU limit (%)[3]	Samples above highest EU limit (%)[3]
		n[1]	%						
Maize									
Aflatoxins[4]	79	39	49.4	0	11	165	379	31.7 (48.7)[5]	20.3 (33.3)[5]
Zearalenone	79	33	41.8	0	17	85	858	3.8	0
Deoxynivalenol	79	33	41.8	0	91	1,211	4,974	8.9	0
T-2 toxin	79	4	5.1	0	0	1.5	61	-	-
Fumonisins[6]	79	73	92.4	1,371	2,387	3,892	10,018	1.3	0
Ochratoxin A	49	11	22.5	0	0	2.4	694	4.1	2.0
Finished feed									
Aflatoxins[4]	145	93	64.1	4.7	48	136	180	48.3 (77.8)[5]	33.8 (54.4)[5]
Zearalenone	146	94	64.4	9.3	43	151	518	9.6	0
Deoxynivalenol	146	91	62.3	154	372	950	1,550	6.2	0
T-2 toxin	145	15	10.3	0	0	2.3	47	-	-
Fumonisins[6]	143	134	93.7	658	1,132	2,859	4,727	0	0
Ochratoxin A	96	28	29.2	0	0	10	207	2.1	0
Other cereals (wheat, barley, rye, triticale, oats)									
Aflatoxins[4]	21	6	28.6	0	0.8	2.1	2.3	0	0
Zearalenone	20	15	75.0	40	59	99	110	5.0	0
Deoxynivalenol	21	17	81.0	467	613	2,724	3,859	19.1	0
T-2 toxin	21	1	4.8	0	0	0	68	-	-
Fumonisins[6]	20	4	20.0	0	0	1,798	10,485	5.0	0
Ochratoxin A	14	5	35.7	0	2.6	4.4	5.3	0	0

[1] Sample number.
[2] Positive samples are defined as >limit of detection, excluding aflatoxins below 0.5 ng/g and other mycotoxins below 1 ng/g.
[3] EU limits in ng/g (including the lowest limit or guidance value stipulated for any commodity or consuming species and higher limits or guidance values stipulated excluding limits for maize byproducts and for oat husks): aflatoxins low: 5, high: 20; zearalenone: low: 100, high: 2,000; deoxynivalenol: low: 900, high: 8,000; fumonisins: low: 5,000, high: 60,000; ochratoxin A: low: 50, high: 250. For T-2 toxin, no EU limits are available. Some mycotoxin groups presented are the sum of more mycotoxins than actually referred to in the regulations (see below).
[4] Sum of aflatoxins B_1, B_2, G_1 and G_2.
[5] Number in bracket refers to samples from Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya and Tanzania only.
[6] Sum of fumonisins B_1 and B_2.

maize and other cereal samples from the other 13 countries exceeded the lowest EU guidance value for fumonisins (Tables 1 and 2). However, all samples complied with the highest EU guidance value.

Prevalence and levels of OTA were generally low and all samples complied with the highest EU guidance value, with the exception of a small fraction (2.0%) of maize samples from 13 African countries (Tables 1 and 2). OTA was most frequently detected in other cereal samples from South Africa (43.1% positive samples).

Year-to-year variation of mycotoxin occurrence in maize from South Africa

To analyse the year-to-year variation of mycotoxin occurrence in a meaningful way, we aimed to compare samples of the same commodity collected from the same country in several consecutive years. For maize from South Africa, we had sufficiently high sample numbers available for the years 2014 to 2017 (2014: n=23; 2015: n=52; 2016: n=39; 2017: n=162). As maize harvest in South Africa starts in April, for the purpose of this analysis a year was defined to start in April and end in March of the subsequent calendar year. As described for the whole dataset of South African maize samples (Table 2), fumonisins, DON and ZEA were prevalent in samples taken from 2014 to 2017, whereas aflatoxins, OTA and T-2 toxin were rarely

Table 2. Occurrence of main mycotoxins in different commodities from South Africa.

Mycotoxin	n[1]	Positive samples[2]		Median concentration (ng/g)	75th percentile (ng/g)	90th percentile (ng/g)	Maximum concentration (ng/g)	Samples above lowest EU limit (%)[3]	Samples above highest EU limit (%)[3]
		n[1]	%						
Maize									
Aflatoxins[4]	282	27	9.6	0	0	0.9	14	0.7	0
Zearalenone	308	145	47.1	0	14	150	6,276	8.4	0.3
Deoxynivalenol	314	253	80.6	290	569	1,436	9,176	13.7	0.3
T-2 toxin	273	2	0.7	0	0	0	80	-	-
Fumonisins[5]	281	225	80.1	177	917	5,020	16,932	5.3	0
Ochratoxin A	269	20	7.4	0	0	0.3	95	0.4	0
Maize silage									
Aflatoxins[4]	109	0	0.0	0	0	0	0	0	0
Zearalenone	102	58	56.9	2.0	35	462	3,975	17.7	1.0
Deoxynivalenol	109	75	68.8	122	360	850	2,943	4.6	0
T-2 toxin	108	0	0.0	0	0	0	0	-	-
Fumonisins[5]	108	43	39.8	0	51	780	1,402	0	0
Ochratoxin A	101	1	1.0	0	0	0	1.3	0	0
Other cereals (wheat, barley, rye, triticale, oats)									
Aflatoxins[4]	63	4	6.4	0	0	0.5	26	3.2	1.6
Zearalenone	62	22	35.5	0	10	144	195	8.1	0
Deoxynivalenol	63	46	73.0	284	627	2,097	11,022	15.9	3.2
T-2 toxin	62	3	4.8	0	0	0	13	-	-
Fumonisins[5]	62	12	19.4	0	0	77	1,119	0	0
Ochratoxin A	51	22	43.1	0	0.9	12	27	0	0
Finished feed									
Aflatoxins[4]	310	18	5.8	0	0	0.5	232	0.7	0.7
Zearalenone	301	173	57.5	5.1	26	116	386	6.3	0
Deoxynivalenol	311	209	67.2	170	373	1,191	9,805	7.4	0.3
T-2 toxin	301	4	1.3	0	0	0	4.5	-	-
Fumonisins[5]	306	255	83.3	146	558	1,807	7,578	1.3	0
Ochratoxin A	259	8	3.1	0	0	0	6.0	0	0

[1] Sample number.

