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RESEARCH PAPERS

Mycotoxin occurrence in maize produced in Northern Italy over the years 2009–2011: focus on the role of crop related factors

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Summary. The occurrence of mycotoxins associated with *Fusarium* spp. and *Aspergillus flavus* in Northern Italy, and the role of cropping systems, were investigated on 140 field samples collected over the years 2009–2011. Samples were analysed for fumonisins B_1 and B_2 (FBs), aflatoxins B_1, B_2, G_1 and G_2 (AFs), deoxynivalenol (DON) and zearalenone (ZEN) using validated analytical methods. Information on: maize hybrid, preceding crop, tillage applied, mineral nutrition, pest and disease control, severity of European Corn Borer (ECB) attack, sowing and harvesting dates, kernel moisture at harvesting and longitude of the sampled province, were also collected. During this period there were distinct differences in FBs and AFs concentrations between years and geographic origins, and very low contamination with DON and ZEN was always found. The incidence of AFs exceeded 75% across all samples, and was almost 100% for FBs. The meteorological trends were quite different in the 3 years surveyed. 2009 was the coldest in June and the warmest in August, 2010 the most humid, and in 2011 cold weather occurred during flowering and dry conditions during ripening. The run of a logistic equation with the backward stepwise approach selected three parameters, (seeding week, ECB severity and longitude of sampling province) to predict AFB_1 contamination and four parameters (year, sowing week, ECB severity and longitude of sampling province) to predict FB contamination. The internal validation gave good results, with 76% correct predictions. The probability of harvesting maize with more than 5 $\mu g\ kg^{-1}$ of AFB_1 varied between 86 and 5%, and the probability of harvesting maize with more than 4,000 $\mu g\ kg^{-1}$ of FBs varied between 81 and 2%, respectively, for conducive and non-conducive environments. Therefore, considerable variability was found even if a limited area and only 3 years were considered.

Key words: aflatoxins, fumonisins, logistic regression, modelling, prediction.

Introduction

Throughout the world, maize is considered one of the most susceptible crops to mycotoxin-producing fungi (Barug *et al*., 2004). Maize can be colonised and contaminated by a wide range of fungi belonging to the *Fusarium* genus, including *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. graminearum*, with consequent fumonisin (FBs) or trichothecene (TCT) contamination, as well as by *Aspergillus* section *Flavi*, producers of aflatoxins (AFs). In Northern Italy, *Fusarium* spp. are the most frequently occurring fungi and FBs are the main mycotoxins detected in maize grain, sometimes at very high levels of contamination (Pietri *et al*., 2004; Battilani *et al*., 2008b; Pietri *et al*., 2012). A limit of 4,000 $\mu g\ kg^{-1}$ as the sum of $FB_1 + FB_2$ for unprocessed maize grain intended for human consumption was fixed by the European Commission (2007). Fumonisin B_1 in particular causes leukoencephalomalacia in horses, pulmonary oedema in swine, liver and kidney cancers in rodents, and is also associated with oesophageal cancer and neural tube defects in humans (Marasas 1996;

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Marasas *et al.*, 2004). Levels of TCTs and zearalenone (ZEN) are generally low, except during rainy years, when *F. graminearum* becomes dominant (Pietri *et al.*, 2004). Significant colonisation by *Aspergillus* section *Flavi* and widespread contamination by AFs in maize was recorded for the first time in Italy in 2003 (Piva *et al.*, 2006; Battilani *et al.*, 2008a). More recently, widespread incidence of AF contamination was observed in 2012 in Southern-eastern Europe (Dobolyi *et al.*, 2013; Levic *et al.*, 2013). This can be considered a climate change effect, associated with persistent dry conditions and increased ambient temperatures (Blaney *et al.*, 2008).

Aflatoxin B_1, the most studied of the aflatoxins, was classified by the International Agency for Research on Cancer (IARC) as a class-1 human carcinogen (Castegnaro *et al.*, 1995). A legal limit of 5 μg kg^{-1} of AFB_1 was set by the European Commission for unprocessed maize grain intended for humans and for feeding stuffs for dairy animals; a limit of 20 μg kg^{-1} was set for all feed materials (European Commission, 2007; 2011).

It is well known that the presence of mycotoxin-producing fungi in host crops is related to year by year meteorological conditions (Battilani *et al*., 2008a, b), and that the cropping system applied can significantly affect toxin occurrence (Munkvold, 2003; Blandino *et al*., 2009; Kos *et al*., 2014). Mineral nutrition and weed control must be managed rationally in maize crops to avoid plant stress, confirmed as related to high levels of mycotoxin contamination. Moreover, fumonisins contamination has been related to ear injuries caused by larvae of *Ostrinia nubilialis* (Hubner; European corn borer (ECB); Papst *et al.*, 2005); damaged maize ears can have several times greater mycotoxin contamination compared to undamaged ears (Alma *et al*., 2005). Pest control is an effective method to reduce FBs contamination (Saladino *et al.*, 2008; Mazzoni *et al*., 2011). Harvest time and related water content of kernels are also relevant; aflatoxin production is facilitated when moisture decreases below 28% (Payne *et al.*, 1998; Marin *et al*., 2004).

