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Safe food and feed through an integrated toolbox for mycotoxin management: the MyToolBox approach

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OPINION ARTICLE

Abstract

There is a pressing need to mobilise the wealth of knowledge from the international mycotoxin research conducted over the past 25-30 years, and to perform cutting-edge research where knowledge gaps still exist. This knowledge needs to be integrated into affordable and practical tools for farmers and food processors along the chain in order to reduce the risk of mycotoxin contamination of crops, feed and food. This is the mission of MyToolBox – a four-year project which has received funding from the European Commission. It mobilises a multi-actor partnership (academia, farmers, technology small and medium sized enterprises, food industry and policy stakeholders) to develop novel interventions aimed at achieving a significant reduction in crop losses due to mycotoxin contamination. Besides a field-to-fork approach, MyToolBox also considers safe use options of contaminated batches, such as the efficient production of biofuels. Compared to previous efforts of mycotoxin reduction strategies, the distinguishing feature of MyToolBox is to provide the recommended measures to the end users along the food and feed chain in a web-based MyToolBox platform (e-toolbox). The project focuses on small grain cereals, maize, peanuts and dried figs, applicable to agricultural conditions in the EU and China. Crop losses using existing practices are being compared with crop losses after novel pre-harvest interventions including investigation of genetic resistance to fungal infection, cultural control (e.g. minimum tillage or crop debris treatment), the use of novel biopesticides suitable for organic farming, competitive biocontrol treatment and development of novel modelling approaches to predict mycotoxin contamination. Research into post-harvest measures includes real-time monitoring during storage, innovative sorting of crops using vision-technology, novel milling technology and studying the effects of baking on mycotoxins at an industrial scale.

Keywords: pre-harvest and post-harvest management, integrated mycotoxin management, thermal processing, decision support, dried distillers' grain soluble

1. Introduction

The occurrence of fungal and subsequently, mycotoxin contamination in various crops is of major concern since it has significant implications for food and feed safety, food security and international trade. The European Commission pointed out that 5-10% of global crop production is lost annually due to mycotoxin contamination (EC, 2015). Considering that the European Union (EU) produces about 133 million tonnes (MT) of wheat (~29,038 M€), 68 MT maize (~13,571 M€) and 8 MT oats (~1,543 M€) annually (Eurostat, 2015a,b), about 1.2-2.4 billion Euro can be lost for wheat alone, thus a reduction in these losses of only 1% could save 12-24 M€. The total costs of mycotoxin contamination due to reduced yields, food and feed losses, increased costs for inspection and analyses, and others, may easily reach billions of Euros annually as estimated by Wu and Munkvold (2008). Moreover, with 'one in eight persons worldwide suffering from chronic undernourishment' (Schmale and Munkvold, 2009) and recent reports of the impact of aflatoxins on stunting of children in Africa (Matacic, 2016; Wild *et al.*, 2015), there is clearly a moral obligation to curb these significant food and feed losses.

In a recent multi-mycotoxin survey, 81% of all samples were contaminated with at least one mycotoxin and 45% contained more than one secondary metabolite of fungi (Nährer and Kovalsky, 2014). The EU's Rapid Alert System for Food and Feed (RASFF) (EC, 2016) showed that of the total border rejections in 2015, 18.3% were due to mycotoxin contamination exceeding the EU legislative limits, accounting for the most frequently reported chemical hazard. The product category with the highest susceptibility of aflatoxin contamination was 'nuts, nut products and seeds'. For example, over the last five years RASFF reported 308 alerts relating to aflatoxin levels exceeding legal limits in peanuts imported from China. However, as illustrated by the RASFF notifications (Figure 1), a significant reduction of about 50% of mycotoxin-related RASFF notifications has been observed over the last 12 years, from 876 notifications in 2004 to 492 notifications in 2015.