[2] Positive samples are defined as > limit of detection, excluding aflatoxins below 0.5 ng/g and other mycotoxins below 1 ng/g.

[3] EU limits in ng/g (including the lowest limit or guidance value stipulated for any commodity or consuming species and higher limits or guidance values stipulated excluding limits for maize byproducts and for oat husks): aflatoxins low: 5, high: 20; zearalenone: low: 100, high: 2,000; deoxynivalenol: low: 900, high: 8,000; fumonisins: low: 5,000, high: 60,000; ochratoxin A: low: 50, high: 250. For T-2 toxin, no EU limits are available. Some mycotoxin groups presented are the sum of more mycotoxins than actually referred to in the regulations (see below).

[4] Sum of aflatoxins B_1, B_2, G_1 and G_2.

[5] Sum of fumonisins B_1 and B_2.

detected. Therefore, we present the year-to-year variation of fumonisin (Figure 2), DON (Figure 3) and ZEA (Figure 4) concentrations only. Fumonisin concentrations were higher in 2016 than in 2014 and 2015 (Figure 2), whereas DON showed the opposite trend (Figure 3). Both mycotoxins occurred in relatively high concentrations in 2017. The levels of ZEA showed little variation between the years (Figure 4).

Co-occurrence of major mycotoxins

To analyse the co-occurrence of major mycotoxins in samples from South Africa and the other 13 countries, we calculated the correlation between mycotoxin concentrations for any combination of two mycotoxins. The concentrations of the *Fusarium* mycotoxins DON and ZEA showed a positive correlation in the samples from Algeria, Tunisia, Morocco, Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya, Tanzania, Zambia and Madagascar

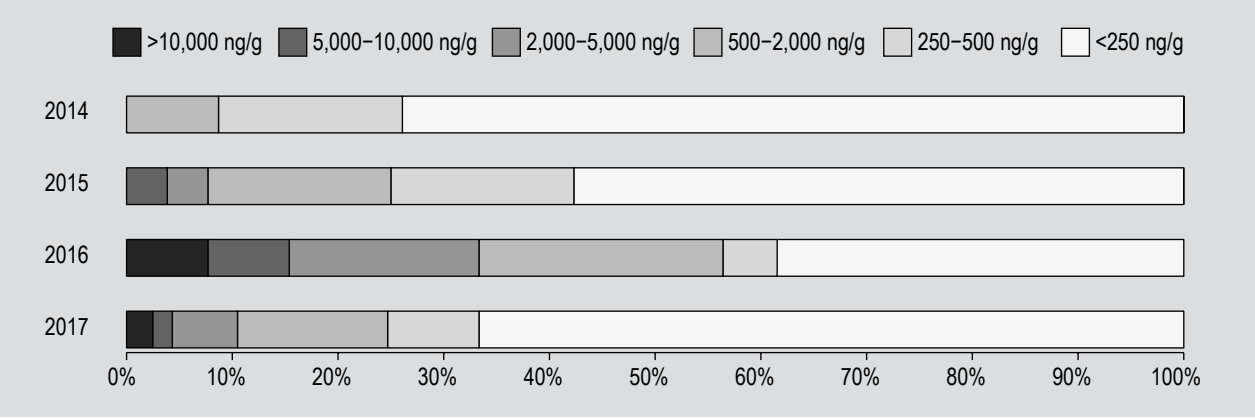

Figure 2. Year-to-year variation of fumonisin concentrations in maize from South Africa. Fumonisin concentrations are a sum of concentrations of fumonisins B_1 and B_2. See text for more details.

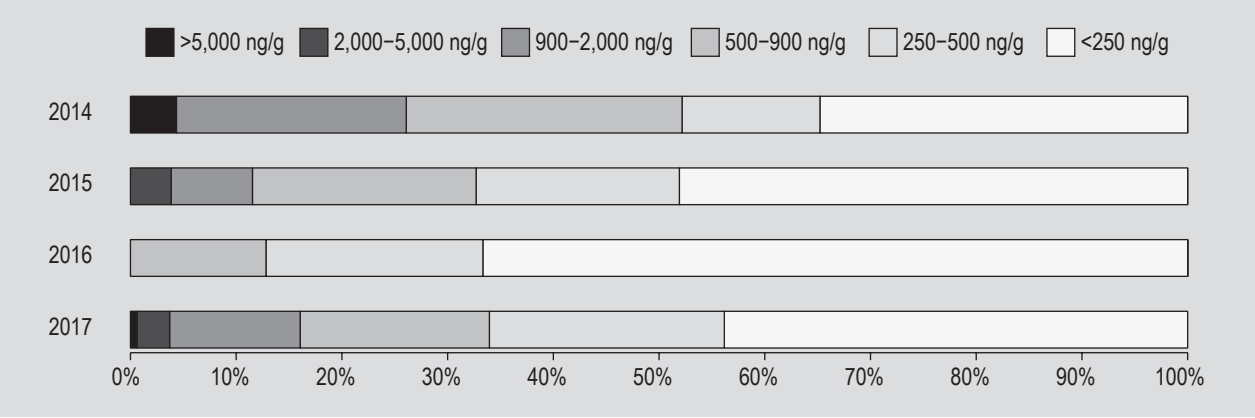

Figure 3. Year-to-year variation of deoxynivalenol concentrations in maize from South Africa. See text for more details.