The use of maize lots, intended for food, feed or other purposes, depends on mycotoxin content. Because of variability in mycotoxin content, it is important to predict which contamination may occur to optimise harvest/post-harvest management, leading to a reduction of consumer and animal exposure. The present study aimed to i) survey the occurrence of all main mycotoxins (AFs, FBs, TCTs and ZEN) in maize produced in Northern Italy (Emilia Romagna region) over 3 years (2009–2011); ii) investigate the role of the cropping system on mycotoxin contamination; and iii) identify crucial operations and describe their effect following a modelling approach.

Materials and methods

Samples

During the years 2009–2011, maize sampling was organised in Northern Italy. The surveyed area included the plain region in the provinces of Bologna (BO; 44°28' N, 11°26' E), Ferrara (FE; 44°48' N, 11°50' E), Modena (MO; 44°30' N, 10°54' E), Piacenza (PC; 44°53' N, 9°35' E), Parma (PR; 44°42' N, 10°05' E) Ravenna (RA; 44°25' N, 11°59' E) and Reggio Emilia (RE; 44°37' N, 10°37' E), located in the Emilia Romagna region. A total of 140 samples were collected and analysed during the three years considered in the study (46 samples in 2009, 48 in 2010 and 46 in 2011).

Field sampling was performed during the combine harvesting discharge, according to Commission Regulation (EC) 401/2006 (European Commission, 2006a). One hundred sub-samples (approx. 100 g each) were collected from the kernel flux, and these contributed to the final sample. The final sample (approx. 10 kg) was sent to the laboratory, where a lab-sample of 100 g was randomly taken from each sample for the determination of fungal incidence. The remaining sample was finely ground using a cyclone hammer mill (1 mm sieve) (Pulverisette, Fritsch GmbH,). For each sample, 2 kg was stored at -20 °C until analysis for mycotoxins.

Meteorological data

Two meteorological stations located in the provinces of FE and PC were selected in this work. Daily data of mean air temperature (T, °C), air relative humidity (RH, %) and rainfall (R, mm) were recorded from the 1st of June to the 30th of August in the years 2009–2011.

Crop phenology and cropping systems

A questionnaire was prepared to collect the relevant information on crop phenology and cropping system relating to each sampled field. In particular, maize hybrid, preceding crop, tillage applied, mineral nutrition, pest and disease control, severity of ECB attack, sowing, silk emergence and harvest dates (weeks of the year), kernel moisture at harvest and georeferences of the sampled province were considered. Data collected were clustered in a database according to the groups defined by Battilani *et al.* (2008a) for data analyses.

Reagents and mycotoxin analyses

Chemicals and solvents used for the extraction and clean-up solutions were ACS grade or equivalent (Carlo Erba). For HPLC analyses, methanol, acetonitrile and acetic acid were HPLC grade (Merck); water was purified through a Milli-Q treatment system (Millipore). Phosphate buffer saline (PBS) was prepared as follows: NaCl 8 g L^{-1}, KCl 0.2 g L^{-1}, Na_2HPO_4 1.15 g L^{-1}, KH_2PO_4 0.2 g L^{-1}; pH 7.4. Analyses and standard preparations were performed according to the methods outlined by Bertuzzi *et al.* (2012) for AFs, Pietri and Bertuzzi (2012) for FBs, Bertuzzi *et al.* (2014) for TCTs and ZEN. Briefly, AFs and ZEN contents were determined using a HPLC instrument with fluorescence detector, FBs using a HPLC-MS/MS system, and TCTs using a GC-MS system. The limits of detection (LOD) and quantification (LOQ) were, respectively: 0.05 and 0.15 μg kg^{-1} for AFs, 10 and 30 μg kg^{-1} for FBs, 3 and 10 μg kg^{-1} for TCTs and ZEN.

Moisture analyses

Moisture content was determined using AOAC Official Method (AOAC, 2005).

Data analyses

The information obtained from questionnaires and mycotoxin content in maize samples was compiled into a database. Samples with mycotoxin content below the respective LOQs were set to zero for data analysis. Data on mycotoxin contamination were ln transformed and univariate analysis of variance was applied using IBM SPSS Statistics 21.

