

1 **Perspectives on global mycotoxin issues and management from the MycoKey Maize Working Group**

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5 **Abstract:** During the last decade, there have been many advances in research and technology that have
6 greatly contributed to expanded capabilities and knowledge in detection and measurement,
7 characterization, biosynthesis, and management of mycotoxins in maize. MycoKey, an EU-funded
8 Horizon 2020 project, was established to advance knowledge and technology transfer around the globe
9 to address mycotoxins impacts in key food and feed chains. MycoKey included several working groups
10 comprised of international experts in different fields of mycotoxicology. The MycoKey Maize Working
11 Group recently convened to gather information and strategize for the development and
12 implementation of solutions to the maize mycotoxin problem in light of current and emerging
13 technologies. This feature summarizes the Maize WG discussion and recommendations for addressing
14 mycotoxin problems in maize. Discussions focused on aflatoxins, deoxynivalenol, fumonisins, and
15 zearalenone, which are the most widespread and persistently important mycotoxins in maize. Although
16 regional differences were recognized, there was consensus about many of the priorities for research
17 and effective management strategies. For pre-harvest management, genetic resistance and selecting
18 adapted maize genotypes, along with insect management, were among the most fruitful strategies
19 identified across the mycotoxin groups. For post-harvest management, the most important practices
20 included timely harvest, rapid grain drying, grain cleaning, and carefully managed storage conditions.
21 Remediation practices such as optical sorting, density separation, milling, and chemical detoxification
22 were also suggested. Future research and communication priorities included advanced breeding
23 technologies, development of risk assessment tools, and the development and dissemination of
24 regionally relevant management guidelines.

25

26 **Introduction**

27 Our perception of the role of mycotoxins in food and feed safety has evolved continuously since the
28 1960s when mycotoxins were first explicitly recognized. Hundreds of toxic fungal metabolites now have
29 been described and many of them occur in maize. Among this myriad of mycotoxins, it has become
30 clear that there are four types that consistently affect maize and the animals that consume it; these
31 are aflatoxins, deoxynivalenol (DON) and its derivatives, fumonisins, and zearalenone (Munkvold et al.
32 2019). As a result, efforts to manage mycotoxin risks in maize have focused on these compounds and
33 the fungi that produce them; primarily, *Aspergillus flavus* (Fig 1a), *Fusarium graminearum* (Fig 1b,c),
34 and *Fusarium verticillioides* (Fig 1d,e, f). Ear rots caused by *F. graminearum* and related species are

35 categorized as “Gibberella ear rot,” “red ear rot,” or “red fusariosis,” whereas those caused by *F.*
36 *verticillioides* and related species are often categorized as “Fusarium ear rot,” “pink ear rot,” or “pink
37 fusariosis.” Ecology, epidemiology, and management of these fungi and their toxins have been
38 reviewed from several perspectives during the past decade (Miller et al. 2014; Munkvold 2014;
39 Munkvold et al. 2019; Palumbo et al. 2020; Perrone and Gallo 2017). Although there are dozens of
40 other mycotoxins and toxigenic fungal species in maize, management aimed at these three species and
41 their toxins has effects that carry over effectively to many of the less common species and mycotoxins.

42 Research and outreach efforts by public and private entities and NGOs are continuously being carried
43 out, addressing all aspects of prevention and mitigation of mycotoxin risks. Among these efforts have
44 been several comprehensive global projects funded by the European Commission, including
45 MycoGlobe (2004 To 2008), MycoRed (2012 To 2016), MyToolBox (2016 to 2020), and MycoKey (2016
46 to 2020). MycoKey (<http://www.mycokey.eu/>) aims at developing and testing smart, integrated,
47 sustainable solutions and innovative tool kits to reduce the major mycotoxins in economically
48 important food and feed chains. Specific objectives include: development and implementation of rapid,
49 reliable, and validated detection tools for toxigenic fungi and multi-mycotoxins; integration
50 of predictive models for aflatoxins, fumonisins, DON and zearalenone contamination in maize;
51 translation of existing monitoring technologies into on-site/storage specific application tools for
52 growers; and communication of mycotoxin management advice with final users. MycoKey is structured
53 into 10 Work Packages encompassing research and communication activities across four food/feed
54 chains (maize, wheat/barley, dried fruit, and grapes) (Fig. 2). Work Package 1 is entitled “Global
55 Mycotoxin Knowledge” and one of the tasks for this Work Package was the establishment of Working

56 Groups corresponding to the four food/feed chains. This review reflects information generated by
57 some activities of the Maize Working Group.

58

59 **Global Ear Rot and Mycotoxin Issues in Maize**

60 Although there are strong commonalities across global maize growing regions regarding the main ear
61 rot diseases and mycotoxins, there are significant regional variations in disease severity, pathogen
62 species composition (Table 1), mycotoxin occurrence, and management options. These variations are
63 climate-driven as a function of latitude, elevation, and rainfall, but also driven by regional crop
64 production and storage practices and resource availability. The magnitude and nature of mycotoxin
65 problems also depends largely on regional differences in maize consumption patterns in the human
66 diet. The following sections highlight the most important issues and management practices for each
67 continent where maize is grown.

68 ***Africa*** - Maize is the critical staple of millions across the African continent. Maize is grown virtually in
69 all of Africa, predominantly by smallholder farmers (Guilpart et al. 2017). In 2018, the highest maize
70 producers in Africa were South Africa, Nigeria, Ethiopia, Egypt, and Tanzania, with 12.5, 10.2, 7.4, 7.3,
71 and 6.0 million tons, respectively (<http://www.fao.org/faostat/>). Maize production has increased
72 significantly in certain countries (e.g., Ethiopia), due to recognition of maize as a high-value food crop,
73 improved varieties, use of fertilizers, and support of extension agents that attend the needs of farmers
74 (Abate et al. 2015). In addition, maize is becoming an important livestock feed ingredient. For example,
75 in Nigeria, about 2 million tons of maize are used by the poultry sector alone.

76 However, maize production in the African continent faces serious challenges preventing it from
77 reaching its maximum potential (Guilpart et al. 2017). Both the productivity and the safety of maize
78 grown in Africa is threatened by climate change (Medina et al. 2017). Diverse mycotoxins, most
79 importantly aflatoxins and fumonisins, frequently contaminate maize throughout the value chain and
80 this is driven by both agronomic and climatic challenges. In addition, sociological and institutional
81 challenges in the continent also contribute to mycotoxin contamination (Ezekiel et al. 2019). Large
82 numbers of scientific papers continuously report single or multiple mycotoxins contaminating maize
83 produced across Africa (Mahuku et al. 2019; Misihairabgwi et al. 2019). In many cases, the detected
84 mycotoxin concentrations are well above tolerance thresholds. Across Africa, mycotoxin contamination
85 standards are either poorly enforced or nonexistent for several reasons that include lack of mycotoxin
86 awareness, little to no human capacity and infrastructure for monitoring, lack of discrimination in
87 markets, copious local markets that are difficult to sample comprehensively, and other constraints
88 (Bandyopadhyay et al. 2016). In addition, contaminated crops that are rejected due to high mycotoxin
89 content often are diverted to informal markets, causing higher exposure among the poor. All these
90 challenges result in chronic mycotoxin exposure among African populations, several health disorders—
91 sometimes death—, and loss of trade opportunities (Kamala et al. 2018; Misihairabgwi et al. 2019; Wu
92 2015).