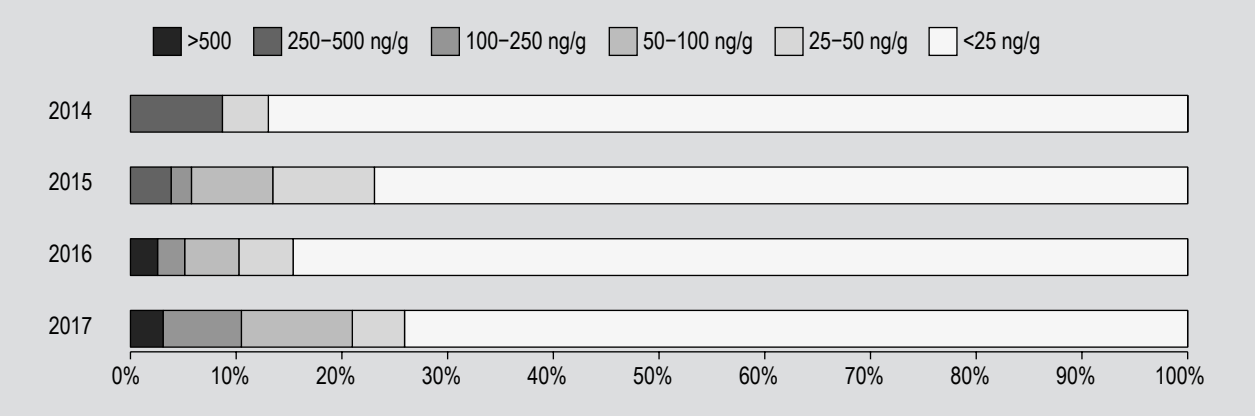

Figure 4. Year-to-year variation of zearalenone concentrations in maize from South Africa. See text for more details.

(r=0.543; Figure 5) and to some extent also in the samples from South Africa (r=0.319; Figure 6). Furthermore, weak positive correlations were observed between concentrations of aflatoxins and OTA, and between concentrations of fumonisins and DON (Figures 5 and 6). For other pairs of mycotoxins, the correlation plot distributions in Figures 5 and 6 were scattered with no clear relationship and low correlation coefficients.

We calculated for each commodity the fraction of samples that contained any combination of two mycotoxins. Aside from DON and ZEA that were also correlated (see above), most frequently observed mycotoxin combinations (detected

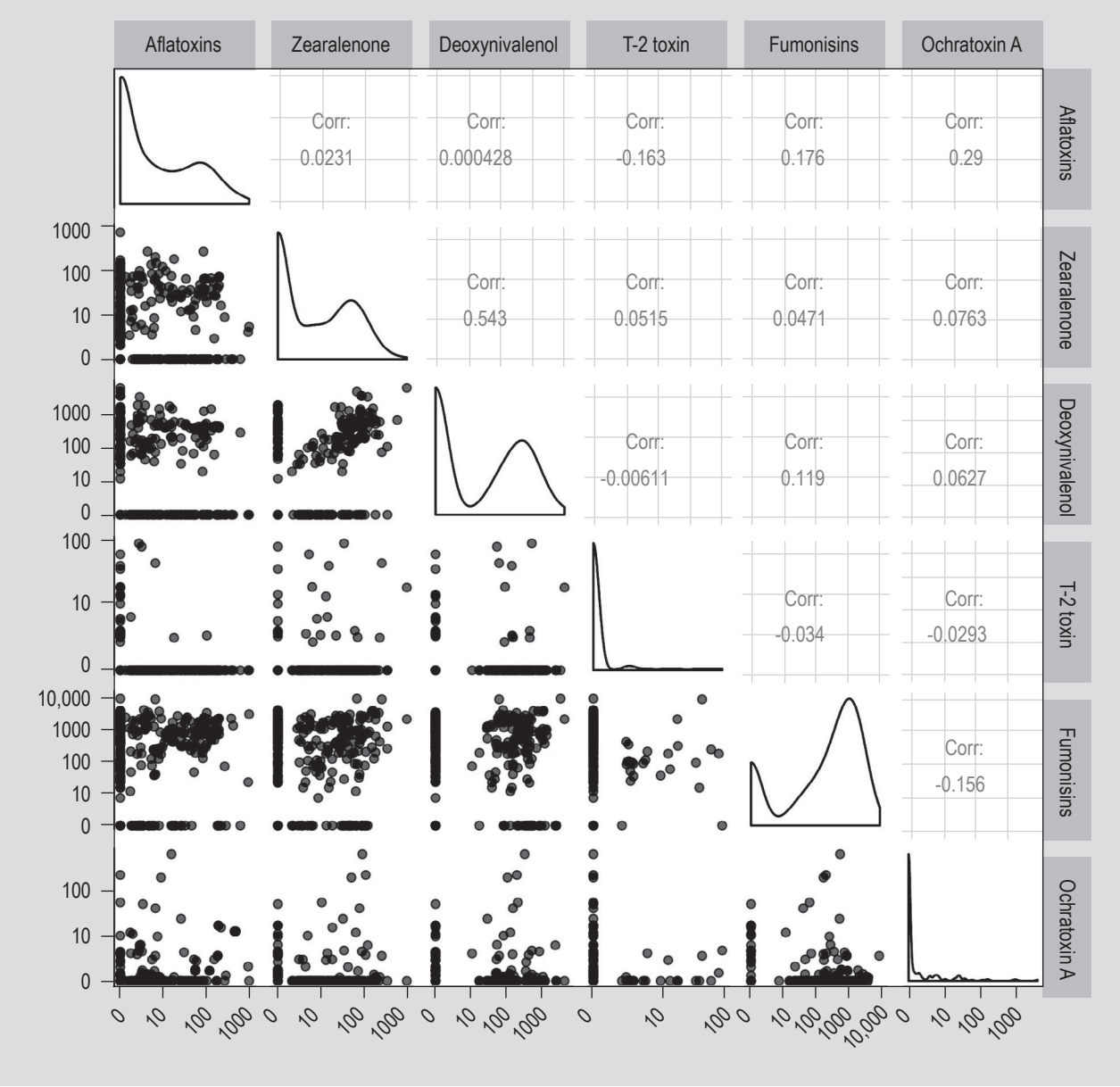

Figure 5. Correlation of mycotoxin concentrations in samples from 13 African countries. Panels on the lower left side show distribution of concentrations (in ng/g) for each combination of two mycotoxins (x- and y-axis are in logarithmic scale). Panels in the diagonal show the distribution of concentrations for each mycotoxin. Panels on the upper right side show the correlation coefficient for each mycotoxin combination. Concentrations of fumonisins and aflatoxins are a sum of concentrations of different compounds as specified in Table 1.