Logistic regressions were developed using cropping system data as independent variables (preceding crops, sowing and harvest weeks, ECB severity and control, and longitude of sampled province), and AFB_1 or FB_1+FB_2 content in grain at harvest as dependent variables, to define the probability to exceed a fixed threshold of contamination depending on the cropping system applied. Contamination data were shared in two exclusive groups, 0 = contamination below the EU legal limits or 1 = contamination equal/above the EU legal limits (European Commission, 2006b; 2007; 2011). The thresholds used to separate data were fixed at 5 μg kg^{-1} for AFB_1 and 4,000 μg kg^{-1} for FBs (as the sum of FB_1 and FB_2).

The probability values of binary logistic regression range from 0 to 1. When $P \geq 0.5$, the event is considered as occurring; when $P < 0.5$, it is considered as not occurring. The logistic regression module PASW of IBM SPSS was used to estimate the parameters of logistic equation. The backward stepwise (conditional) method was applied for the selection of relevant independent variables with probability levels of F = 0.05 and F = 0.10, respectively, for variables to enter and exit, and a maximum number of iterations = 20. Results predicted by the logistic regressions developed for AFB_1 and FBs were validated using the data collected in the study (internal validation).

Results and discussion

Meteorological data

Summer meteorological conditions were very different in the 3 years considered. As an example, Piacenza and Ferrara are highlighted. These are located, respectively, in the North-Western and North-Eastern parts of the region. Ferrara was more humid than Piacenza, with more rain days and more rain in 2009 and 2010, but the rainfall trend observed during the maize growing seasons in the 3 years considered was similar in the two areas. Regarding temperature, in 2009, the lowest mean daily temperature for June and the highest for August were registered in both provinces considered. 2010 was the most humid year in June and August, when the greatest number of rain days and amount (mm) of rainfall were observed. July, the month of maize flowering, was the coldest in 2011 (1–3°C less than the other two years), with more rain days, and with no rain in August (Table 1) in both Piacenza and Ferrara.

Crop phenology and cropping system

Most of the fields sampled were seeded with 500 and 600 FAO class hybrids (40 and 42%, respective-

Table 1. Mean monthly temperature (T, °C), mean monthly relative humidity (RH, %), mean monthly summation of rainfall (R_mm,) and days with rain (R_days) in June, July and August of 2009, 2010 and 2011 measured at two meteorological stations located in the provinces of Piacenza and Ferrara, Northern Italy.

Meteo data	2009			2010			2011		
	June	July	August	June	July	August	June	July	August
Ferrara									
T	21	24	26	22	25	23	22	23	25
RH	72	72	65	75	70	74	73	70	63
R_days	6	2	3	8	3	6	7	4	0
R_mm	59	29	27	124	51	74	38	42	0
Piacenza									
T	23	25	26	22	26	23	21	23	25
RH	60	60	61	61	59	67	71	63	56
R_days	3	2	1	5	4	3	6	5	0
R_mm	25	11	4	131	9	69	92	31	0

ly). Short season hybrids, FAO class 300-400, were grown only in 24 fields (18%) during the 3 years of the study. Fifty per-cent of the fields were sown during mid-April, and the remaining samples were from crops sown in late-March or late-April (respectively, 35 and 15% of the samples). Silk emergence was observed from 15 June and ended around 25 June in 2010, and 12 July in 2009 and 2011. The most recurrent harvest period (66% of total samples) was September, while 31% of the crops were harvested at the end of August and the remaining 3% during the first week of October. Control of ECB during maize growing was limited; 37% of sampled fields were sprayed in 2009, only 17% in 2010 and 30% in 2011. Moisture of maize kernels at harvest was less than 20% for 53% of samples, between 20% and 25% for 33% of samples and greater (>25%) for the remaining 14% of samples collected during the three years. Only a few response from farmers were received regarding other cropping parameters, so these were not considered in the analyses.

Mycotoxin contamination

An overview of mycotoxin contamination found in maize in the three years is presented in Table 2. AFs and FBs were often/always detected in the maize fields sampled, while TCTs and ZEN were less regularly detected. Among TCTs, only DON was found; DON occurred in 30 samples, and only in two samples was the contamination greater than 200 μg kg^{-1} (maximum value: 428 μg kg^{-1} in 2010). ZEN occurred only in one sample. These mycotoxins were not considered further in this study.

Aflatoxins

Occurrence. The region-wide incidence of AFs was greater than 75%, with levels above 200 μg kg^{-1} in some samples (560 μg kg^{-1} was the maximum level for AFB_1). However, the occurrence varied between years. In particular, an AFB_1 contamination greater than 5 μg kg^{-1} was observed, respectively, in 28, 30 and 16% of samples collected in 2009, 2010 and 2011. (Figure 1). Twelve percent of samples exceeded 20 μg kg^{-1}, the legal limit for feed (European Commission, 2011). The occurrence of AFs in maize produced in Northern Italy has already been reported in previous studies. In the years 1995-1999, AFB_1 was detected in 57% of samples, with an average level of 1.7±10.2 μg kg^{-1}: the contamination was less than 5 μg kg^{-1} in 97% of samples and exceeded 20 μg kg^{-1} only in seven samples (1.4%; Pietri *et al.*, 2004). Higher levels of contamination were detected in samples collected

Table 2. Descriptive statistics of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), fumonisins (considered as the sum of FB_1 and FB_2), deoxynivalenol (DON) and zearalenone (ZEN) contamination (μg kg^{-1}) for maize grain samples collected in Emilia-Romagna over the years 2009, 2010 and 2011.