Nonetheless, prevention and control of these toxic secondary metabolites remains difficult and the agriculture and food industries continue to be vulnerable to problems of contamination. For example, from February to March 2013, Romania, Serbia and Croatia reported high aflatoxin M_1 contamination of milk. Severe droughts in Serbia in 2012 resulted in 70% of the maize crop being contaminated with aflatoxins (Anonymous, 2013). Feeding this maize to dairy cattle led to high levels of aflatoxin M_1 in milk, up to twice the EU legal limit of 0.05 μg/kg. The milk scandal was fuelled when the permitted level of aflatoxin M_1 in milk in Serbia was temporarily raised to 0.5 μg/kg milk, 10 times the EU legal limits. However, the catastrophic floods and the rainy summer in 2014 resulted in low levels of aflatoxin B_1 with high levels of dexoynivalenol (DON) in Serbia (Jakšić *et al.*, 2015). Obviously, extreme weather conditions as a result of climate change are increasingly affecting the mycotoxin map in Europe and also world-wide (Battilani *et al.*, 2016).

Furthermore, a dramatic increase in DON contamination in grains cultivated in Nordic countries has been observed in recent years. The Norwegian Scientific Committee for Food Safety concluded in a study published in 2013 that '[*t*]*he estimated mean and high (95-percentile) exposures to DON* [...] *were in the range of, or exceeded the TDI* [Tolerable Daily Intake] *by almost 2 times in 1-year-old infants and 2-year-old children and up to 3.5 times in years with high DON levels which has been especially true for oat based products* (Norwegian Scientific Committee for Food Safety, 2013). In general, grain-based foods (e.g. pasta, bread, bakery products) account for the largest contribution to mycotoxin exposure in all age classes of the EU population,

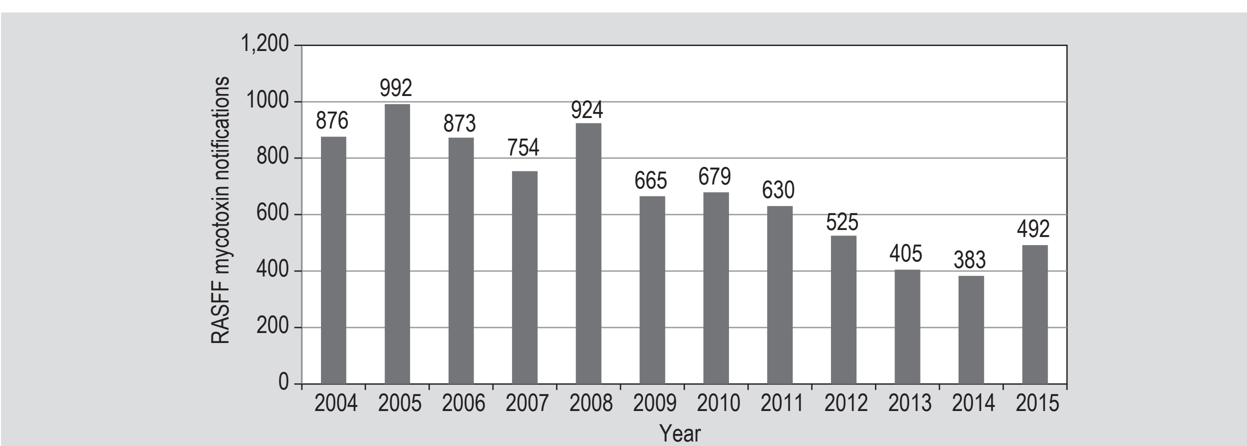

Figure 1. RASFF mycotoxin notifications from 2004-2015.

in particular due to the mycotoxins produced by *Fusarium* spp. (DON, T-2/HT-2 toxins, zearalenone, ochratoxin A and fumonisins). However, as shown above, contamination with these mycotoxins is difficult to predict and manage, due to new trends in mycotoxin occurrences.

Exposure of livestock to mycotoxins is also estimated to result in substantial economic losses (Wu and Munkvold, 2008). While mycotoxicoses in farmed animals are regularly reported (Jovanović *et al.*, 2015), it is difficult to obtain an accurate estimate of the burden of market loss associated with animal health and productivity, and loss of animal feed. However, several publications from different parts of the world provide some insight into the potential magnitude of the problem. In the USA, Vardon *et al.* (2003) estimated a loss of 225 million US$ per annum for feed maize due to contamination with aflatoxins, and an additional loss of livestock of 4 million US$ per annum. Wu and Munkvold (2008) estimated the losses due to decreased weight gain in pigs in the USA, resulting from feeding dried distillers' grain solubles (DDGS) contaminated with fumonisins, at 147 million US$ annually (assuming 20% DDGS in the diets and market coverage is 100%). A study conducted in Thailand (Lubulwa *et al.*, 2015) revealed that aflatoxins in maize can be expected to cause losses for the livestock sector between 7 and 110 million US$ per annum. These estimates show that, although limited to aflatoxins and fumonisins in two countries only, the losses in the livestock sector will be significantly multiplied if other commodities and other mycotoxins are taken into account.