in >50% of samples) were fumonisins and DON, fumonisins and ZEA and fumonisins and aflatoxins. Fumonisins and DON co-occurred in 58.5% and 55.9% of finished feed samples from South Africa and the other 13 countries, respectively. Fumonisins and ZEA co-occurred in 49.8 and 58.7% of finished feed samples from South Africa and the other 13 countries, respectively. Fumonisins and aflatoxins co-occurred in 60.8% of finished feed samples from the other 13 countries.

Multi-mycotoxin analysis

To determine the occurrence of a greater variety of fungal metabolites and of modified mycotoxins, 377 feed samples from South Africa and 113 feed samples from the other 13 African countries were subjected to multi-mycotoxin analysis using LC-MS/MS. In total, 240 and 173 compounds were detected in samples from South Africa and from the other 13 countries, respectively. Between 7 and 82 (average=36) and between 13 and 79 (average=46) metabolites were detected per sample in the dataset from South Africa and the other

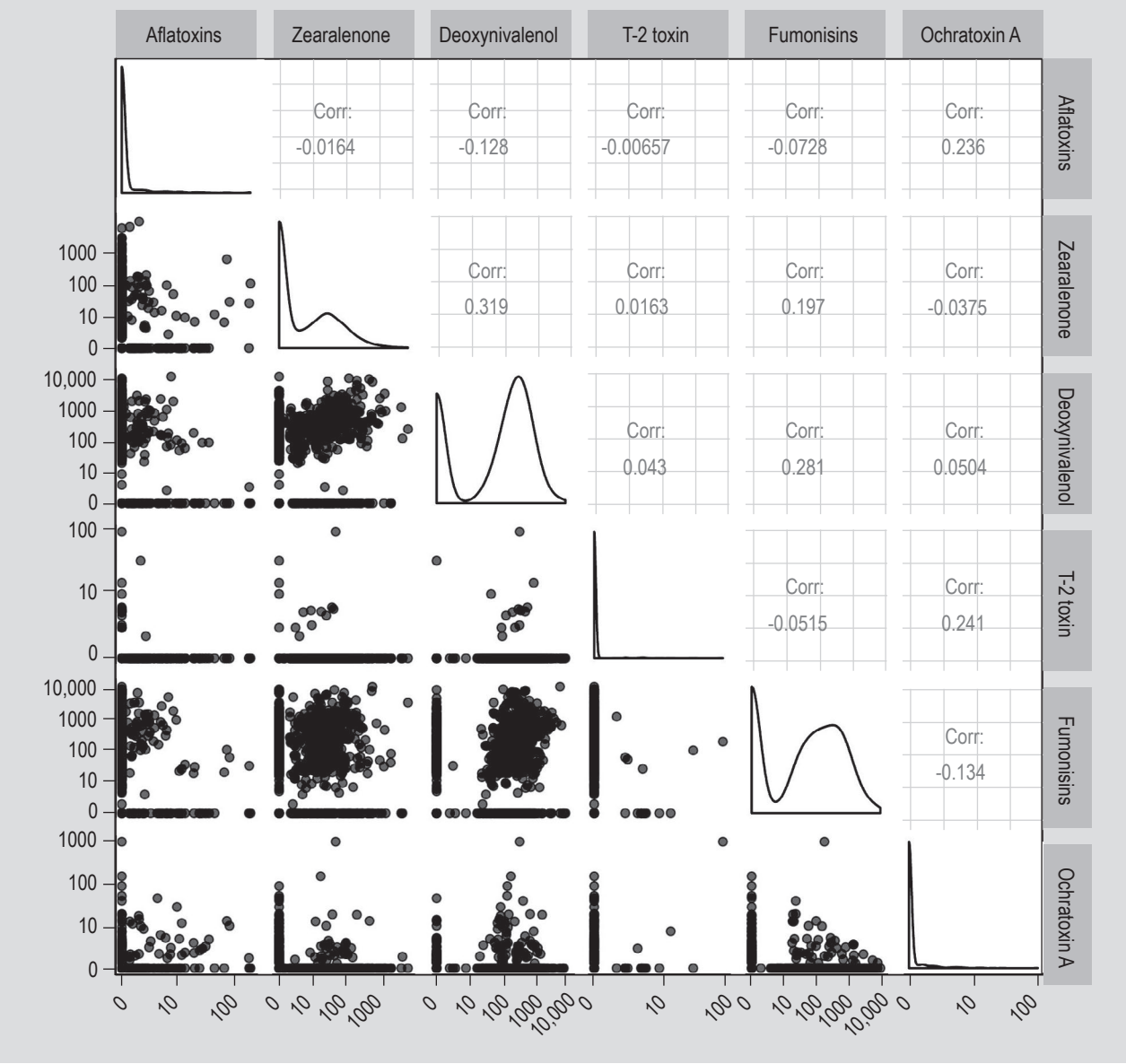

Figure 6. Correlation of mycotoxin concentrations in samples from South Africa. Panels on the lower left side show distribution of concentrations (in ng/g) for each combination of two mycotoxins (x- and y-axis are in logarithmic scale). Panels in the diagonal show the distribution of concentrations for each mycotoxin. Panels on the upper right side show the correlation coefficient for each mycotoxin combination. Concentrations of fumonisins and aflatoxins are a sum of concentrations of different compounds as specified in Table 1.