Year	Statistics	AFB_1 (μg kg^{-1})	AFB_2 (μg kg^{-1})	AFG_1 (μg kg^{-1})	AFG_2 (μg kg^{-1})	FB_1+ FB_2 (μg kg^{-1})	DON (μg kg^{-1})	ZEN (μg kg^{-1})
2009 (n=46)	% positives	95.6	71.7	30.4	13.0	100	32.6	/
	Mean ± dev std	34.7±115	2.2±7.1	1.4±4.6	0.1±0.2	3,040±2,830	16±43	/
	Median	2.2	0.2	<0.1	<0.1	2098	<3	/
	Maximum value	560	38.4	22.6	0.9	10604	266	/
2010 (n=48)	% positives	77.1	54.2	41.3	14.6	100	22.9	2.1
	Mean ± dev std	15.9±42.9	1.5±4.1	1.8±4.6	0.1±0.2	3,781±2,848	27±72	/
	Median	1.7	0.2	<0.1	<0.1	3,003	<3	/
	Maximum value	213	22.0	23.1	0.9	12,637	428	53
2011 (n=46)	% positives	58.7	28.3	15.2	4.3	97.8	8.7	/
	Mean ± dev std	9.8±48.2	0.7±3.5	0.7±3.5	<0.1	2,181±3,184	4±19	/
	Median	0.1	<0.1	<0.1	<0.1	1,356	<3	/
	Maximum value	335	24.2	21.4	0.6	21,007	55	/

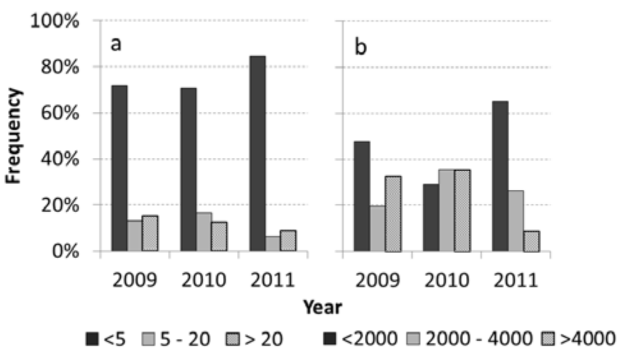

Figure 1. Frequency classes of occurrence (%) for aflatoxin B_1 (a) and fumonisin B_1+B_2 (b) in maize samples collected in seven provinces of Emilia-Romagna in 2009 (46 samples), 2010 (48 samples) and 2011 (46 samples).

in 2004–2007, particularly in the Emilia Romagna region (Battilani *et al.*, 2008a); mean (median) values of 16.6 (3.2) μg kg^{-1} were found in 2004, 2.3 (<LOD) μg kg^{-1} in 2005, 20.0 (3.6) μg kg^{-1} in 2006 and 15.1 (0.6) μg kg^{-1} were found in 2007. A high AFB_1 incidence (87%) was reported in maize produced in the Emilia Romagna region in 2008; the average level was 7.7±15.3 μg kg^{-1}, and 33% of samples exceeded 5 μg kg^{-1} (Pietri *et al.*, 2012). Considerable contamination (42% of samples exceeding 5 μg kg^{-1}) was found in maize samples collected in the Central-East area of the region, corresponding to the provinces of RE, MO, BO, FE and RA.

In the present study, the trend was similar in 2009 and 2010; more than 40% of samples collected in this area showed amounts of AFB_1 above 5 μg kg^{-1}; only two samples (one in 2009 and one in 2010) collected in other provinces showed a concentration greater than 5 μg kg^{-1}. In 2011, the contamination was low throughout the region.

Role of meteorological conditions and cropping systems. Because of the increasing contamination trend observed in recent years in Northern Italy, the roles of meteorological data and cropping systems were considered, to underline the most relevant factors and possibly predict their effects on final AFB_1 content.

Considering the meteorological data collected from the two selected stations (Table 1), a remarkably higher RH was recorded in the area of FE compared with PC (east and west of the region, respectively), particularly in 2009 and 2010.