93 There are several mycotoxin management strategies available for use in different African contexts. It
94 is important to note that, in many cases farmers do not employ available management strategies
95 because they are not aware of the mycotoxins, are unaware of the available technologies, or the
96 technologies are labor- and cost-prohibitive. Management practices include adhering to recommended
97 planting dates to avoid stress conditions, planting adapted disease-resistant hybrids, tillage practices

98 to bury residues, rotating with non-susceptible crops, correct use of fertilizers, maintaining optimal
99 plant densities, controlling other plant pathogens and weeds, harvest on time, rapid grain drying,
100 avoiding mechanical and insect damage, and storing the maize at $\leq 13\%$ moisture in a clean, cool, well-
101 ventilated place with no insects (Hell and Mutegi 2011). However, in some regions, even if all
102 recommended practices are followed, mycotoxin contamination still occurs. In Tanzania, for example,
103 it was found that climate, soil characteristics, and geographic conditions are more likely to influence
104 *Fusarium* communities and both fumonisin and DON levels than farming practices (Degraeve et al.
105 2016). Intrinsic characteristics of the maize agroecosystems coupled with unexpected events such as
106 drought, insect attack, or flooding, have a large influence on the final mycotoxin accumulation.

107 Use of Bt maize in the African continent is allowed only in South Africa and Sudan (ISAAA 2017); political
108 opposition and insufficient regulatory processes hinder its approval in other African nation . Studies
109 conducted in South Africa have reported that insect-resistant Bt maize hybrids consistently contain
110 significantly less fumonisin and trichothecene concentrations compared to conventional maize hybrids
111 (Alberts et al. 2017; Pellegrino et al. 2018). However, despite its benefits, adoption of insect-resistant
112 Bt maize by smallholder farmers in South Africa remains a challenge because of the high cost and the
113 preference of farmers to pay for herbicide-tolerant transgenic maize hybrids (Alberts et al. 2017).

114 Maize germplasm with resistance to aflatoxin contamination has been identified in Nigeria (e.g.,
115 (Meseka et al. 2018), Kenya, and South Africa. The aflatoxin-resistant germplasm from Kenya and South
116 Africa was found to be resistant to fumonisin accumulation as well (Rose et al. 2017). Other research
117 efforts in South Africa have identified inbred lines with superior resistance to fumonisin accumulation
118 (Small et al. 2012) and hybrids with reduced susceptibilities to both aflatoxin and fumonisin
119 contamination have been developed (Chiuraise et al. 2016). Although germplasm with superior

120 resistance to aflatoxin and/or fumonisin is widely being used in breeding pipelines of different
121 organizations, significantly more efforts are needed to make improved materials reach the farmers.
122 Additionally, biofortification (enhancement of nutritional value through genetic manipulation) is now
123 part of several breeding programs across Africa, including maize. Relatively recently, it was reported
124 that certain provitamin A maize materials have reduced susceptibility to aflatoxin contamination
125 (Suwarno et al. 2019). Large-scale use of maize germplasm with increased provitamin A may contribute
126 to reduce aflatoxin exposure in African nations and elsewhere.

127 Mycotoxin-predictive models for use in Africa and elsewhere have been difficult to achieve because of
128 large number of variables—both biotic and abiotic—that influence contamination events (Mahuku et
129 al. 2019; Munkvold 2003c). A model developed in South Africa took into account maximum
130 temperature and minimum humidity as determinants for fumonisin-producing fungi to infect maize
131 grains but this was also conditioned by weather conditions after flowering and maize dough stage (Rose
132 et al. 2018). Models should also take into account the role of diverse insects attacking the maize ears.

133 Aflatoxin-predictive models have been developed for use in Kenya (Chauhan et al. 2015). It is unclear
134 whether the models have reached large-scale use as decision support tools by farmers or agribusiness.

135 Biocontrol has become an important tactic for prevention of aflatoxins in several African nations. The
136 International Institute of Tropical Agriculture (IITA) and the United States Department of Agriculture –
137 Agricultural Research Service (USDA–ARS), and local national institutes have successfully adapted and
138 improved aflatoxin biocontrol technology for use in sub-Saharan Africa under the trade name Aflasafe
139 (Bandyopadhyay et al. 2016). Within each nation, Aflasafe product contains four native atoxigenic
140 isolates of *A. flavus*, which contain several SNPs, deletions, or insertions in the aflatoxin biosynthesis
141 gene cluster, preventing them from producing aflatoxins (Adhikari et al. 2016). Aflasafe products have

142 been developed, carefully tested, and registered with national biopesticide regulatory authorities for
143 use in maize in Nigeria, Kenya, Tanzania, Senegal, The Gambia, Burkina Faso, Ghana, Zambia,
144 Mozambique, and Malawi (Moral et al. 2020). Products for use in another 10 nations are currently
145 being developed. To increase production and distribution of Aflasafe, IITA has transferred the
146 technology to public or private entities in Kenya, Nigeria, Senegal, and Tanzania, for manufacture and
147 distribution, and to raise awareness about the aflatoxin problem and its impact on health. More
148 transference agreements are being discussed for other countries. The use of biocontrol products to
149 limit aflatoxin content of maize is reducing aflatoxin exposure and is resulting in increased income and
150 trade benefits to smallholder farmers (Agbetiameh et al. 2019; Bandyopadhyay et al. 2019; Senghor
151 et al. 2020).

152 Prior to storage, sorting discolored, damaged, or irregularly shaped kernels is useful to remove grains
153 with high mycotoxin content (Matumba et al. 2015). Drying and storing grain at safe moisture levels
154 are important challenges in Africa. Adequately drying outside of the field, off the ground and on
155 platforms, or using solar dryers, has been shown to reduce the growth of toxigenic fungi (Hell et al.
156 2008; Ogunkoya et al. 2011). Packaging materials or enclosed structures for stored grain that prevent
157 absorption of moisture or provide a controlled atmosphere (hermetic storage with high CO₂ and low
158 O₂) have been shown to inhibit *A. flavus* growth and reduce aflatoxin production in maize (Hell and
159 Mutegi 2011; Walker et al. 2018) . Several storage technologies have been developed that are suitable
160 for smallholder farmers. Hermetic storage bags are becoming increasingly popular as several
161 manufacturers are producing them, and more than a million bags have been sold in Africa. However,
162 uptake of many other technologies has been slow due to their cost or availability (Hell and Mutegi
163 2011; Walker et al. 2018).

164 **Asia** - In Asia, represented by as many as 48 countries, maize is one of the most important crops for
165 food, feed, and industrial uses, and it is an important source of income for millions of farmers. Maize
166 ranks third after rice and wheat both in area and production. The major maize-producing countries in
167 Asia are China, India, Indonesia, Philippines, Nepal, Thailand and Vietnam. In 2018 maize production
168 was 257 million tons in China, 27 million tons in India, and 30 million tons in Indonesia
169 (<http://www.fao.org/faostat/>). In Nepal, maize was adopted in the 17th century and by the 19th century
170 was the major food grain for populations, particularly the poor, throughout the Nepal foothills
171 (Desjardins and Busman 2006). Today Nepal produces about 2.5 million tons of maize for a population
172 of about 28 million. Asia represents a wide range of climate zones, and mycotoxin problems are
173 regionally variable.