Previous success in reducing mycotoxin contamination in food and feed now necessitates the mobilisation of the wealth of knowledge gained over the past 25-30 years, to merge in cutting-edge research and the closure of knowledge gaps. With practical and affordable tools that build on existing research-results, stakeholders along the food and feed chain can reduce the risk of mycotoxin contamination in crops, food and feed. These tools prevent losses and waste along the food and feed chain, and provide traceable information to the supply chain and consumers using mainstream information and communication technology (ICT). Existing knowledge combined with novel findings need to be adapted and integrated to provide the vehicle needed to practically implement this knowledge into tools for use along the food and feed chain. This is the mission of MyToolBox (www.mytoolbox.eu), a four year project, which has been recently funded by the European Commission (EC) and which was officially launched in March 2016 (Figure 2). The project applies a multi-actor and multi-disciplinary approach throughout the food and feed chain with 40% industry participation including five end users and three Chinese institutions who collaborate closely with farmers and other relevant stakeholders from the industry.

2. Objectives

The overall objective of the MyToolBox project is to develop a series of integrated measures, which will enable a significant reduction of different kind of losses due to mycotoxin contamination. Besides a field-to-fork approach along the food and feed chain, MyToolBox also considers safe use options of mycotoxin-contaminated batches such as microbial energy conversion to efficiently produce biogas and bioethanol. The consideration of the entire production chain (soil-field-crop-food processing-waste management-alternative energy-government) is a major motivation behind MyToolBox to ensure food and feed security and safety within a sustainable economic environment. The mycotoxin commodity combinations that are specifically addressed are the most prevalent *Fusarium* mycotoxins (DON, T-2/HT-2 toxins, zearalenone and fumonisins) in wheat, oats, maize and animal feed chains, ochratoxin A in wheat, and aflatoxins in maize, peanuts and dried figs.

The pre- and post-harvest interventions that are being developed within the MyToolBox project for these mycotoxins build on, and integrate, the wealth of knowledge resulting from the substantial EU-, nationally and internationally, currently and previously funded research projects on mycotoxin prevention and control, such as the Mycotoxin Prevention Cluster (FP5 projects), MycoGlobe (FP6 project, MycoRed and MycoKey [www.mycored.eu/d/84/MycoKey/]). Consequently, existing knowledge, novel findings and technologies developed in MyToolBox will be integrated into a comprehensive series of tools and applications. These will be accessible via a dynamic web-based MyToolBox e-platform (MyToolBox; Figure 2) that will also be accessible with mobile devices, which will provide the end users along the whole food and feed chain with the recommended measures for mycotoxin reduction.

By collaborating with partners from industry, farming communities, agronomists, manufacturers and other professionals in agriculture and food processing, SMEs and academia from the EU and beyond, the usability and applicability of the MyToolBox decision support system is being ensured.

The MyToolBox project also anticipates legislative implications of its project outputs. As such, it is cooperating with institutions experienced in standard setting, such as the European Food Safety Authority (EFSA) and the EC's Directorate-General DG Santé. In addition, it aims to develop a sound scientific basis for setting authorisation standards of mycotoxin detoxifying feed additives in China.

The following major objectives are pursued over the course of the 48-month project.