13 countries, respectively. The most prevalent *Fusarium* metabolites in both datasets (detected in ≥77.7% of samples) were moniliformin (MON) and beauvericin (Tables 3 and 4). Furthermore, aurofusarin (ARF) was detected frequently in both datasets (≥75.3% of samples) and reached levels up to 26.2 µg/g (Tables 3 and 4). Fusaproliferin (FUSA) was not detected with high frequency (13.3%) but reached levels up to 17.2 µg/g in samples from South Africa. Aflatoxins and OTA showed a low prevalence in samples from South Africa (0.3-6.9%; Table 3), but were more prevalent in samples from the other 13 countries (2.7-36.3%; Table 4). Likewise, other *Aspergillus* metabolites, such as tryptophol, 3-nitropropionic acid (NPA) and asperglaucide were more frequently detected in this dataset and reached a high prevalence of 91.2, 89.4 and 87.6%, respectively. The non-toxic compound kojic acid reached a high maximum level of 148.4 µg/g in a sample from South Africa. The *Alternaria* metabolite tenuazonic acid (TeA) was frequently detected in samples from South Africa (62.2%) and from the other 13 countries (69.0%).

Several modified forms of major mycotoxins were detected in feed samples from South Africa and the other 13 countries. A small number of maize, soy oil cake and finished feed samples from South Africa (1 sample) and

the other 13 countries (10 samples) contained low levels (≤32 ng/g) of aflatoxin M_1 (AFM_1). The ZEA metabolites α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL) were detected in a small number of samples from South Africa (6.1 and 6.6%, respectively) and the other 13 countries (4.4 and 8.8%, respectively). Median levels of α-ZOL (2.9 and 2.3 ng/g in samples from South Africa and the other 13 countries, respectively) and β-ZOL (4.1 and 2.6 ng/g in samples from South Africa and the other 13 countries, respectively) were in a similar range as median levels detected for ZEA (13 and 11 ng/g in samples from South Africa and the other 13 countries, respectively). DON-3-glucoside was the most frequently detected modified form of DON with a prevalence of 55.5 and 40.7% and levels up 1.0 and 1.0 µg/g in samples from South Africa and the other 13 countries, respectively. 15-Acetyl-DON and 3-acetyl-DON were detected in a lower fraction of samples (≤10.4%) in both datasets (Tables 3 and 4).

Table 3. Multi-mycotoxin analysis of 377 feed samples collected from South Africa.

Compound[1]	Positive samples		Median (ng/g)	90th percentile (ng/g)	Maximum (ng/g)
	n[2]	%			
Fusarium metabolites					
Moniliformin	300	79.5	56	306	2,599
Beauvericin	293	77.7	16	106	599
Aurofusarin	284	75.3	296	1,432	26,209
Culmorin	272	72.1	59	262	24,229
Deoxynivalenol (DON)	**265**	**70.2**	**289**	**921**	**9,176**
Zearalenone	**261**	**69.1**	**13**	**83**	**6,276**
15-Hydroxyculmorin	258	68.4	178	629	9,319
Fumonisin B_1	**255**	**67.6**	**127**	**1,269**	**9,115**
Fumonisin B_2	**232**	**61.4**	**60**	**605**	**3,077**
DON-3-glucoside	**209**	**55.5**	**24**	**143**	**971**
Equisetin	209	55.3	8.6	57	1,076
Bikaverin	167	44.4	61	257	626
Fusarinolic acid	161	42.8	288	1,679	9,201
Butenolid	157	41.8	52	202	1,817
Fumonisin B_3	**156**	**41.2**	**42**	**281**	**1,014**
Fumonisin B_4	**141**	**37.5**	**45**	**338**	**2,091**
Nivalenol	**108**	**28.7**	**21**	**169**	**1,760**
Fusaric acid	91	24.2	165	1,287	3,227
15-Hydroxyculmoron	69	18.4	99	288	1,038
5-Hydroxyculmorin	54	14.4	162	505	3,020
Fusaproliferin	50	13.3	275	1,951	17,172
Fumonisin A_2	**41**	**10.9**	**29**	**140**	**430**
15-Acetyldeoxynivalenol	**39**	**10.4**	**88**	**232**	**725**
β-Zearalenol	**25**	**6.6**	**4.1**	**20**	**232**
α-Zearalenol	**23**	**6.1**	**2.9**	**7.9**	**81**
HT-2 toxin	**10**	**2.7**	**32**	**46**	**58**
T-2 toxin	**10**	**2.7**	**3.7**	**5.2**	**8.3**
3-Acetyldeoxynivalenol	**7**	**1.9**	**11**	**48**	**71**
Fumonisin A_1	**6**	**1.6**	**9.6**	**15**	**17**
Fumonisin B_6	**4**	**1.1**	**28**	**45**	**49**
Nivalenol glucoside	**2**	**0.5**	**35**	**47**	**50**
Aspergillus metabolites					
Tryptophol	245	64.9	140	781	5,958
Asperglaucide	229	60.9	16	158	3,404
Kojic acid	182	48.4	131	2,034	148,400
3-Nitropropionic acid	145	38.6	4.1	55	1,558
Aflatoxin B_1	**26**	**6.9**	**1.8**	**36**	**109**
Aflatoxin G_1	**10**	**2.7**	**2.5**	**14**	**87**

Table 3. Continued.