No significant differences in AFs contamination were found between FAO classes of maize hybrids; the overall mean (median) values were 4.7±7.2 (1.2) μg kg^{-1} for 300-400 class hybrids, 4.6±9.6 (1.0) μg kg^{-1} for 500 class hybrids and 8.9±18.5 (1.1) μg kg^{-1}, for 600 class hybrids. The FAO class has already been suggested as an improper parameter to distinguish between hybrids for mycotoxin susceptibility (Battilani *et al.*, 2011). The water activity content of kernels, which is certainly crucial for fungal life, is related to the hybrid, but not to the FAO class. The percentage of uncontaminated samples for the three classes was, respectively, 17%, 30% and 24%, while the percentage of samples showing contamination levels greater than 5 μg kg^{-1} was 29% for 300-400 hybrids, 26% for 500 hybrids, and 17% for 600 class hybrids.

No significant differences in contamination were observed between the sowing weeks; similarly, no difference was found between samples harvested in August and those in the first 2 weeks of September. Samples harvested after September 15 (n = 30) showed a low AFs contamination; only in six samples was the AFB_1 level above 5 μg kg^{-1}. In the years 2009–2010 (in 2011 the contamination was negligible), the average AFB_1 contamination was 9.3±14.5 (median 2.5) μg kg^{-1} for the samples collected before September 15, and 4.9±12.8 (median 1.1) μg kg^{-1} for the samples collected after September 15. Other reports regarding maize sowing periods generally agree on the positive effect of early sowing, both on plant well-being and mycotoxin contamination levels, while comments related to harvest period are more variable (Battilani *et al.*, 2008b; Blandino *et al.*, 2009; Parson and Munkvold, 2012). This is not surprising, because many factors influence harvest time, including season length for different hybrids, sowing period, irrigation and fertiliser supply, to mention those playing major roles (Tedihou *et al.*, 2012).

Measurements of kernel moisture at harvest indicated that samples were in three classes: less than 20%, between 20 and 23.5%, greater than 23.5% water content. Samples harvested with low moisture were generally highly contaminated (about 15% of samples showed mycotoxin levels >20 μg kg^{-1}). On the other hand, no sample harvested with moisture greater than 23.5% exceeded 20 μg kg^{-1}, and only two samples showed contamination between 5 and 20 μg kg^{-1}. These data confirmed that the risk of high mycotoxin contamination increases when kernel moisture at harvest is low (Homdork *et al.*, 2000).

Control of ECB was performed in only 28% of the fields; no differences in AFs content were found in grain from treated or untreated crops, but the low number of treated fields possibly influenced this result.

Prediction of AFs contamination. All these data were considered for a logistic regression approach, previously used for the prediction of AFs contamination in maize using an aridity index as the independent variable (Battilani *et al.*, 2008a). The logistic equation, run with the backward stepwise approach, selected three parameters, sowing week, ECB severity and longitude of sampling province, to predict AFB_1 contamination. According to the internal validation (Table 2a), which compared the output of the logistic regression and field collected data used as input for parameter definitions, 76% of samples were correctly classified, 70% correctly predicted as not contaminated, and 6% as contaminated above 5 μg kg^{-1} of mycotoxin. False negative predictions were 21% and false positives were 3%. The most conducive condition for mycotoxin development occurred with maize sown in late April in RA with severe ECB attack. Conversely, non-conducive conditions resulted for maize sown in late March in PC, with light ECB attack. The probability of harvesting maize with more than 5 μg kg^{-1} mycotoxin content was 86% for conducive environments, and 5% for non-conducive environments (Table 3).

Fumonisins

Occurrence. The overall incidence of FBs (FB_1 + FB_2) was close to 100%, with maximum values above 10,000 μg kg^{-1} in each year. The percentage of sam-

Table 3. Results of the internal validation of logistic regression for: a) Aflatoxin crop-related factors, and b) Fumonisin crop-related factors. Values (%) in bold represent correct predictions; predicted 0 and observed 1 are underestimates and predicted 1 and observed 0 are overestimates.

	Aflatoxin		Fumonisin	
	a Predicted		*b* Predicted	
Observed	0	1	0	1
0	***70***	3	***72***	4
1	21	***6***	22	***4***

ples exceeding 4,000 μg kg^{-1} was 33%, in 2009, 35% in 2010 and 9% in 2011 (Figure 1).

In this study, mean annual contaminations were less than recorded for previous surveys. Several studies reported high levels of incidence and contamination in maize collected in Northern Italy. In 2002-2007 (Battilani *et al.*, 2008b) FBs were detected in almost all maize fields sampled. With the exception of the year 2007, which showed low contamination, the mean annual contamination of FB_1 ranged from 4,018 to 6,910 μg kg^{-1}. Comparable results were reported by Berardo *et al.* (2011) over the years 2006–2008; a mean FBs contamination of 4,800 μg kg^{-1} was reported for the samples collected in Emilia Romagna.

