Recent advances in modelling the risk of mycotoxin contamination in crops

Short Title: **Modelling Mycotoxins in Crops**

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Abstract

Recent advances confirm the focus in modelling mycotoxin in few crops; cereals, both small grains and maize, deserve major attention. Deoxinivalenol is still the mycotoxin of major interest in wheat, with some studies developed on T2 and HT2 toxins. Regarding maize, after fumonisins, the interest moved to aflatoxins. Minor efforts were devoted to aflatoxin contamination in nuts and ochratoxin A in grapes. Weather data are the main driving variables used as input in all modelling approaches, in empiric models commonly supported by cropping data. Few example of mechanistic models were developed, but they seems more promising in the future, when climate change is expected to significantly impact on mycotoxins.

Introduction

The dynamic of scientific paper publication has confirmed mycotoxin as an increasing trend. Since 1965, the year when aflatoxin was the first mycotoxin identified [1], 21,973 papers were recorded in Scopus (www.scopus.com, August 2016), with more than 800 per year starting from 2012. Efforts devoted to improve the knowledge of mycotoxins are totally justified by: i) the considerable global occurrence of mycotoxins (72% of the samples analysed worldwide; [2]), ii) the crucial role played by mycotoxins as the highest chronic health foodborne risk [3]), and iii) the increasing attention paid to food and feed safety in the 21st century.

The impact of mycotoxins on agriculture and the agri-food market increased when legal rules, imposing maximum amounts of mycotoxin content in raw and processed agricultural products, were settled and involved a growing number of compounds, crops and countries worldwide [4,5]. The interest towards the dynamic of mycotoxin contamination in crops during the growing season, and especially at harvest, increased too. In fact, it has been confirmed that almost all mycotoxins come from the field, even if their content in crop derived products can rise with improper postharvest management. Weather conditions are always the crucial driving variables for mycotoxin contamination in all crops, and they determine if fungi can grow and produce toxins, while the cropping system modulates the amount of contamination [6].

The combination of "mycotoxin" and "model" still shows a positive trend in papers published since 1969, but they represent around 10% of available literature (2,079 papers) and dropped down to less than 1% (145 papers) including "crops." Therefore, "mycotoxin" is a hot topic, but efforts to model the risk of contamination in crops is still limited (**Figure 1**). There can be several reasons behind this: the low interest in modelling, the unclear view on support they can give, the limited trust in their benefit, and/or the troubles faced in their development.

The aims of this review is therefore to check recent advances in modelling the risk of mycotoxin contamination in crops and highlight future needs.

Mycotoxins Modelling in Crops

Modelling mycotoxin is focused on a few crops and cereal grains, but both small grains and maize deserve major attention because of their worldwide distribution and their potential risk of contamination.

Fusarium head blight (FHB) is a serious wheat disease of worldwide interest, caused by a complex of Fusaria able to compromise grain production due to consistent negative effects on the harvested amount of grain and its quality. Deoxynivalenol (DON) and zearalenone (ZEA) are the main toxins commonly associated with this disease and are regulated in many countries worldwide, including all of the European Union [7].

The dynamic of FHB in small cereals and DON accumulation during ripening, or only at harvest, are the output of available predictive models (7 papers revised by [6,8-11]), with a tentative modelling in field variations of DON contamination in oats using proximal and remote sensing [12]. T2 and H-T2 toxins were recently considered [13,14] to be toxins of emerging interest, supposed to be comprised in EC regulation in a short time, with recommendations just settled.

Maize hosts several mycotoxin producing fungi belonging both to *Fusarium* and *Aspergillus* spp., with DON, ZEA, fumonisins (FUM) and aflatoxins (AF) as compounds of concern. They are all regulated in Europe and AF is regulated in most countries worldwide. Among the mentioned mycotoxins, one is commonly dominant with respect to the others in a maize crop, depending on the growing area and the year. Efforts devoted to modelling resulted in 8 papers: 4 for FUM (revised by[6]), 1 for DON [15] and 3 for AF [16,17]. Regarding other relevant crops, the interest in peanuts or grapes is focused in geographic areas where related mycotoxins are important. AF in peanuts in Australia[17] and ochratoxin A (OTA) in grapes in Italy [18].

Modelling Mycotoxins Without Crops

Most papers regarding modelling and mycotoxins, not related to crops, investigate how ecological factors influence fungi grown in controlled ecological conditions, on artificial or natural media. Several examples, published after 2010, regard toxigenic fungi important for cereals, grapes and nuts. Most of them focus on fungal growth and related mycotoxin production in different temperature and water activity regimes. Aflatoxin B_1 production by *A. flavus* was considered by S Marin, et al. [19],D Garcia, et al. [20],L Aldars-Garcia, et al. [21], and *A. parasiticus* by D Garcia, et al. [22]. The potential impact of key environmental factors, including extreme temperature and CO_2 (possible in future scenarios), and their interactions on the molecular ecology, growth and AF production by *A. flavus* was revised by A Medina, et al. [23].