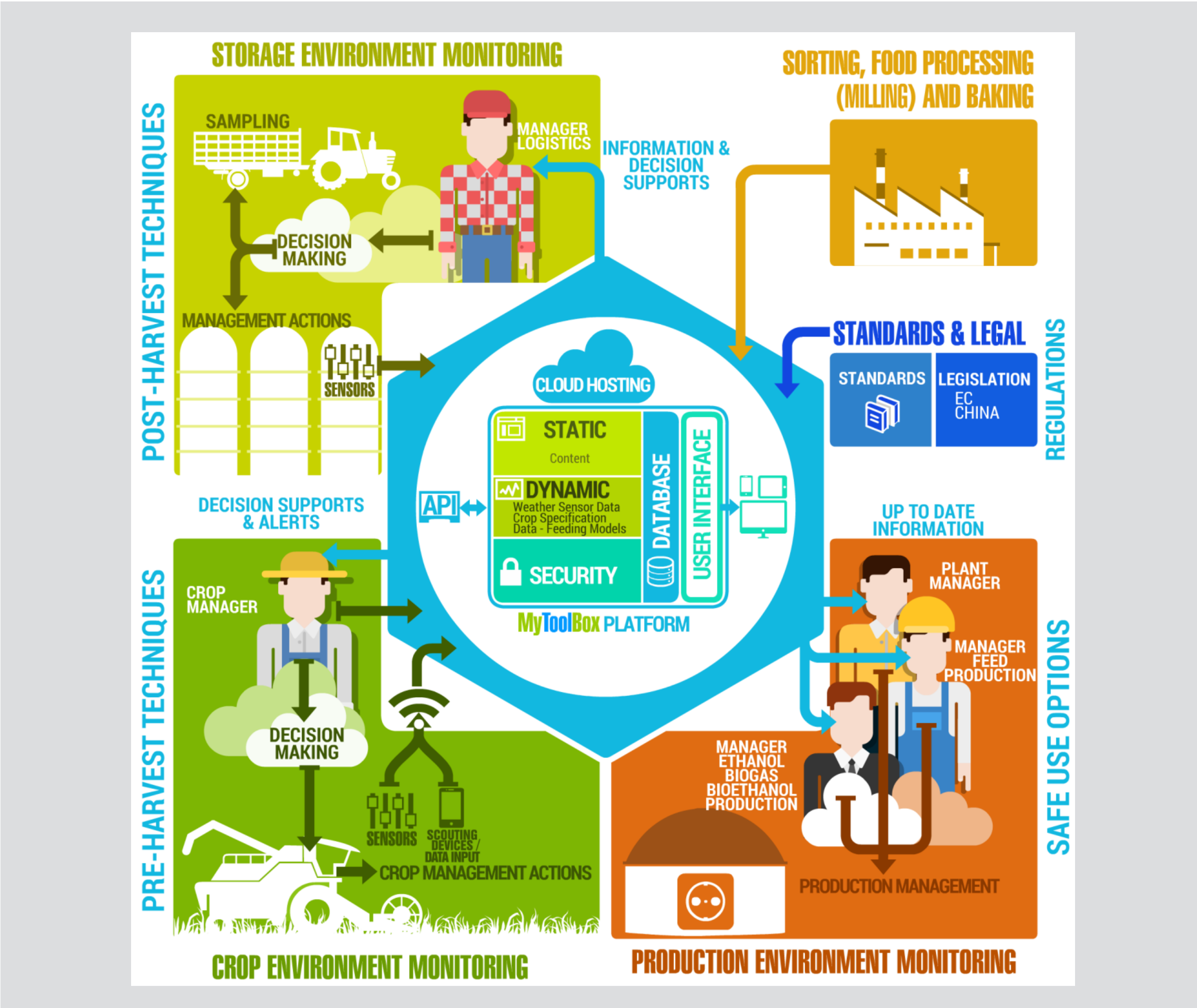

Figure 2. Overview of the MyToolBox project approach (© IRIS).