174 Many recent studies have shown that *Fusarium* spp. are the main pathogens of corn ear rot in most
175 temperate-zone Asian countries and regions (Table 1). In China, like other parts of the world, the most
176 important ear rot pathogens include *F. verticillioides* and the *F. graminearum* complex, though at least
177 10 other species have been reported. *F. verticillioides* is mainly distributed in the provinces of
178 Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Henan, Shandong, Anhui, Sichuan, Hunan, Hubei
179 and others, while the *F. graminearum* complex is mainly distributed in Shanxi, Shaanxi, Gansu, Yunnan
180 and Guizhou province (Dong et al. 2015; Ren et al. 2012; Zhang et al. 2012). In Gansu Province in 2011-
181 2012, 516 *Fusarium* isolates were identified as eight different species. The *F. graminearum* species
182 complex was dominant in 2011, but in 2012, *F. verticillioides* was the dominant species (Guo et al.
183 2014).

184 In China, fumonisins, DON, and zearalenone are the most common mycotoxins of economic
185 importance. (Wang et al. 2006) collected 284 maize samples from 6 provinces and measured DON

186 contents using gas chromatography. Results showed an incidence of 67%, a range of positive samples
187 at 10 to 3800 µg/kg, an average content of 26 µg/kg, and a mean of positive samples at 52 µg/kg for
188 all 6 provinces. Zhang et al (2012) investigated the contamination of maize samples from three areas
189 in eastern China by *Fusarium* and fumonisin-producing fungi as well as their fumonisin-producing
190 potential. The results showed that both the contamination by fumonisin-producing *Fusarium* species
191 and their fumonisin-producing potential were highest in samples from the mideastern area, and the
192 northeastern area had the lowest incidence and concentration of fumonisin contamination. The
193 contamination by fumonisin-producing *Fusarium* species was more serious in samples from this area
194 than in samples from the southeastern area. About 30.5% and 50.9% of maize samples were positive
195 for fumonisins with the mean levels of 175 µg/kg and 224 µg/kg in 2011 and 2012, respectively, in
196 Gansu province (Guo et al. 2014). In India, maize poultry feed samples from Haryana were analyzed,
197 indicating widespread prevalence of fumonisin B₁. Ninety one percent of maize samples and 84% of
198 poultry feed samples contained fumonisin B₁, ranging from 100 to 87000 µg/kg in maize and 20 to
199 28000 µg/kg in poultry feed (Jindal et al. 1999). On smallholder farms in the foothills of the Himalayan
200 Mountains of Nepal, members of the *F. graminearum* complex cause Gibberella ear rot of maize and
201 contamination with nivalenol and DON. Gibberella ear rot of maize in Nepal is associated with a several
202 members of the *F. graminearum* complex - mainly *F. asiaticum* and *F. meridionale*, but also *F. boothii*
203 and a putative new lineage. *F. graminearum* sensu stricto, which dominates in maize elsewhere in Asia
204 and worldwide, was not detected in Nepal (Desjardins and Proctor 2011). Many Nepalese maize
205 farmers are not aware that grain contaminated with fungi also can contain toxic metabolites. As a
206 result, farmers continue to consume the grain themselves, or use it as animal feed, leading to
207 mycotoxicoses that are sometimes lethal.

208 In parts of tropical and sub-tropical Asia, maize is a dietary staple, *A. flavus* is common, and associated
209 aflatoxins in maize are a major human health problem. For example, 6% of maize samples collected in
210 the Philippines contained over 300 µg/kg aflatoxins. In recent surveys in several SE Asian countries,
211 mean levels of aflatoxins in maize consistently exceeded safe limits, especially in the Philippines,
212 Thailand, and Indonesia, where means were always above 20 µg/kg (Benkerroum 2020). In a 10-year
213 summary of global mycotoxin data in animal feeds, the South Asia region had the second-highest mean
214 level of aflatoxins (20 µg/kg), and the highest percentage of samples exceeding 20 µg/kg (41.1%)
215 (Gruber-Dorninger et al. 2019). Estimates of market losses in maize due to aflatoxin contamination in
216 Thailand vary from approximately 7 to 100 million USD per year (Lubulwa et al. 2015). Other
217 mycotoxins also are important in southeast Asia. A survey of zearalenone in Indonesian maize-based
218 food and feed indicated that 32 samples (36.0%) were contaminated in a range from 5.5 to 526 µg/kg.
219 Only one highly contaminated sample was observed in a category of home-made food samples (>500
220 µg/kg) and the highest percentage of contaminated samples (>85.7%) was noted in a category of
221 poultry feed (Nuryono et al. 2005). Another survey of maize for feed in Bogor, west region of Indonesia,
222 showed that zearalenone was detected in 21% of 52 samples in a wide range from 1 to 13,500 µg/kg
223 (Widiastuti et al. 1988). (Aksoy et al. 2009) analyzed by ELISA the occurrence of aflatoxin B1, T-2 toxin
224 and zearalenone in forty compound animal feed samples collected in Turkey and these mycotoxins
225 were reported to be present in 95, 65 and 87.5% of tested samples, respectively.

226 In recent years, more and more attention has been paid to the breeding of maize ear rot resistant
227 varieties in China and other parts of Asia. However, the development of maize genotypes with
228 resistance to *Fusarium* or *Aspergillus spp.* is difficult because of polygenic inheritance, incomplete
229 information about the underlying resistance mechanisms, and lack of highly resistant germplasm.

230 Numerous studies have been published describing efforts in China to develop resistance to *F.*
231 *verticillioides* and *F. graminearum* in different parts of the country using various sources of germplasm,
232 often using artificial inoculation methods (Xu et al. 2019; Zhang et al. 2019; Zou et al. 2017). In most
233 cases, moderate levels of resistance have been identified in a subset of inbred lines or varieties, and
234 these are being incorporated into public and private breeding programs. In some cases, the resistant
235 phenotype in highly-resistant and highly-susceptible inbred lines was reproducible over years, while
236 phenotypes of inbred lines with moderate resistance were largely influenced by environmental factors.
237 In one study using a double-toothpick inoculation technique, a total of 366 maize accessions from China
238 and abroad were evaluated for resistance against *F. verticillioides* and *F.graminearum*. A total of 90
239 accessions with resistance to *F. verticillioides* and 32 accessions with resistance to *F. graminearum* were
240 found. There was no significant difference in resistance to ear rot between resources collected in China
241 and abroad (Xu et al. 2019). Taken together, these results provided insight in genetic improvement of
242 resistance to ear rot in maize in China.

243 Other management practices in Asian countries include insect management, fungicides, cultural
244 practices, and improvement of drying and storage practices. The influence of cotton bollworm and corn
245 borer on ear rot in corn was studied on widely grown varieties in North China. The results showed that
246 corn borers had more effect on ear rot under conditions of high rainfall and high humidity; however,
247 cotton bollworm had more destructive effects under conditions of low rainfall and low humidity (Wei
248 et al. 2013). In Thailand, management has included on biological control with atoxigenic strains of *A.*
249 *flavus* (Pitt et al. 2015).

250 Toxicity of nine fungicides to *F. verticillioides* and *F. graminearum* were tested in the lab and in the field
251 for efficacy of control of maize ear rot in China. Difenoconazole and thiophanate-methyl showed strong

252 toxicity against both species. Control efficacies of iprodione and Jingangmeisu (a microbial fungicide)
253 in the field were 92.3% and 80.64%, and control efficacy of spraying fungicides after inoculation was
254 better than that of inoculation after spraying fungicides (Sui et al. 2014).