Role of meteorological conditions and cropping system. No rain occurred in August 2011. This probably enhanced water loss with rapid reduction of water activity in kernels. Fusaria activity and FBs production during ripening was consequently limited. The temperature measured in July 2011 was lower compared with 2010 and 2009 in the same month, and these low temperatures were probably a further limiting factor for FBs synthesis. Hooker *et al.* (2005) published data from 1993 to 2000 on FBs occurrence in maize cultivated in Ontario, Canada. They also reported low incidence of FBs in combination with low rainfall periods from the end of July to mid-September.

No significant differences in effects of cropping systems were found between hybrid season length. This is not in accordance with results from a previous study, in which significantly lower contamination was reported for hybrids belonging to 3-400 FAO classes (Pietri *et al.*, 2012) than other classes. The low number of 3-400 class hybrids in the present study (18%) could have influenced the statistical data analysis. Furthermore, other characteristics of hybrids, such as chemical composition and fatty acid content, as suggested by Dall'Asta *et al.* (2012), could have played a major role. No significant differences regarding sowing and harvesting weeks were observed. As for AFs, low moisture content of kernels at harvest increased the risk of high contamination. For samples harvested at less than 20% moisture, 37% exceeded 4,000 μg kg^{-1}, while only 16% of samples at greater moisture content exceeded this amount of mycotoxin. Control of ECB reduced FBs contamination; the percentage of samples exceeding 4,000 μg kg^{-1} was 30% without control and 16% where control had been applied.

Prediction of FBs contamination. The factors selected by the logistic regression, run using the backward stepwise procedure to compute the probability of exceeding the fixed threshold for FBs, were year, sowing week, ECB control and longitude of sampling province. These factors were only partially in agreement with those selected in a previous similar approach. In particular, in Battilani *et al.* (2008b), sowing week and longitude of sampling place were between the selected parameters, in agreement with this study, but FAO hybrid class and growing weeks (number of weeks from sowing to harvest) were also included. ECB control, selected in the present study, was not considered by Battilani *et al.* (2008b), because that practice was not yet commonly applied.

As reported in Table 2b, according to the internal validation managed in this study, 76% of samples were correctly classified, 72% correctly predicted as not contaminated and 4% were predicted as contaminated above 4,000 μg/kg. The samples classified as false negatives, predicted uncontaminated but observed with FBs above the legal limit, were 22% of the total dataset. The residual 4% included "false alarms", which means samples below, but predicted above, the fixed threshold. This result is better than those reported previously, when only 58% of samples were correctly classified (Battilani *et al.*, 2008b).

The most conducive conditions for maize grain mycotoxin production occurred in the BO growing area, in crops sown late in April and not sprayed against ECB. Moreover, ECB control and early sowing (before the end of March) resulted in a significant reduction of the contamination risk in the RE province. The probability of harvesting maize with more than 4,000 μg kg^{-1} was 69% and 3% when the most conducive or unfavourable conditions, respectively, were used as data input in the logistic regression as input (Table 4).

Concluding remarks

This 3-year investigation and computation of an epidemiological regression model confirmed the important role played by agronomic practices during the maize growing season for AFs and FBs contamination of kernels. Both for AFB_1 and FBs, cropping system data, in particular sowing period and ECB attack, together with longitude, gave 76% correct predictions in terms of probability of overcoming the fixed legal mycotoxin limits. Longitude clustered

Table 4. Cropping system factors selected by the logistic equations to define conducive/not-conducive scenarios for AFB_1 and FBs contamination in maize grain.

Cropping system factor	Conducive	Not conducive
Aflatoxin (AFB_1)		
Sowing week	Late (end of April)	Early (March)
ECB severity	High	Light
Longitude of sampling province	RA (11°59' E)	PC (9°35' E)
	86%[a]	5%[a]
Fumonisin (FBs)		
Sowing week	Late (end of April)	Early (March)
ECB control	Not-sprayed	Sprayed
Longitude of sampling province	BO (11°26' E)	RE (10°37' E)
	69%[a]	3%[a]

[a] Probability to exceed the fixed threshold of contamination depending on the cropping system applied.

different aspects such as meteorological conditions, soil type or the cropping system commonly applied, which are typically dependent on the habits of farmers. These factors contributed significantly, as expected.

The major role played by meteorological conditions is confirmed, both for AFB_1 and FBs, and weather cannot be ignored in predictions. A mechanistic model using meteorological data as input to predict AFB_1 contamination in maize was recently published (Battilani *et al.*, 2013) and a similar one regarding FBs is currently in preparation. However, some parameters, i.e. sowing week and longitude, were selected for their relevant contribution to FBs prediction in this study, in agreement with a previous report (Battilani *et al.*, 2008b). ECB control, applied to reduce the severity of pest attack, was also included among the significant model parameters in this study. Cropping system data are difficult to consider factor by factor, due to their likely interactions. The logistic regression should therefore represent a good alternative, to take into account these interacting parameters. The logistic regression developed here, accounting for the role of cropping system, should support previously cited mechanistic models, using meteorological data as inputs, to improve their predictive performances. This holistic approach could better support farmers, stakeholders and others involved in the management of mycotoxins along the maize chain (Battilani and Camardo Leggieri, 2014). The logistic regression developed, even if based on data collected in a particular geographic area (Emilia Romagna, North Italy), can be used in other areas after validation/recalibration of parameters with data of cropping system and mycotoxin contamination collected in the areas of interest (Camardo Leggieri *et al.*, 2013).