Pre-harvest objectives

- To reduce fungal infection of grains on field, biopesticides (e.g. Forrer *et al.*, 2014) permitted within organic production systems are used in combination with agro-management tools such as water and soil conservation.
- To increase organic matter within the soil, more sustainable crop production strategies in combination with minimum tillage are being utilised. To determine the most promising available novel cultural control strategies targeting the *Fusarium* inoculum within crop debris, *Brassica* biofumigation (Ngala *et al.*, 2015; Perniola *et al.*, 2014), as well as accelerated biodegradation (Hofgaard, 2012) with an extract or competitive fungi (Matarese *et al.*, 2012) are being explored.
- To increase the climate change resilience of EU-maize farmers and thus reduce susceptibility of maize to aflatoxin contamination, MyToolBox is testing two measures: (1) selecting appropriate genotypes of maize with various traits (e.g. drought resistance, early mature plant cultivars) in field conditions that are likely to reduce aflatoxin contamination; and (2) applying atoxigenic *Aspergillus* strains that are out-competing toxigenic *Aspergillus* strains, which is a novel and proven technology developed in the USA and Africa (Atehnkeng *et al.*, 2008; Bandyopadhyay *et al.*, in press; Brown *et al.*, 1991; Probst *et al.*, 2011), adapted to Europe in collaboration with the international experts from those continents.
- To assist short term decision making regarding fungicide application, harvest timing, and long term planning, available forecasting models regarding DON contamination in wheat and maize are being validated, combined and extended to cover the most important wheat and maize growing areas in Europe. Previously developed weather-driven forecasting models were validated to a limited extent, mainly in the regions where they have been developed, and mostly to predict *Fusarium* head blight or DON contamination specifically in wheat. By synergising and extending existing models,

MyToolBox will therefore significantly improve the forecasting of potential DON contamination in major grain crops in Europe.

Post-harvest objectives

- To facilitate effective integrated control of moulds and mycotoxins and to provide an early warning of risks, and thus enable implementation of effective prevention/remedial actions, real-time post-harvest decision support systems for storage of cereals and peanuts are being developed. They are based on the sensitive indicator of CO_2, as well as temperature and relative humidity, and will be tested for local conditions in European and Chinese cereal and peanut silos.
- To achieve significant improvements in sorting of dried figs and to also improve the work environment dramatically, MyToolBox develops a non-invasive system for the real-time sorting of contaminated dried figs, based on visible and near infrared spectral imaging and appropriate algorithms. Traditionally, to remove units contaminated with aflatoxins, dried figs are sorted by hand (Benalia *et al.,* 2013; Oezlueoymak, 2014; Patel *et al.*, 2012). These require working conditions in the dark, under UV light, which is potentially harmful to the health of the employees (Ichihashi *et al.*, 2003; Kammeyer and Luiten, 2015; Lavker *et al.*, 1995). Therefore, besides contributing to reduced contamination of mycotoxins in dried fig batches (with 5% or less probability of the batch average concentration of aflatoxin B_1 failing to meet the EU limits), MyToolBox will also contribute to occupational health improvements.
- Innovative pre-milling and milling strategies are combined and scaled-up, which will lead to more accurate separation of grain tissues with characterised (different) mycotoxin contamination levels. Milling strategies, such as debranning and micronisation, are efficient, but individual tissues of the grains are often inhomogeneous and not well confined. By-products, usually intended for animal feed but also exploitable for wholegrain food products, contain about 15% proteins, 40% starch and 40% fibre with levels of mycotoxins which can exceed legal limits (Delcour *et al.*, 2012). The synergistic potential of debranning, micronisation and dry- or wet-turbo separation will minimise the mycotoxin concentration in raw materials and the final wheat products destined for the consumer.
- Within MyToolBox, the fate of mycotoxins as well as their modified forms and co-occurrences in naturally contaminated raw materials (with simultaneous co-occurrence of different mycotoxins) will be monitored during thermal food processing at an industrial scale with Barilla (Parma, Italy) . These measures are expected to provide (1) structural information of altered chemical structures (using newest LC-HRMS and metabolomics techniques (Bueschl *et al.*, 2014)); and (2) novel bakery production protocols, controlling for instance fermentation, temperature/time and recipes, to develop a sound basis to establish process factors to reduce risks as part of a post-harvest intervention strategy (Bergamini *et al.*, 2010; Generotti *et al.*, 2015). These efforts cater to the need to obtain further data on the fate of masked/modified mycotoxins, as defined by EFSA in 2014 (EFSA CONTAM Panel, 2014), during food and feed processing. Hence, MyToolBox complements previous studies in this area by adding results of in-situ tests with practical applicability to lab-scale experiments that have used artificially contaminated material at high levels (Meca *et al.*, 2013; Valle-Algarra *et al.*, 2009; Voss and Snook, 2010).
- To study the use of mycotoxin contaminated batches in biofuel production MyToolBox partners Südzucker (Mannheim, Germany) and BIOMIN (Getzersdorf, Austria) are investigating the reduction of the mycotoxin content in the by-product DDGS, for which recently available novel enzymes will be utilised in technical scale fermentation processes. Maize-based DDGS can be contaminated with high levels and a broad spectrum of mycotoxins (US Grains Council, 2012; Rodrigues and Chin, 2011; Schaafsma *et al.*, 2009; Zachariasova *et al.*, 2014; Zhang and Caupert, 2012), resulting in losses in feed production. Micro-organisms can degrade mycotoxins but the efficacy for a safe use to efficiently produce biogas (Hanschmann, 2008) and bioethanol (Kłosowski *et al.*, 2010) by fermentation of mycotoxin-contaminated batches must still be determined. Therefore, the performance of specific mycotoxin-degrading microorganism strains (Vekiru *et al.*, 2010) and novel enzymes provided by partner BIOMIN, on the detoxification of DON (EC, 2013) and fumonisins (EC, 2014) during biogas and bioethanol production are being evaluated.