255 **Europe** - Grain maize is grown across nearly all of Europe, with early-maturing hybrids reaching North
256 Germany, South Scandinavia and the Moscow region where 30 years ago only silage maize was grown.
257 Climate change and the adoption of very early maturing hybrids have aided the Nordic expansion of
258 the crop and altered the range of mycotoxigenic species infecting grain. Yearly maize production in the
259 European Union is variable and in 2018 69.1 million tons were produced
260 (<https://ec.europa.eu/eurostat/web/products-datasets/-/tag00093>). Romania was the largest maize producer
261 (around 28%), and together with France (17%), Hungary (12%) and Italy (10%) these four Member
262 States covered 67% of the total European grain maize production (ec.europa.eu). Conditions vary from
263 the humid and mostly cool Atlantic region to the strongly continental Eastern Europe to the mostly hot
264 and dry Mediterranean area, so maize hybrids, production practices, and fungal populations are
265 diverse. In the Mediterranean area maize is mostly irrigated, and in Central Europe droughts are
266 frequent, so yields are highly variable, ranging from 3.5 to 12 tons/ha. The majority of maize utilization
267 in Europe is for animal feed, with most of the remaining crop used for biofuel or processing into food
268 ingredients.

269 In Europe, “red ear rot” or “red fusariosis” is mainly caused by *F. graminearum* and *F. culmorum*, and
270 “pink ear rot” or “pink fusariosis” is caused by *F. verticillioides*, *F. proliferatum*, and *F. subglutinans*.
271 Other species may be involved in causing both symptom types (Table 1). Both types can be found
272 throughout Europe, but red ear rot is more predominant at higher latitudes and pink ear rot at lower
273 latitudes (Bottalico 1998; Munkvold 2003c, 2017). Aspergillus ear rot and aflatoxins are less prevalent

274 than *Fusarium* spp. in Europe, but can cause significant problems during outbreak years characterized
275 by heat and drought (Battilani et al. 2013). In Hungary, aflatoxin outbreaks occurred in 2007, 2012 and
276 2017, with levels reaching 300 to 400 µg/kg on the most susceptible hybrids. Climate change is likely
277 to increase aflatoxin problems throughout Europe (Battilani et al. 2016). Mycotoxin occurrence is
278 highly variable across different geographic areas and years (Palumbo et al. 2020), and even between
279 very close farms due to microclimatic variation (Leggieri et al. 2015).

280 Genetic resistance is a major component of the mycotoxin management strategy throughout Europe.
281 In each region the optimal hybrids with resistance to toxigenic species should be identified. Highly
282 susceptible hybrids may have serious fungal infection and toxin contamination in the absence of insect
283 damage. Intense breeding efforts are required and the most effective programs rely on artificial
284 inoculation-mediated selection where yield and resistance to toxigenic fungi have to be combined. A
285 set of QTLs and candidate genes that could accelerate breeding for resistance of maize lines to *F.*
286 *verticillioides* has been identified (Maschietto et al. 2017), but work is still in progress to transfer these
287 results to farmers. Investigation of the international germplasm collection in Hungary demonstrated
288 that there can be a very large 10-20 fold difference in resistance to individual toxigenic species among
289 maize genotypes. Ten to 15 % of the hybrids show some resistance to the three main pathogens, while
290 another 10-15% is highly susceptible to all three (Mesterhazy et al. 2012; Szabo et al. 2018). Because
291 toxin sensitivity differs among animal species, hybrid choice is important and can differ based on
292 intended end-use; the necessary hybrid database should be at hand to make the best decision.
293 Resistance must be monitored during the registration process and the hybrids in the commercial
294 production should be checked for resistance to the given pathogens. A similar process should be made

295 in every maize growing area. In this way farmers can decide optimize hybrid selection and balance the
296 different priorities.

297 Even though resistance has a central role, full resistance does not exist for any of the pathogens, and
298 therefore, an integrated plant management strategy should be planned for every field. Optimizing
299 fertilization, tillage, sowing time and seedbed quality, uniform plant stand, insecticide use, harvest and
300 storage is necessary to maintain safety of the crop from mycotoxins (Blandino et al. 2009). In particular,
301 management of European corn borer (*Ostrinia nubilalis*) (ECB) with insecticide treatment or transgenic
302 Bt maize hybrids has been shown to decrease levels of fumonisins and other *Fusarium* toxins in several
303 European countries (Blandino et al. 2010; Folcher et al. 2010; Ostry et al. 2015; Ostry et al. 2010;
304 Pellegrino et al. 2018; Regnault-Roger et al. 2010). However, Bt maize can be planted on a limited basis
305 only in a few European countries; in 2017, only Spain and Portugal produced Bt maize (ISAAA 2017).
306 Because of these regulatory constraints on the planting of transgenic maize, heavy use of insecticides
307 is required to effectively control ECB in most European countries.

308 Biocontrol with atoxigenic strains of *A. flavus* was considered in Italy and positive results from field
309 trials (Mauro et al. 2018) opened the way for both giving the product to farmers with a temporary
310 authorization and for registration, expected before 2021.

311 Support for farmers coming from predictive modelling (Battilani et al., 2015) has been pursued for
312 fumonisin and aflatoxin management (Battilani et al. 2013; Battilani et al. 2003; Maiorano et al. 2009).
313 The dynamic risk of contamination is predicted during the growing season, starting from silk
314 emergence, and the output reports the probability of producing maize grain contaminated above the

315 legal limit in force in Europe. Even if pre-harvest remedial actions are not available, predictions are
316 appreciated by farmers for the harvest and the post-harvest grain management.

317 **North America/Central America** - The United States is the leading maize producer in the world and the
318 largest exporter (<http://www.fao.org/faostat/>). Maize production occurs to some extent in 49 of the 50
319 states, but its intensity is greatest in the so-called “corn belt” consisting of about 10 states in the Central
320 US. The leading maize producing state is Iowa, with production of about 63 million tons in 2018, more
321 than any other nation except China and Brazil. Canadian maize production is limited by climate, with
322 annual production of about 14 million tons concentrated in the southernmost latitudes of the country.
323 Maize produced in the US and Canada is nearly 100% commercial hybrid varieties and the majority is
324 utilized for animal feed or ethanol production. Mexico is a significant maize-producing country and the
325 center of origin of maize. In contrast to the US, maize production in Mexico, as in many Central
326 American countries, involves a range of operations from large, irrigated, commercial hybrid production
327 to households growing open-pollinated land races on small, rainfed plots for subsistence. A large
328 proportion of maize produced in Mexico and Central America is for direct human consumption, which
329 makes the mycotoxin issue a critical one for human health. Mycotoxins, especially fumonisins, are
330 linked to several human health problems in this part of the world (Van der Westhuizen et al. 2013).