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Literature cited

AOAC, 2005. Grain. Moisture. Method 925.10, Chapter 32: 1. In: *Official Methods of Analysis of the International Association of Official Analytical Chemists* (W.H.a.M.W. Trucksess, ed.), Gaithersburg, MD, USA.

Alma A., F. Lessio, A. Reyneri and M. Blandino, 2005. Relationships between *Ostrinia nubilialis* Hübner (lepidopter Cramidae) crop technique and mycotoxin contamination of corn kernel in northwestern Italy. *International Journal of Pest Management* 51, 165–173.

Barug D., H. van Egmond, R. López Garzía, T. van Osenbruggen and A. Visconti, 2004. *Meeting the Mycotoxin Menace*. Wageningen Academic Publishers, The Netherlands.

Battilani P. and M. Camardo Leggieri, 2015. Predictive modelling of aflatoxin contamination to support maize chain management. *World Mycotoxin Journal, Special Issue "Aflatoxin in maize"* 8:2, 161–170.

Battilani P., C. Barbano and G. Piva , 2008a. Aflatoxin B_1 contamination in maize related to the aridity index in North Italy. *World Mycotoxin Journal* 1, 449–456.

Battilani P., A. Pietri, C. Barbano, A. Scandolara, T. Bertuzzi and A. Marocco. 2008b. Logistic regression modeling of cropping systems to predict fumonisin contamination in maize. *Journal of Agricultural and Food Chemistry* 56, 10433–10438.

Battilani P., S. Formenti, C. Ramponi and V. Rossi, 2011. Dynamic of water activity in maize hybrids is crucial for fumonisin contamination in kernels. *Journal of Cereal Science* 54, 467–472.

Battilani P., M. Camardo Leggieri, V. Rossi and P. Giorni, 2013. AFLA-maize, a mechanistic model for *Aspergillus flavus* infection and aflatoxin B_1 contamination in maize. *Computers and Electronics in Agriculture* 94, 38–46.

Berardo N., C. Lanzanova, S. Locatelli, P. Laganà, A. Verderio and M. Motto, 2011. Levels of total fumonisins in maize samples from Italy during 2006-2008. *Food Additives and Contaminants: Part B: Surveillance* 4, 116–124.

Bertuzzi T., S. Rastelli, A. Mulazzi and A. Pietri, 2012. Evaluation andiImprovement of extraction methods for the analysis of aflatoxins B_1, B_2, G_1 and G_2 from naturally contaminated maize. *Food Analytical Methods* 5, 512–519.

Bertuzzi T., M. Camardo Leggieri, P. Battilani and A. Pietri, 2014. Co-occurrence of type A and B trichothecenes and zearalenone in wheat grown in northern Italy over the years 2009-2011. *Food Additives and Contaminants: Part B Surveillance* 7(4), 273–281

Blandino M., A. Reyneri, F. Vanara, M. Pascale, M. Haidukowski and C. Campagna, 2009. Management of fumonisin contamination in maize kernels through the timing of insecticide application against the European corn borer *Ostrinia nubilalis* Hübner. *Food Additives and Contaminants* 26, 1501–1514.

Blaney B.J., K. O'Keeffe and L.K. Bricknell, 2008. Managing mycotoxins in maize: case studies. *Australian Journal of Experimental Agriculture* 48, 351–357.

Camardo Leggieri M., H.J. Van Der Fels-Klerx , P. Battilani, 2013. Cross-validation of predictive models for occurrence of deoxynivalenol in wheat at harvest. *World Mycotoxin Journal* 6, 389–397.

Castegnaro M. and C.P. Wild, 1995. IARC activities in mycotoxin researcher. *Natural Toxins* 3, 327–331

Dall'Asta C., C. Falavigna, G. Galaverna and P. Battilani, 2012. Role of maize hybrids and their chemical composition in Fusarium infection and fumonisin production. *Journal of Agricultural and Food Chemistry* 60, 3800–3808.

Dobolyi C., F. Sebok, J. Varga, S. Kocsube, G. Szigeti, N. Baranyi, A. Szecsi, B. Toth, M. Varga, B. Kriszt, S. Szoboszlay, C. Krifaton and J. Kukolya, 2013. Occurrence of aflatoxin producing *Aspergillus flavus* isolates in maize kernel in Hungary. *Acta Alimentaria* (Budapest) 42, 451–459.