Objectives throughout the food and feed chain including dissemination

- To provide decision-support in mycotoxin management to different end-users along the food and feed chain, an integrated, dynamic, MyToolBox e-platform is being designed, tested and validated. This e-platform will provide users with information, tools and guidance concerning actions that can be taken to reduce fungal contamination, leading to reductions in contamination-levels of mycotoxins in the food and feed chains. The MyToolBox e-platform will be accessible to farmers, food processors and other actors along the chain through all mainstream interfaces (PCs, tablets or smartphones).
- To ensure economic feasibility, cost-effective sampling and monitoring strategies for mycotoxin management applied over the entire food and feed chain are adapted and integrated in the MyToolBox e-platform.
- To strengthen international cooperation in research and innovation for mycotoxins, all work packages reflect

collaborative actions and tasks between partners from two or more different countries. To invigorate the ties between China and Europe in the area of mycotoxin reduction, MyToolBox has initiated a formal EU-China dialogue through an EU-China Mycotoxin Forum.

3. Expected impact

Overall, the impacts of an integrated toolbox for mycotoxin management, as developed within MyToolBox shall be a reduced number of incidents and more transparent supply chains. The developed and applied pre- and post-harvest measures, including reliable monitoring of crops and proper risk assessment strategies, should lead to significant economic and food safety benefits and improvements in mycotoxin management along the food and feed chains. Moreover, MyToolBox shall contribute to improved consumer trust in imported food and feed. Members of EFSA will be on the advisory board to ensure European citizens are well informed regarding risks in the food and feed chain.

Building on the existing knowledge, MyToolBox integrates the wealth of knowledge on mitigation and reduction of mycotoxins in food and feed in one single online decision support platform, leading to more efficient use of resources. In particular unexpected contamination of grains can cause huge losses: For example, the long term costs for maize based feed contaminated with aflatoxins are estimated to cost Serbia hundreds of millions of Euros per year. Thus, it is expected that farmers and other stakeholders along the food and feed chain will benefit financially by saving resources (e.g. fertilisers, seeds, etc.) and by lowering the risk of rejection of their products due to unexpected contamination. As such, mycotoxin-related border rejections noted by RASFF are expected to decrease further (Anonymous, 2014).

Pre-harvest impacts

The pre-harvest impact of the MyToolBox work will be measured by comparing the achieved reduction in mycotoxin content and increase in grain yield, based on field trials, between the conventional agricultural systems to the newly designed systems. The use of biopesticides and biofumigation or debris treatment in EU field trials each should reduce the DON level in wheat by 50% (Paul *et al.*, 2008). However, the combined effect is yet to be explored. In EU field trials the aflatoxin content of resistant maize cultivars will be compared to susceptible cultivars, which is expected to be lower than previously achieved 70% (Windham and Williams, 1998). The aflatoxin contents of maize (EU) and peanuts (China) after treatment with atoxigenic fungal strains will be compared to non-treated crops in field trials. The aim is to achieve a 90% reduction in aflatoxin contamination (Dorner, 2004).