Compound[1]	Positive samples		Median (ng/g)	90th percentile (ng/g)	Maximum (ng/g)
	n[2]	%			
Aflatoxin B_2	**7**	**1.9**	**2.8**	**11**	**12**
Ochratoxin A	**5**	**1.3**	**1.8**	**39**	**62**
Aflatoxin G_2	**1**	**0.3**	**14**	**14**	**14**
Aflatoxin M_1	**1**	**0.3**	**3.4**	**3.4**	**3.4**
Alternaria metabolites					
Tenuazonic acid	234	62.2	119	757	4,791
Alternariol	185	48.9	4.4	55	236
Alternariolmethylether	183	48.4	3.1	19	263
Macrosporin	163	43.1	3.8	14	101
Infectopyron	112	29.8	112	1,329	2,600
Penicillium metabolites					
Emodin	232	61.4	5.1	35	199
Rugulusovin	225	59.6	47	389	1,658
Brevianamid F	219	58.0	58	272	991
Curvularin	144	38.3	6.0	32	1,254
Other fungal metabolites					
cyclo(L-Pro-L-Tyr)	296	78.5	237	2,994	34,910
Questiomycin A	207	55.1	14	52	257
Neoechinulin A	190	50.5	12	95	14,320
cyclo(L-Pro-L-Val)	187	49.5	174	6,244	20,589
Citreorosein	153	40.4	5.0	47	673
Diplodiatoxin	102	27.1	168	954	5,623
Flavoglaucin	52	13.8	19	188	4,572

[1] All compounds are listed that (1) are major mycotoxins (i.e. aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin A) or their metabolites (in bold), (2) were present in >40% of samples, or (3) were present in >10% of samples at a concentration of >1 µg/g.
[2] Sample number.

Table 4. Multi-mycotoxin analysis of 113 feed samples collected from 13 African countries.

Compound[1]	Positive samples		Median (ng/g)	90th percentile (ng/g)	Maximum (ng/g)
	n[2]	%			
Fusarium metabolites					
Moniliformin	103	91.2	47	332	2,469
Beauvericin	100	88.5	6	82	842
Fumonisin B_1	**94**	**83.2**	**242**	**1,723**	**3,007**
Fumonisin B_2	**94**	**83.2**	**83**	**606**	**1,009**
Equisetin	94	83.2	13	63	640
Zearalenone	**91**	**80.5**	**11**	**80**	**858**
Aurofusarin	87	77.0	185	1,063	15,056
15-Hydroxyculmorin	85	75.2	58	331	1,961
Culmorin	84	74.3	27	77	1,281
Epiequisetin	75	66.4	3.4	13	161
Fumonisin B_3	**67**	**59.3**	**84**	**279**	**526**
Fumonisin B_4	**65**	**57.5**	**52**	**201**	**501**
Bikaverin	63	55.8	67	310	516
Enniatin B	58	51.3	6.7	50	386

Table 4. Continued.

Compound[1]	Positive samples		Median (ng/g)	90th percentile (ng/g)	Maximum (ng/g)
	n[2]	%			
Fusarinolic acid	56	49.6	430	2,026	4,338
Deoxynivalenol (DON)	**55**	**48.7**	**137**	**497**	**4,974**
Enniatin B_1	55	48.7	7.5	40	260
Nivalenol	**54**	**47.8**	**41**	**162**	**291**
Fusapyron	50	44.2	9.5	46	170
DON-3-glucoside	**46**	**40.7**	**15**	**35**	**1,043**
Fusaric acid	44	38.9	146	558	3,564
Fusaproliferin	23	20.4	448	939	10,076
T-2 toxin	**22**	**19.5**	**3.4**	**27**	**47**
HT-2 toxin	**17**	**15.0**	**12**	**37**	**72**
5-Hydroxyculmorin	12	10.6	99	275	1,152
β-Zearalenol	**10**	**8.8**	**2.6**	**7.2**	**9.5**
Fumonisin A_2	**9**	**8.0**	**24**	**89**	**115**
Nivalenol glucoside	**7**	**6.2**	**5.9**	**8.1**	**10**
3-Acetyldeoxynivalenol	**6**	**5.3**	**4.9**	**7.6**	**8.8**
α-Zearalenol	**5**	**4.4**	**2.3**	**8.9**	**12**
15-Acetyldeoxynivalenol	**2**	**1.8**	**292**	**506**	**559**
Fumonisin B_6	**1**	**0.9**	**3.7**	**3.7**	**3.7**
Aspergillus metabolites					
Tryptophol	103	91.2	177	441	1,636
3-Nitropropionic acid	101	89.4	10.9	48	506
Asperglaucide	99	87.6	60	230	491
Kojic acid	75	66.4	124	773	8,440
Asperphenamate	64	56.6	5.7	26	148
Aflatoxin B_1	**41**	**36.3**	**11**	**70**	**819**
Aflatoxin G_1	**30**	**26.5**	**7.8**	**47**	**397**
Aflatoxin B_2	**28**	**24.8**	**2.0**	**15**	**63**
Aflatoxin G_2	**20**	**17.7**	**3.4**	**13**	**59**
Aflatoxin M_1	**10**	**8.8**	**2.0**	**7.1**	**32**
Ochratoxin A	**10**	**8.8**	**2.6**	**6.4**	**15**
Ochratoxin B	**3**	**2.7**	**20**	**37**	**41**
Alternaria metabolites					
Tenuazonic acid	78	69.0	62	280	1,010
Infectopyron	54	47.8	97	192	1,163
Tentoxin	50	44.2	3.0	13	52
Alternariol	47	41.6	3.6	15	253
Penicillium metabolites					
Brevianamid F	103	91.2	29	123	2,054
Emodin	71	62.8	3.9	18	198
Rugulusovin	61	54.0	7.0	76	166
Other fungal metabolites					
cyclo(L-Pro-L-Tyr)	102	90.3	141	759	11,823
Neoechinulin A	86	76.1	17	205	828
cyclo(L-Pro-L-Val)	74	65.5	104	594	9,183
Questiomycin A	70	61.9	17	59	296
Citreorosein	49	43.4	3.5	28	296
Cercosporin	23	20.4	41	375	4,261

[1] All compounds are listed that (1) are major mycotoxins (i.e. aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin A) or their metabolites (in bold), (2) were present in >40% of samples, or (3) were present in >10% of samples at a concentration of >1 µg/g.
[2] Sample number.