Aspergillus carbonarius [22,24], together with other black aspergilli [25,26] and *A. ochraceus* [27] were studied for OTA production, as were *F. langsethiae* and *F. sporotrichioides* for T-2 and HT-2 production [13]. Other steps of toxigenic fungi infection cycles are sometimes investigated and modelled. Like sporulation of *F. langsethiae* and *F. sporotrichioides* [28], sporulation of *A. flavus* sclerotia [29], conidia germination of *A. carbonarius* [30] or *F. graminearum* ascospore release in different temperature and humidity regimes [31].

All these results, and similar papers published before 2010, define and sharply describe, with mathematical functions, the effect of each variable considered, such as temperature or water regimes, on fungal life. Even if they do not directly model mycotoxin production in crops, they contribute significantly as tassels in predictive modelling of mycotoxin risk in crops.

The Modelling Approach

An empiric or mechanistic approach can be applied in modelling (**Figure 2**).

The **empiric** approach describes the relation between the driving variables (i.e. rain during flowering) and the event of interest (i.e DON contamination in grain at harvest) using statistical analysis. A good example was developed by HJ Van der Fels-Klerx, et al. [32] in the Netherlands and by S Landschoot, et al. [10] in Belgium. They collected data on DON concentrations in many fields of mature winter wheat for several years and related cropping information and weather data. Based on a univariate analysis of variance, the set of variables able to give the best description of DON variability was selected. Both weather (mainly from about three weeks before flowering to harvested crop) and cropping data (i.e. wheat resistance level against *Fusarium* spp., flowering date, fungicide spray) were included. Then, applying multiple regression analyses, the best function able to relate the selected variables and DON contamination at harvest was settled.

The cause and effect relationship between variables is the basis for **mechanistic** models [33]. Mathematical functions to describe the relationship between driving variables and each step of fungi infection cycle (i.e. sporulation, infection or toxin production) are developed and mainly obtained by trials managed in controlled conditions, as previously mentioned. All mathematical functions are linked to obtain the mechanistic model (accounting for all the steps of the infection cycle), whose output is the risk index. Then, mycotoxin contamination in the field is compared to the risk index and a probability function is developed to determine the risk of contamination over a fixed threshold- commonly the legal limit [16,34].

Both empiric and mechanistic models are used to predict mycotoxin contamination in crops. The boundaries between the two approaches are not always well defined. For example, empiric elements are commonly used in mechanistic models, mainly in linking the risk index with contamination in the field [34]. Mycotoxin prediction in crops was mainly managed with empiric models, while the list of papers following the mechanistic approach is limited and focuses on FHB and DON contamination in wheat [35], *A. flavus* and AF in maize[16] and nuts [17], and *A. carbonarius* and OTA in grapes [18].

Empiric *Versus* Mechanistic Approach

Models developed according to the aforementioned modelling approaches differ in their **complexity of the data input** (unprocessed hourly meteorological data for mechanistic models or more complex and processed data, including cropping systems, for empiric models), **recalibration request** when applied in relevantly different conditions, i.e. a different geographical area (needed only for empiric models), and **time period** of predictions (during the crop growing season for mechanistic models, or only at harvest for empiric models) [16,32,36].

There are probably no specific reasons to be in favor of one approach or the other. Surely the combined use of more than one model can give more concrete predictions if the outputs are in agreement and highlight uncertainty where they disagree. There has been only one attempt to compare the two modelling approaches. It evaluates FHB and DON contamination in wheat, with the two models developed in Italy and The Netherlands (mechanistic and empirical, respectively). Satisfactory prediction resulted when both models were validated with external data collected in both countries, the empiric model after recalibration. This comparison led to the conclusion that the joint application of models developed according to diverse approaches can give practical advantages, with mechanistic contributing more in understanding plant-pathogen interaction and empiric more driving variables. Therefore, the two approaches should be compensative more than exclusive [37] as well experienced in meteorological forecast to reduce uncertainty [38].

Model Application

"Modelling is a dynamic simulation by numerical integration of component processes, accounting for variation in climatic factors, supported by computers, used to construct a comparatively transparent surrogate of reality based on existing knowledge," as summarized by Kumar [39] regarding crop modelling. This concept is perfectly applicable to modelling mycotoxins in crops when aimed to describe how mycotoxin producing fungi respond to key drivers, mainly intended as weather variables.

The input of weather variables can regard the ongoing season, past years, and the future (based on historical data).

The most common application regards the use of data from the ongoing season. Model output consists of the prediction of mycotoxin contamination during the growing season and at harvest. Very few choices are possible in season, in order to prevent mycotoxin contamination, and they are commonly based on agronomic or growth stage criteria. Therefore, the output of predictive models cannot support operational decisions. Nevertheless, they optimize harvest time and post-harvest management, including the correct way of production to food/feed/energy purposes, according to legislation compliance [40]. An interesting aid for farmers in pre-season decisions regarding the cropping system comes from past scenarios, the output generated by historical data input, where the risk associated to a geographic area is highlighted based on historic meteorological data, commonly shown as risk maps. Finally, predicted meteorological data allows the prediction of future scenarios (also presented as risk maps) with increasing interest, due to the confirmation of the ongoing climate change and its impact on mycotoxin contamination[41,42].