331 Occurrence of ear rots and mycotoxins in the US and Canada mimics the pattern observed for similar
332 latitudes in Europe (Munkvold et al. 2019). The most widespread toxigenic species is *F. verticillioides*
333 and fumonisins are the most common mycotoxins. Similar symptoms caused by *F. proliferatum*, *F.*
334 *subglutinans*, and *F. temperatum*, are associated with fumonisins and other mycotoxins. Fumonisin
335 contamination in the Central US is highly correlated with insect injury in the field (Munkvold 2014;
336 Munkvold 2003b). Incidence of DON in US maize can be similar to that of fumonisins, but average

337 contamination levels are lower for DON (Munkvold et al. 2019), and are more concentrated at higher
338 latitudes in the US and in Canada, especially around the Great Lakes. Contamination by DON in North
339 America is mostly associated with *F. graminearum sensu stricto*. Unsafe levels of aflatoxin
340 contamination occur sporadically in the Central US and Canada, but aflatoxins are a chronic problem in
341 the southern states of the US. In Central America and Mexico, *F. verticillioides* and *A. flavus* are the
342 most important toxigenic species, and most prevention and mitigation efforts target fumonisins and
343 aflatoxins. At higher elevations in Mexico, *F. subglutinans* becomes more important as a cause of
344 Fusarium ear rot (Reyes-Velazquez et al. 2011).

345 In the US and Canada, mycotoxin management efforts focus on hybrid selection for resistance to ear
346 rot diseases and transgenic resistance to insects (Munkvold 2014). Most widely planted commercial
347 hybrids have partial resistance to Fusarium and Gibberella ear rots, and some have moderate partial
348 resistance to *A. flavus*. Most resistance breeding programs are in the private sector, but public-sector
349 breeding programs have reported significant progress toward improved resistance to Aspergillus ear
350 rot (Womack et al. 2020), Fusarium ear rot (Holland et al. 2020; Morales et al. 2019), Gibberella ear
351 rot (Butron et al. 2015; Kebede et al. 2016), and their associated mycotoxins. The use of multiple
352 “stacked” insect resistance genes in commercial hybrids is standard practice, to mitigate yield losses
353 and mycotoxin risks associated with injury by ECB and other lepidopteran insects. Populations of ECB
354 have plummeted dramatically in the US due to the widespread planting of insect-resistant hybrids,
355 providing significant economic benefit to maize producers and consumers (Hutchison et al. 2010). In
356 Mexico and Central America, management practices place a greater emphasis on cultural practices and
357 endemic resistance in land races, although commercially bred resistance is important in hybrids.
358 Transgenic maize is not allowed in Mexico or in Central America, except for Honduras, where the

359 majority of maize is insect-resistant (ISAAA 2017). For this reason, managing insects that promote
360 mycotoxin contamination in this part of the world relies heavily on insecticides. Various cultural
361 practices have been shown to influence mycotoxin levels in maize grown in North and Central America;
362 however, implementation of these practices (or lack thereof) is often driven by economic forces that
363 are independent of mycotoxin concerns. Thus, decisions about planting date, crop sequence,
364 fertilization, irrigation, tillage, etc., are usually based on optimizing return on investment in terms of
365 yield, or resource availability. On the other hand, biological control for aflatoxins using atoxigenic *A.*
366 *flavus* strains has been effective and is increasingly implemented in the southern US (Isakeit 2011).

367 **South America** - In South America, maize is a major source of food used in human consumption in raw
368 form or in maize based-products. Maize also is the main ingredient in feed intended for swine, poultry
369 and dairy cattle. Brazil and Argentina are among the five major maize producing and exporting
370 countries in the world. In 2018 maize production in Brazil was estimated at 82 million tons and 43
371 million tons in Argentina. Among the world's leading maize exporters, Brazil and Argentina occupy the
372 second and third place, after USA, with around 29 and 24 million tons, respectively, in 2017
373 (<http://www.fao.org/faostat/>).

374 Both in Brazil and Argentina, maize is cultivated in different agro- ecological areas; however, the main
375 concern related to fungal and mycotoxin contamination is due to *Fusarium* species and fumonisin
376 contamination. *F. verticillioides* is the predominant *Fusarium* species associated with maize (Castanares
377 et al. 2019; Iglesias et al. 2010). In Brazil, *F. verticillioides* was common throughout all geographical
378 regions, and *F. proliferatum* and *F. subglutinans* are uncommon pathogens in maize in Brazil (Silva et
379 al. 2017). In Argentina *F. subglutinans* was the predominant species in cold and temperate areas such

380 as the Northwest of the country (Torres et al. 2001), and *F. temperatum* was also common in this area
381 (Fumero et al. 2020).

382 Brazilian mycotoxin surveys have reported high frequencies and levels of fumonisin
383 contamination. The second most commonly detected mycotoxin is zearalenone, present in 74-95% of
384 maize samples (de Oliveira et al. 2009). All studies performed in maize and maize based products
385 agreed in the low frequency of aflatoxin contamination. Low rates of contamination and levels of DON
386 were also found (Franco et al. 2019; Oliveira et al. 2017). In Argentina a survey carried out from 1999
387 to 2010 showed that the percentage of maize samples contaminated by fumonisins was also between
388 90 and 100%. The percentages of positive samples with zearalenone and DON in maize were similar
389 and did not exceed 10%. As was mentioned for Brazil, except in one year, aflatoxins levels were low
390 (Garrido et al. 2012).

391 Other maize-producing countries in South America are, in decreasing order, Paraguay, Venezuela,
392 Colombia and Peru with a maize production volume amounting to about 3.2 (Paraguay) to 1.5 (Peru)
393 million tons. In these countries there have been few studies related to the presence of mycotoxins in
394 maize; publications are limited to case reports, as in Colombia in 2005 when unusually high aflatoxin
395 contamination occurred, affecting nearly 50,000 hectares planted with white and yellow maize,
396 associated with an unusual precipitation pattern in the region and a pest infestation (Acuña et al. 2005).

397 Mycotoxin management strategies in South American maize focus on the preharvest stage. These
398 include cultural practices, use of Bt maize, resistant hybrids, and prediction modelling. Chemical control
399 is widely used for foliar diseases but the effectiveness of this measure for ear rot control is very
400 inconsistent (Lanza et al. 2016).

401 Techniques to reduce mycotoxin levels include those associated with the control of ear rot in the field,
402 such as the rotation of maize with non-host crops. However, common rotation schemes in Argentina
403 are limited to two-year rotations of maize/soybean or wheat/soybean/maize (three crops in two years).
404 In Brazil, a key driver in the expansion of maize production has been double cropping, which means
405 planting two crops in a field in the same year, soybean in the summer (rainy season) and a winter crop
406 of maize. Additionally, destruction of stubble, the use of disease-free seeds to prevent the further
407 spread of the causative agents, seed treatment with fungicides, precision agriculture cropping systems
408 with balanced fertility by use of precision fertilizer management systems are practiced to promote
409 lower disease incidence and lower mycotoxin contamination.

410 Transgenic maize varieties expressing *Bacillus thuringiensis* proteins (Bt maize) have been a widely
411 adopted alternative to insecticides and, have been the primary technology for insect control in Brazil
412 and Argentina with approximately 85% of Brazilian maize and 87% of Argentinian maize planted with
413 Bt traits in 2017 (ISAAA 2017). Studies have shown that Bt maize presented lower incidence of *F.*
414 *verticillioides* and fumonisin levels, presumably through the reduction of insects, which act as vectors
415 of fungi (Barros et al. 2009; de la Campa et al. 2005), although another study in Brazil found no
416 statistical difference in fumonisin contamination between Bt and non-Bt samples (Barroso et al. 2017).
417 Resistance to Bt maize recently has been described in Argentina and Brazil in some insect populations
418 including *Diatraea saccharalis* (sugarcane borer) and *Spodoptera frugiperda* (fall armyworm) (Fatoretto
419 et al. 2017; Grimi et al. 2018).