4. Discussion

Occurrence of major mycotoxins in different commodities

Aflatoxins showed a low prevalence in feed samples from South Africa and detected levels were mostly unproblematic as the majority of the samples complied with the regulatory limits that are in effect in the EU. By contrast, significant fractions of finished feed (52.3%) and maize (34.2%) samples from Senegal, Côte d'Ivoire, Nigeria, Ghana, Namibia, Uganda, Kenya and Tanzania contained more than 20 ng/g total aflatoxins which corresponds to the EU limit for AFB_1 in finished feed destined for the most aflatoxin resistant animal species, such as poultry and ruminants, and is also the EU limit for AFB_1 in maize intended for feed. High aflatoxin levels in finished feed from African countries have been detected in other recent surveys. In a survey of 58 commercial poultry feed samples from Nigeria, 62% of samples were contaminated with a total aflatoxin level of >20 ng/g (Ezekiel *et al.*, 2012a) and in a survey of 83 chicken feed samples collected from Cameroon, 24% of samples contained >10 ng/g total aflatoxins (Kana *et al.*, 2013). Furthermore, high levels of aflatoxins have been detected in maize from African countries. Of 339 maize samples collected from 18 sub-Saharan African countries, 46 (13.5%) contained >20 ng/g total aflatoxins (Probst *et al.*, 2014). AFB_1 levels in 51 maize flour samples from Côte d'Ivoire showed a high median level of 75.4 ng/g and a high maximum level of 309 ng/g (Kouadio *et al.*, 2014). In a survey of 70 stored maize samples from Nigeria, 51.4% of samples contained more than 10 ng/g total aflatoxins (Adetunji *et al.*, 2014). In a survey of 90 maize samples intended for human consumption in Malawi, 29% contained more than 10 ng/g total aflatoxins (Matumba *et al.*, 2015). These examples illustrate that unsafe levels of aflatoxins in animal feed and maize from different African countries are a recurring issue that deserves attention.

For the overwhelming majority of samples, levels of OTA and *Fusarium* mycotoxins did not exceed the highest EU guidance values stipulated for the most resistant animal species. However, a higher fraction of samples did not comply with the lowest EU guidance values stipulated for the most sensitive animal species. These samples may still safely be used for animal nutrition if they are allocated to less sensitive species.

Year-to-year variation of mycotoxin occurrence in maize from South Africa

Fumonisin contamination in South African maize samples was higher in 2016 compared to the other years, which may be due to differences in climatic conditions during the growing season. In the summer of 2015/2016, South Africa suffered a severe drought that led to low crop yields. In the period from July to December, which is the critical period for maize establishment, rainfall was very low in 2015. It amounted to 141 mm, which was just 67% of the mean from 1991 to 2014 (211 mm) according to data from the Climate Change Knowledge Portal (The World Bank Group, 2018). Drought stress is known to favour the infection of maize with *Fusarium verticillioides* and concomitant accumulation of fumonisins (Munkvold, 2003; Rheeder *et al.*, 2016). Still, in 84.6% of samples fumonisin levels were below the EU guidance value for feedingstuffs destined for the most sensitive animal species (5 µg/g). Rainfall during the silking period of maize favours infection with the DON producer *Fusarium graminearum* and consequently accumulation of DON in the crop (Munkvold, 2003; Van Asselt *et al.*, 2012). Accordingly, DON levels were lower in samples from 2016 compared to the other years. In the period from July to December 2016, the amount of rainfall in the maize growing areas of South Africa was normal compared to historical data (South African Weather Service, 2018) and the crop yield in 2017 was higher than in previous years (Crop Estimates Committee, 2017). The data of our survey suggest that for the 2017 harvest, fumonisin levels were lower and DON levels were higher than in 2016, likely due to higher humidity. ZEA levels were similar to the previous years. In addition to climatic conditions during the growing season, timing of harvest and post-harvest handling and storage of crops may have affected the formation of mycotoxins.

Co-occurrence of major mycotoxins

DON and ZEA were found to frequently co-occur and their concentrations were positively correlated in feed samples from South Africa (Figure 6) and from the other 13 African countries. Both mycotoxins are produced by *F. graminearum* and *Fusarium culmorum*. Accordingly, co-contamination of crops with DON and ZEA has been frequently observed, especially in the temperate regions of Europe and North America, but less so in surveys conducted so far in other parts of the world (Smith *et al.*, 2016). Therefore, co-exposure of animals to DON and ZEA may be common. DON and ZEA have been reported to cause additive, synergistic or antagonistic effects in experiments with cultured cells (e.g. Kouadio *et al.*, 2007; Vejdovszky *et al.*, 2016; Xia *et al.*, 2017), while *in vivo* in mice and pigs mainly additive and synergistic effects have been observed on different endpoints such as different parameters of immune function (Dabrowski *et al.*, 2016; Pestka *et al.*, 1987; Ren *et al.*, 2016b) and liver health (Sun *et al.*, 2014), and oxidative stress in the spleen (Ren *et al.*, 2016b), brain (Ren *et al.*, 2016a) and kidneys (Liang *et al.*, 2015). In light of these reports of additive and synergistic effects, co-occurrence of DON and ZEA may be relevant to feed safety.