Post-harvest impacts

The post-harvest impact of the real-time sensors developed in MyToolBox will be measured by comparing the achieved reduction in mycotoxin content of cereals in trials in grain silos compared to conventional practices. This is expected to contribute to approximately 15% savings (~100 M€/year). While it is impossible to accurately determine the reduction in costs, it is estimated that costs for monitoring could be reduced by at least 30% at all stages of the food and feed supply chain. This impact will be measured by comparing the estimated current costs for monitoring by EU governments and industry to the newly designed monitoring systems. Overall, MyToolBox aims to reduce post-harvest losses, due to mould spoilage and mycotoxins, pests in food/feed chains, in the EU and its associated countries by at least 15%.

The novel non-invasive real-time sorting of dried figs is expected to result in maximum 5% probability of the batch average concentration of aflatoxin B_1 failing to meet the EU limits as compared to conventional sorting. The developed innovative milling and baking techniques aim to achieve a 50 and 15% mycotoxin reduction in the finished food products respectively. New information on the presence of mycotoxins and their modified forms resulting from food processing will be forwarded to EFSA as a relevant contribution for its overall risk assessment of mycotoxins in foods.

The effect of the use of enzymes on mycotoxin reduction during biofuel production is being assessed in trials with and without addition of the enzymes. The impact will be a significant reduction of the DON- and fumonisin-content in DDGS as opposed to conventional production. In 2013, about 5.2 million m^3 of bioethanol were produced from grains in Europe (and 88.8 million m^3 worldwide), resulting in a production of about 4.2 million tons of DDGS in Europe. It is estimated that the losses in feed production due to mycotoxin-contaminated DDGS to be in the range of 15-20 million Euro annually for the swine industry alone. Therefore, a reduction of losses is expected due to (1) significantly lowering mycotoxin levels in DDGS; (2) improved efficiency of the bioethanol production of 10-30%; and (3) increased market value (>10%) of DDGS of low mycotoxin content. The outcome of these MyToolBox studies could also positively contribute to the current food vs fuel debate, as mycotoxin contaminated batches could then be safely employed to produce biofuels on the one hand, and high quality DDGS on the other hand.

Impacts throughout the food and feed chain including dissemination

Finally, the web-based MyToolBox platform will represent a one-stop-shop consisting of information and advisory modules, management tools, as well as technologies that equip farmers and all other actors in the food and feed supply chain with systematic, pro-active, cost effective and affordable approaches for effective monitoring and reduction of the incidences of mycotoxin contamination in crops, food and feed. In addition, it will contribute to the reduction of stakeholders' dependence on expensive and wasteful end-point testing and segregation.

The EU-China partnership within MyToolBox is aimed to contribute to the standard settings for authorisation of mycotoxin-detoxifying feed additives in China, whereby current EU guidelines for the registration of detoxifying feed additives shall serve as examples for possible adaptions of Chinese legislation. This is important as currently about 475 million pigs are farmed in China, representing approximately half of the world's total pig population. Since up to 24% of complete feed for pigs have exceeded the maximum limits set for DON levels by the Chinese authorities (Li *et al.*, 2014). The need for more than 54,000 metric tons of detoxifying agents can be estimated in China, thus contributing to more efficient pork-meat production in China.

4. Conclusions

The MyToolBox project does not only pursue a field-to-fork approach to reduce mycotoxins in wheat, oats, maize, peanuts and dried figs, but also considers safe use options of mycotoxin contaminated batches such as microbial energy conversion for efficient production of biogas, bioethanol and safe DDGS. Up to now, different agricultural strategies including crop rotation, tillage practices, fungicide application and the use of resistant plant cultivars have been used to reduce the impact of fungal infection of crops and subsequent mycotoxin formation. As none of these strategies by themselves have been able to efficiently reduce the impact of fungal infection, a novel integrated approach is pursued, which also takes into account issues related to climate change. Consequently, MyToolBox combines a series of integrated pre- and post-harvest measures, which could enable a 20-90% reduction (depending on the type of commodity and intervention) in losses of crops due to fungal and mycotoxin contamination.

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