420 Developing and using resistant hybrids may prevent both ear rot progress and mycotoxin
421 contamination. Although genetic variation for resistance to *Fusarium* ear rot exists among inbred lines
422 and hybrids in field maize, there is no complete resistance to either ear rot or fumonisin accumulation.

423 Efforts are directed towards the search for genetic material resistant to both parameters, since the
424 correlation between grain infection and fumonisin levels in kernels is variable (Munhoz et al. 2015).
425 High levels of disease resistance were observed in Argentinean landraces that are being used to
426 improve elite germplasm (Campos-Bermudez et al. 2013).

427 The use of models to predict fumonisin accumulation in maize may become an integrative tool; several
428 predictive models have been proposed for *F. verticillioides* infection and fumonisin contamination by
429 including different combinations of climatic, agronomic and maize genotype factors. In Argentina,
430 (Sancho et al. 2018) developed weather-based logistic models as tools for estimating seasonal
431 contamination levels, with the goal of improving kernel sampling efficiency at export terminals and
432 mills as part of an integrated system for fumonisin management in the maize value chain.

433 The efficacy of bacteria antagonistic to *F. verticillioides* has been demonstrated under greenhouse and
434 field conditions with the intention to exploit them as potential biocontrol agents suitable for
435 widespread use in maize in Argentina. *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas*
436 *cepacia* have been used to suppress *Fusarium verticillioides*, reduce fumonisin accumulation in maize
437 kernels, and promote plant growth (Cavaglieri et al. 2005; Pereira et al. 2011). A promising strategy to
438 reduce aflatoxin accumulation is the biological control based on competitive exclusion; reduction of
439 aflatoxin B₁ content in maize kernels by 54 to 83% was reported in Argentina (Alaniz Zanon et al. 2018;
440 Camiletti et al. 2018).

441 **MycoKey Maize Working Group – Mycotoxin Research and Management Priorities**

442 On June 6, 2018, the Maize Working Group of the MycoKey Project met in Bucharest, Romania, to
443 undergo a brainstorming session on the most important priorities for research and management of

444 mycotoxins in maize. The session was guided through the use of the Nominal Group Technique, a
445 moderated discussion approach that has been used previously to identify priorities for research and
446 management of mycotoxin problems. (Bandyopadhyay et al. 2008; Leslie et al. 2018) focused on
447 research ideas related to chemical detection methods, genetics and biodiversity of mycotoxins. The
448 Nominal Group Technique is designed to generate a diversity of ideas and to facilitate equal input from
449 all participants, as well as incorporating a ranking process. The resulting ideas and rankings are useful
450 for identifying trends as well as novel innovations. In the Maize Working Group session, six questions,
451 formulated by the MycoKey project leadership, were posed separately to two groups of experts from
452 Europe, North and South America, and Africa. Questions addressed pre- and post-harvest management
453 priorities for fumonisins, aflatoxins, DON, and zearalenone; effective decontamination/detoxification
454 strategies; and priorities for technology development and information transfer (Suppl. Table 1). The
455 Nominal Group Technique results in a structured discussion with five stages: 1) Silent generation of
456 individual responses; 2) Sharing ideas, during which members of the group share their responses and
457 all responses are recorded; 3) Idea explanation, which consists of a moderated discussion during which
458 responses are clarified and refined; 4) Individual voting and ranking, when each participant ranks the
459 five most important responses for the question and these ranks are collected and summarized; 5)
460 Presentation of results, when results from the discussions are presented on a question by question
461 basis. There were 10 participants in the Nominal Group activity, which was carried out in two
462 iterations. First, two separate subgroups of five participants simultaneously considered each question
463 through Stage 4; then, results were combined, Stages 2 through 4 were repeated with all 10
464 participants, and Stage 5 was completed. All responses are listed in Supplementary Table 1; very similar
465 responses were combined to prepare the summary charts (Fig. 3).

466 *Pre-harvest Management Actions*

467 *Fumonisin*. When queried about the most important pre-harvest actions to limit fumonisin
468 contamination in maize, participants identified 20 potentially effective actions, although all 20
469 alternatives were not mutually exclusive (Suppl. Table 1). The most highly ranked practice was maize
470 genotype selection (Fig. 3a), incorporating the concepts of genetic resistance to infection by *F.*
471 *verticillioides* or fumonisin production, and local adaptation. This practice was identified as the most
472 important one by 50% of the working group members. The second most highly ranked practice was
473 insect management, which was scored as the most important practice by 30% of the working group
474 members. The third-ranking response to this question was knowledge of good agricultural practices,
475 which combines concepts of crop management to optimize plant health with the importance of
476 information and technology transfer to ensure implementation of these practices. Other highly ranked
477 actions were water management (irrigation and drainage) and fertilization.

478 *Aflatoxins*. When queried about the most important pre-harvest actions to limit aflatoxin
479 contamination in maize, participants identified 15 non-mutually exclusive actions (Suppl. Table 1).
480 Again, maize genotype selection was the most highly ranked action (Fig. 3b), with 50% of working group
481 members ranking it as the most important one. Biological control was ranked 2nd, with 40%
482 considering it the most important action. The third-ranked response was irrigation/water
483 management, followed by insect management and fertilization.

484 *Deoxynivalenol and zearalenone*. Actions for pre-harvest management of DON and zearalenone were
485 queried in a single question, because these two toxins are typically produced by the same fungi.
486 Eighteen responses were generated (Suppl. Table 1). Genotype selection was again the most highly

487 ranked option (Fig. 3c), with 90% of the members ranking this action as the most important. This
488 selection emphasized genetic resistance but also encompassed the importance of hybrid maturity class,
489 with earlier-maturing hybrids typically having a lower risk of contamination. Crop rotation was the 2nd
490 most highly ranked action; and crop residue management (tillage) was tied for 5th. Together these two
491 tactics reflect the importance of the previous crop's residue as a source of inoculum for fungi in the *F.*
492 *graminearum* complex. Fungicide application was ranked 3rd and insect management was ranked 4th,
493 followed by planting date, with earlier planting in most regions resulting in a lower risk of mycotoxin
494 contamination.

495 These results indicate some similarities in the approaches considered effective across the different
496 types of mycotoxins, and also indicate some important differences. The predominance of genotype
497 selection as an important action reflects the fact that there are many promising published studies
498 identifying effective resistance to infection or mycotoxin production by *A. flavus*, the *F. fujikuroi*
499 complex (primarily *F. verticillioides*), and the *F. graminearum* complex (Gaikpa and Miedaner 2019;
500 Hawkins et al. 2018; Holland et al. 2020; Lanubile et al. 2017; Szabo et al. 2018), and it also reflects
501 field observations of marked differences among genotypes. This option was the highest ranked one for
502 all the mycotoxins mentioned, but it was nearly unanimously chosen as the most important action only
503 for the toxins (DON and zearalenone) associated with the *F. graminearum* complex. This can be
504 attributed to two main factors. Major-gene resistance has been identified for *F. graminearum*, whereas
505 resistance to *A. flavus* and the *F. fujikuroi* complex is based on QTL with moderate effects and more
506 complex inheritance (Mesterhazy et al. 2012; Szabo et al. 2018), thus incorporation of resistance to
507 the *F. graminearum* complex has been more successfully implemented in commercial hybrid
508 development. Secondly, the geographic scope of DON and zearalenone impacts is narrower than those

509 of aflatoxins and fumonisins, and this scope is predominantly in areas of North America, Europe, and
510 China, where maize production consists almost entirely of commercial hybrids. In contrast, aflatoxin
511 and fumonisin impacts are widespread across areas of the developing world where maize production
512 is a mixture of commercial hybrids and open-pollinated varieties.