Modelling With a Changing Climate

The rise in air temperature and CO_2, different rain distribution throughout the year, with an increase in rain frequency and intensity during the winter and a decrease in summer, seem like certainties in the future of our climate. Change in the frequency of extreme events, including changes in climate variability are uncertainties we will face in the future, with the expectation of a strong impact on mycotoxins [43-45]. Crop geographic distribution and crop production, such as the phyllosphere mycoflora of crops, are expected to be strongly affected by climate change [41,42]. The alternation, in the same growing season, of weather conditions favorable to different mycotoxin producing fungi is the most reliable event, strongly suggesting focus in future studies be placed on the consequences of mycotoxin production and modification[46].

Research regarding mycotoxin modelling and climate change is still in the beginning stages. Very recent experiences considered the prediction of mycotoxin risk in cereals cultivated in Europe in climate change scenarios. Three studies followed an empiric approach. Two focused on DON in wheat, predictions regarded in north west Europe [47] and the UK [48], while the other considered the changing interaction between host, pathogen and environment with focus on primary inoculum and species composition in FHB in Scotland [8]. The mechanistic model, was applied to predict AF contamination of maize and wheat grown in Europe, but applicable worldwide with climate data available [41].

Re-calibration in changing conditions deserves consideration. We are headed towards a changing world and, in this context, mechanistic models should be more suitable than empiric ones.

Efforts are mandatory to face future changes and challenges. Mycotoxin-crop combinations considered in modelling are very limited [49] whereas the expected effects quite relevant. Therefore, modelling approaches that combine data on climate, pathogen and host, including cropping systems, could provide great support by giving estimates of disease and mycotoxin risk under the anticipated scenarios, as stressed by M Vaugham, et al. [42].

Conclusions

The global occurrence of mycotoxins is a matter of concern and the efforts devoted to improve knowledge of such have not yet marked the beginning of a downward trend in detected contamination. Moreover, it has been confirmed that we cannot escape, but only mitigate, mycotoxin contamination.

Research is focusing in pre-harvest, being the growing period of crops crucial to determine “if” mycotoxin contamination will take place and “how high” it will be at harvest.

Microbiome associated with host crops and its complex interactions, including the cross talk with the host plant in a certain environment, are the objects of deep studies to improve information and support the best prevention strategies. The complexity of these interactions is increasing in the ongoing climate change and the crucial support that can come from modelling is undoubtable, as stressed by several authors.

Nevertheless, the number of predictive models for mycotoxin contamination in crops is still low and needs to be enlarged. Data collection in controlled conditions has been greatly enriched since 2000. This is very good, but georeferenced field data is still particularly very poor, especially in certain areas of the world.

Furthermore, modelling approaches were faced by research groups separately and hesitant of comparing or merging their models. As a result, validating different areas from those of their development are almost absent.

Finally, food for thought: Many authors agree that the crucial point is that studies need to include climate projection, crop phenology and fungal/mycotoxin prediction. Equally important, the economic impact cannot be neglected. Holistic and interdisciplinary approaches are the key words to make predictive models a true support to start the downward trend in mycotoxin contamination in crops, and it would be even better if included in a decision support system, as described by V Rossi, et al. [50] for FHB in durum wheat.

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Figure 1. Proportion of papers found in Scopus (www.scopus.com; August 2016) using as key words "mycotoxin" alone or combined with "model" and with "crop". Starting from around 20,000 paper regarding mycotoxin, the combination of "mycotoxin" and "model" gives around 2,000 papers (10% of available literature on mycotoxins), and less than 1% (145 papers) including "crops."

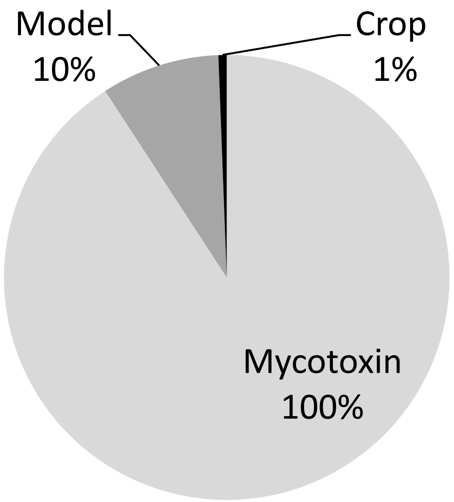

Figure 2. Summary of approach and data input for empiric and mechanistic models, including crops and mycotoxins considered in recent literature. Only field data are considered for empiric model, while in vitro data significantly contribute to mechanistic model development. They are based on a cause-effect relationship and therefore account for the infection cycle of involved mycotoxin producing fungi. Cereals are the key crops for mycotoxin problems, with deoxynivalenol (DON) and aflatoxins (AF), but nuts and AF, so as grapes and ochratoxin (OTA) are considered.