Multi-mycotoxin analysis

Several modified forms of major mycotoxins were detected using LC-MS/MS-based multi-mycotoxin analysis. The AFB_1 metabolite AFM_1 was detected in a small number of samples. AFM_1 is most often found in the milk of lactating animals, but it was also occasionally detected in plant derived products such as maize (Ezekiel *et al.*, 2012a; Matumba *et al.*, 2015), sorghum (Chala *et al.*, 2014), or peanuts (Ezekiel *et al.*, 2012b; Oyedele *et al.*, 2017), where it was suggested to have been produced by fungi or insects. The ZEA metabolites α-ZOL and β-ZOL were detected in a smaller number of samples than ZEA, but at similar median concentrations. As their parent compound, α-ZOL and β-ZOL are oestrogenic toxins. While β-ZOL has a reduced oestrogenic potency compared to ZEA, α-ZOL is 60 times more potent than ZEA (EFSA, 2016). Therefore, co-occurring α-ZOL and β-ZOL can be expected to add to the oestrogenic effect of ZEA.

Several less investigated fungal metabolites were detected with high frequency or at high levels. Many of these metabolites are likely no cause for concern for animal nutrition. For others, toxic effects in animals have been reported. For selected metabolites, possible consequences of their occurrence for animal health are discussed in the following.

MON was the most frequently detected *Fusarium* metabolite in feed samples from South Africa and the other 13 countries. MON has been shown to be toxic to different animal species, e.g. 25 mg MON/kg feed was shown to be cardiotoxicity and hepatotoxicity to poultry (Broomhead *et al.*, 2002) and 3 mg MON/kg body weight (BW) exerted immunotoxic effects in rats (Jonsson *et al.*, 2015). Furthermore, dietary FB_1 (200 µg/g) increased the cardiotoxic effect of dietary MON (100 µg/g) in Japanese quail (Sharma *et al.*, 2012), suggesting that co-occurrence of MON and FB_1, two of the most prevalent compounds detected in our survey, may be problematic. However, MON and FB_1 dosages administered in these studies could not be reached by exposure to the dietary MON and FB_1 levels detected in this survey (maximum of 2.6 µg/g and 9.1 µg/g, respectively). The consequences – if any – of chronic (co-)exposure to such low dietary MON and FB_1 levels are currently unclear.

The *Fusarium* metabolite ARF was frequently detected in feed from South Africa and from the other 13 countries at high maximum levels of 26.2 and 15.1 µg/g and at high 90^{th} percentile levels of 1.4 and 1.1 µg/g, respectively. The maximum levels may be problematic as contamination of feed with 26.4 µg/g ARF was shown to cause negative effects in poultry. Dietary exposure of quails to 26.4 µg/g ARF altered the antioxidant composition and fatty acid profile of their eggs and thereby reduced egg nutritional quality (Dvorska *et al.*, 2001) and compromised the antioxidant system of the embryo (Dvorska *et al.*, 2002). In intestinal porcine epithelial cells (IPEC-J2) 10 µM ARF decreased the transepithelial electrical resistance (TEER), indicating reduced intestinal barrier integrity (Springler *et al.*, 2016). Furthermore, in the same study, a combination of 5 µM ARF and 1.5 or 3 µM DON decreased TEER, whereas individual application of these compounds at the respective concentrations did not. These *in vitro* results suggest an additive or synergistic effect of two of the most frequently detected compounds in our survey on intestinal barrier integrity.

FUSA was detected in a smaller fraction of samples (13.3% of samples from South Africa and 20.9% of samples from the other 13 countries), but reached a high maximum level of 17.2 and 10.1 µg/g and a high 90^{th} percentile level of 2.0 and 0.9 µg/g in samples from South Africa and the other 13 countries, respectively. Occasional occurrence of high FUSA levels is in accordance with earlier reports (Santini *et al.*, 2012). The consequences of dietary exposure to such high FUSA levels are unclear. While toxicity in the brine shrimp (*Artemia salina*) larvae (Logrieco *et al.*, 1996) and chick embryo (Ritieni *et al.*, 1997) bioassays was reported, data on *in vivo* toxicity of FUSA, especially after dietary exposure, are lacking.

Of the non-regulated *Aspergillus* metabolites frequently detected in this survey, NPA has the highest toxic potential. As NPA causes brain lesions that resemble those caused by Huntington's disease (Brouillet *et al.*, 2005), the toxin is extensively used to investigate neurodegeneration. Based on a no observed adverse effect level (NOAEL) of 2.5 mg/kg BW/day in rats, an acceptable daily intake (ADI) of 25 µg/kg BW/day has been proposed for humans (Burdock *et al.*, 2001). Based on this ADI, the maximum NPA level detected in this survey (1.6 µg/g) might not be considered safe for human consumption. Still, a NOAEL of 2.5 mg/kg BW/day in rats indicates that negative effects of 1.6 µg/g dietary NPA on farm animals can largely be excluded.

TeA was the most frequently detected *Alternaria* metabolite and reached a high maximum level of 4.8 µg/g and a 90^{th} percentile level of 0.8 µg/g in South Africa. These levels may be a cause for concern, as negative effects of 5-10 µg/g dietary TeA in chickens have been reported. Dietary exposure of chickens to 10 µg/g TeA for 3 weeks caused lesions in different organs and led to decreased weight gain and feed efficiency and oral administration of 0.63 mg TeA/kg BW (which corresponds to 5 µg/g in the diet) did not affect performance but caused pathological changes in spleen and gizzard (Giambrone *et al.*, 1978). Furthermore, a recent toxicokinetic study showed that TeA is rapidly absorbed and slowly eliminated in chickens (Fraeyman *et al.*, 2015) suggesting that the toxin may accumulate upon frequent dietary exposure.

High levels of diplodiatoxin (maximum: 5.6 µg/g; 90th percentile: 1.0 µg/g) detected in some samples from South Africa may be a cause for concern. In ruminants, ingestion of mouldy maize infected by *Stenocarpella maydis* causes diplodiosis, a neuromycotoxicosis most common in Southern Africa and diplodiatoxin may be a causative agent of this disease (Masango *et al.*, 2015).

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