513 Insect management was identified as an important practice for all three mycotoxin groups, but Working
514 Group members (30%) chose it as the most important action only for fumonisins. This reflects the
515 existing data that tie fumonisin contamination very closely with insect damage (Munkvold et al. 2019),
516 whereas other environmental factors are more important influencers of contamination by aflatoxins,
517 DON, or zearalenone (Fountain et al. 2014; Munkvold 2003c). Nevertheless, insect damage is
518 universally recognized as an important factor in the epidemiology of diseases associated with most
519 mycotoxins in maize, and reductions in aflatoxins and DON have been associated with transgenic insect
520 protection (Folcher et al. 2012; Schaafsma et al. 2002; Yu et al. 2020).

521 The other practices recommended across mycotoxin groups were water management and fertilization.
522 Water management is a complex issue in relation to mycotoxin risk; in the case of aflatoxins, drought
523 stress is a known risk factor (Chauhan et al. 2015), whereas excess moisture is a known risk factor for
524 infection by *F. graminearum* and subsequent DON and zearalenone contamination (Schaafsma and
525 Hooker 2007), and fumonisin contamination is favored by drought stress followed by humid conditions
526 (Miller 2001). From a management perspective, irrigation systems can be designed to avoid drought
527 stress while also avoiding excess moisture in the crop canopy, especially during and after flowering,
528 and drainage issues can be remediated, but water management continues to be a challenge in rainfed
529 agriculture. Fertilization was mentioned as an important practice across mycotoxin groups, but was

530 ranked no higher than 5th, indicating that overall plant health is a component of mycotoxin risk, and
531 proper plant nutrition is one factor that can contribute to reducing the risk.

532 One of the most striking differences in recommendations for effective management actions is related
533 to biological control, which was ranked very highly for aflatoxin management but was not mentioned
534 in relation to the other mycotoxins. Application of atoxigenic strains of *A. flavus* to suppress aflatoxin
535 contamination has been researched extensively and implemented widely in North America and Africa.
536 This approach has been very successful (Bandyopadhyay et al. 2016), but analogous efforts to
537 successfully suppress *Fusarium* mycotoxins with atoxigenic strains have not been pursued.

538 Crop rotation and crop residue management were ranked as relatively important practices for DON
539 and zearalenone, but not for aflatoxins or fumonisins. This reflects some differences in the biology of
540 the fungi involved. *A. flavus* and *F. verticillioides* are ubiquitous occupants of the agricultural landscape,
541 and they produce vast numbers of airborne conidia that can travel long distances. As a result, crop
542 residue management in individual fields has little impact. *Fusarium graminearum* spreads by rain-
543 splashed macroconidia from in-field residues, and by airborne ascospores and macroconidia that can
544 be dispersed over long distances. Reducing in-field inoculum sources can be valuable, but the impacts
545 of crop rotation and residue management are limited by the scale of maize and wheat production
546 contributing to regional atmospheric inoculum (Ponte et al. 2003).

547 Fungicide applications were ranked as an important practice only in relation to managing DON and
548 zearalenone. Although fungicides are not widely used to manage mycotoxins in maize, they are
549 commonly used to manage DON contamination in small grain cereals, and this success has stimulated

550 similar interest in maize. Research on prevention of *F. graminearum* infection by protecting susceptible
551 silks with fungicides has shown promise (Limay-Rios and Schaafsma 2018).

552 *Post-harvest Management and Processing/Decontamination Actions*

553 Questions were posed to the Working Group to assess the most effective post-harvest management
554 and processing or decontamination actions for all mycotoxins. There were 21 responses for postharvest
555 management and 21 responses for processing and decontamination, some of which overlapped with
556 post-harvest management responses (Suppl. Table 1). There was less consensus in these results than
557 in the pre-harvest results. The most highly ranked post-harvest management action was rapid grain
558 drying, which included elements of transportation logistics from the field and the actual drying method
559 (Fig. 3d); 30% of Working Group members ranked this as the most important practice. The 2nd most
560 highly ranked action was grain cleaning or sorting, to remove damaged/contaminated kernels; 20% of
561 Working Group members ranked this as the most important practice. The use of good harvesting and
562 grain handling equipment was ranked 3rd; only 10% of members ranked it as most important, but others
563 ranked it 2nd or 3rd. This practice focuses on preventing grain damage during harvest and handling.
564 Good storage facilities and harvest timing tied for 4th rank, both with 20% of members ranking them as
565 most important. Harvesting at the proper time also was included as a response in the pre-harvest
566 management query, illustrating the wide recognition of this approach as a way to prevent elevated
567 mycotoxin levels that can occur if maize remains in the field, drying naturally. The 5th-ranked response
568 was control of relative humidity and temperature in storage; 20% ranked it as most important. This
569 response is somewhat confounded with the storage facilities response, considering that “good” storage
570 facilities should include sanitation, exclusion of pests, and some level of environmental control.

571 For the processing and decontamination question, the highest ranking response was optical sorting (Fig
572 3e). Although only 10% of Working Group members ranked it as most important, several others ranked
573 it highly. Optical sorting has repeatedly been demonstrated to be effective at removing mycotoxin-
574 contaminated maize kernels from a moving grain stream (Pearson et al. 2004; Stasiewicz et al. 2017),
575 although the use of this technology for this purpose is largely limited to human food uses in
576 industrialized countries. The 2nd highest ranking response was removal of fine material through sizing
577 or sieving operations. This was ranked highest by 30% of members. This response is very similar to the
578 grain cleaning/sorting response that also ranked 2nd in the post-harvest management actions. The 3rd-
579 ranking response was density separation, with 30% of members ranking it as most important. All three
580 of these top responses are related to different physical methods of identifying and separating damaged
581 grain and other material from healthy grain. The 4th ranked response was milling, which allows for the
582 separation of the more contaminated components of the grain (such as the pericarp) from the typically
583 less-contaminated components such as endosperm and embryo. Chemical detoxification ranked 5th;
584 this category includes various approaches including ozone treatment, nixtamalization, ammoniation,
585 and others, but does not include adsorbent materials, which was a separate response that ranked 6th.

586 *Research/Information Needs*

587 Finally, the Working Group responded to a question about the most pressing needs for new research
588 and information that will most effectively contribute to improvements in mycotoxin management or
589 reduction in risk. Twenty-six responses were generated (Suppl. Table 1). The top-ranked response was
590 the category of “risk assessment tools,” which included the development of risk maps, real-time and
591 long-term prediction models, and guidelines for susceptibility of maize genotypes (Fig. 3f). These ideas
592 were ranked as most important by 40% of the Working Group members. The 2nd-highest ranking

593 response was improved breeding technologies, including the development of transgenes, the use of
594 genome editing, and improved knowledge about the relationship between genetic resistance and toxin
595 accumulation. The 3rd-ranking (“geo-referenced management guidelines”) and 4th-ranking
596 (“information transfer”) responses both relate to the effective communication of existing and new best
597 practices for mycotoxin management. Collectively, these two responses were ranked as most
598 important by 50% of Working Group members. The 5th-ranked response was improvement in
599 technology availability/adoption. This response refers to the role of biotechnology in the battle against
600 mycotoxins in maize; including tools such as transgenic insect protection (already available but not
601 globally adopted), biotechnology-derived resistance to infection or mycotoxin production, or bio-
602 engineered in-plant detoxification mechanisms (not yet available).