European Commission, 2006a Commission regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union* L 70, 12–34.

European Commission, 2006b. Commission regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of European Union* 364, 5–24.

European Commission, 2007. Commission regulation (EC) N° 1126/2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. *Official Journal of European Union* 255, 14–17.

European Commission, 2011. Commission Regulation (EC) N° 574/2011 of 16 June 2011 amending Annex I to Directive 2002/32/EC. *Official Journal of the European Union* 159, 7–24.

Homdork S., H. Fehrmann and R. Beck, 2000. Influence of different storage conditions on the mycotoxin production and quality of Fusarium-infected wheat grain. *Journal of Phytopathology* 148, 7–15.

Hooker D.C. and A.W Schaafsm, 2005. Agronomic and environmental impacts on concentration of deoxynivaleonl and fumonisin B_1 in corn across Ontatio. *Canadian Journal of Plant Pathology* 27, 347–356.

Kos J., J. Mastivolic, E.J. Hajnal and B. Saric, 2014. Natural occurrence of aflatoxins in maize harvested in Serbia during 2009-2012. *Food Control* 34, 31–34.

Levic J., S. Gosic-Dondo, D. Ivanovic, S. Stankovic, V. Krnjaja, A. Bocarov-Stancic and A. Stepanic, 2013. An outbreak of *Aspergillus* species in response to environmental conditions in Serbia. *Pesticidi i Fitomedicina* 28, 167–179.

Marin S., N. Magan, A.J. Ramos and V. Sanchis, 2004. Fumonisin-producing strains of Fusarium: a review of their ecophysiology. *Journal of food potection* 37, 1792–1805.

Marasas W.F., 1996. Fumonisins: history, world-widw occurrence and impact. In: *Fumonisins in Food* (Jackson J.W.D.L.S., Bullerman L.B., eds). New York, USA: Plenum Press, 1–18.

Marasas W.F., R.T. Riley, K.A. Hendricks , V.L. Stevens, T.W. Sadler, J. Gelineau-van Waes, S.A. Missmer, J. Cabrera, O. Torres, W.C. Gelderblom, J. Allegood, C. Martinez, J. Maddox, J.D. Miller, L. Starr, M.C. Sullards, A.V. Roman, K.A. Voss, E. Wang and A.H. Merrill, 2004. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *Journal of Nutrition* 134, 711–716.

Mazzoni E., A. Scandolara, P. Giorni, A. Pietri and P. Battilani, 2011. Field control of Fusarium ear rot, *Ostrinia nubilalis* (Hübner) and fumonisins in maize kernels, *Pest Management Science* 67, 458–465.

Munkvold G.P., 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology* 109, 705–713.

Parson M.W. and G.P. Munkvold, 2012. Effects of planting date and environmental factors on fusarium ear rot symptoms and fumonisin B_1 accumulation in maize grown in six North American locations. *Pland Pathology* 61, 1130–1142.

Papst C., H.F. Utz, A.F. Melchinger, J. Eder, T. Magg, D. Klein and M. Bohn, 2005. Mycotoxins producend by *Fusarium* spp. in isogenic Bt vs. non maize hybrids under European corn borer pressure. *Agronomy Journal* 97, 730–742.

Payne G.A., W.M. Hagler and C.R. Adkins, 1998. Alfatoxin accumation in inoculated ears of field-grown maize. *Plant Disease* 72, 422–424.

Pietri A. and T. Bertuzzi, 2012. Simple phosphate buffer extraction for the determination of fumonisins in masa, maize and derived products. *Food Analytical Methods* 5, 1088–1096.

Pietri A., T. Bertuzzi, L. Pallaroni and G. Piva, 2004. Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northen Italy. *Food Additives and Contaminants* 21, 479–487.

Pietri A., P. Battilani, A. Gualla, and T. Bertuzzi, 2012. Mycotoxin levels in maize produced in northern Italy in 2008 as influenced by growing location and FAO class of hybrid. *World Mycotoxin Journal* 5, 409–418.

Piva G., P. Battilani and A. Pietri, 2006. Emerging issues in Southern Europe: aflatoxins in Italy. In: *The Mycotoxin factbook. Food and Feed Topics* (Barug D., Bhatnagar D., eds) Wageningen Academic Publisher, The Netherlands.

Saladini M.A., M. Blandino, A. Reyneri and A. Alma, 2008 Impact of insecticide treatments on *Ostrinia nubilalis* (Hbner) (Lepidoptera: Crambidae) and their influence on the mycotoxin contamination of maize kernels. *Pest Management Science* 64,1170–1178.

Tedihou E., R. Olatinwo, K. Hell, B. Hau and G. Hoogenboom, 2012. Effects of variety, cropping system and soil inoculation with *Aspergillus flavus* on aflatoxin levels during storage of maize. *Tropical Plant Pathology* 37, 25–36.

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