603 **Conclusion**

604 The safety of maize as a food and feed component is challenged everywhere across the globe. The four
605 major mycotoxin groups (aflatoxins, DON and related trichothecenes, fumonisins, and zearalenone)
606 that threaten the maize supply are common to all maize-producing areas, but their relative importance
607 and the severity of the challenge are geographically dependent. The MycoKey Maize Working Group
608 has served to guide research and management priorities, and their recommendations emphasize the
609 importance of genetic resistance, insect management, grain drying and cleaning methods, and the
610 development of risk assessment tools that account for the impacts of climate change on evolving
611 mycotoxin risks in maize. As warming climate increases the risk of several mycotoxins, higher levels of
612 resistance and well adapted cultural practices can contribute to sustainable production with reduced
613 mycotoxin levels.

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Table 1. Toxigenic species of *Aspergillus* and *Fusarium* associated with maize ear rot by continent (adapted from (Munkvold 2003a)).^a

Species	Disease symptoms ^b	Africa	Asia	Europe	North/Central America	South America	Oceania
<i>Aspergillus flavus</i>	AER	X	X	X	X	X	X
<i>Aspergillus parasiticus</i>	AER	X	X	X	X	X	X
<i>Fusarium acuminatum</i>	FER, GER		X	X	X	X	
<i>F. asiaticum</i>			X				
<i>F. avenaceum</i>	FER, GER		X	X	X		
<i>F. boothii</i>			X				
<i>F. cerealis</i>	GER			X			
<i>F. chlamydosporum</i>	FER, GER		X				
<i>F. crookwellense</i>	GER		X	X			X
<i>F. culmorum</i>	GER		X	X	X		X
<i>F. equiseti</i>	FER, GER		X	X	X	X	
<i>F. fujikuroi</i>	FER		X				
<i>F. graminearum</i>	GER	X	X	X	X	X	X
<i>F. meridionale</i>	GER		X				
<i>F. poae</i>	GER			X	X		X
<i>F. proliferatum</i>	FER	X	X	X	X	X	
<i>F. pseudograminearum</i>	GER		X				
<i>F. semitectum</i>	FER		X	X	X		
<i>F. sporotrichioides</i>	GER		X	X	X		
<i>F. subglutinans</i>	FER	X	X	X	X	X	X
<i>F. temperatum</i>	FER			X	X	X	
<i>F. verticillioides</i>	FER	X	X	X	X	X	X

^a X indicates that the species is associated with ear rot; **X** indicates that species is a major cause of ear rot

^b AER = *Aspergillus* ear rot, FER = *Fusarium* ear rot (pink ear rot), GER = *Gibberella* ear rot (red ear rot)

Figure captions

Figure 1. The most important globally occurring, mycotoxin-producing, ear rot pathogens of maize: 1a, *Aspergillus flavus* usually appears as a powdery olive-green or yellow-green mold scattered across the ear or associated with insect injury; 1b, *Fusarium graminearum* appears as a dense pink to red mold that colonizes the tip of the ear and moves basally, sometime involving the whole ear; 1c, blue-black perithecia of *F. graminearum* may develop on ears, husks, or ear shoots; 1d, *Fusarium verticillioides* symptoms are cottony white to pink, purple, or salmon-colored mold, often associated with insect injury or scattered across the ear; 1e, maize ear showing *F. verticillioides* mold and vivipary symptoms around insect injury; 1f, maize ear showing scattered moldy or “starburst” symptoms, also typical of *F. verticillioides*. In each case, related species can cause similar symptoms.

Figure 2. Cross-functional structure of the MycoKey project. Work Packages (numbered 1 to 10) are organized to work on research, communication, and technology transfer activities across four major crop utilization chains that are the most heavily impacted by mycotoxin contamination.

Figure 3. Summary of MycoKey Maize Working Group Round Table on prioritization of research and management efforts for mycotoxins, based on Nominal Group discussion, June, 2018: a, priorities for pre-harvest management of fumonisins; b, priorities for pre-harvest management of aflatoxins; c, priorities for pre-harvest management of deoxynivalenol and zearalenone; d, priorities for post-harvest management of mycotoxins; e, priorities for decontamination/detoxification of mycotoxin-contaminated maize grain; f, priorities for new research and information that will most effectively contribute to improvements in mycotoxin management or reduction in risk.



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368x328mm (150 x 150 DPI)

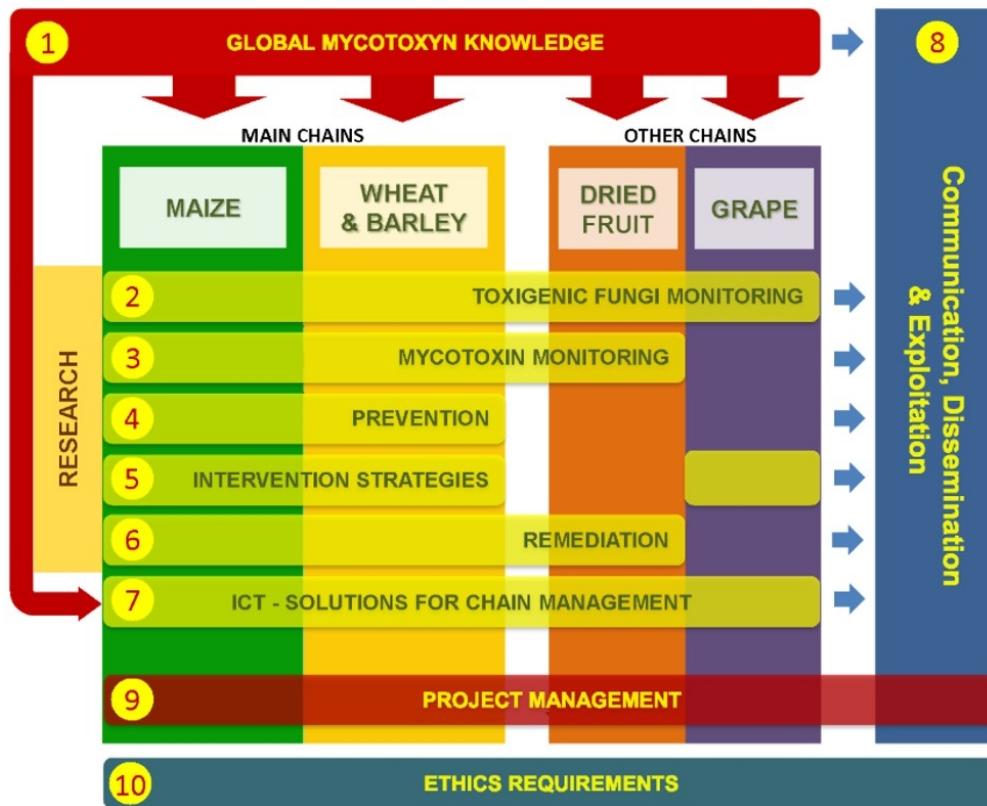


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165x133mm (150 x 150 DPI)

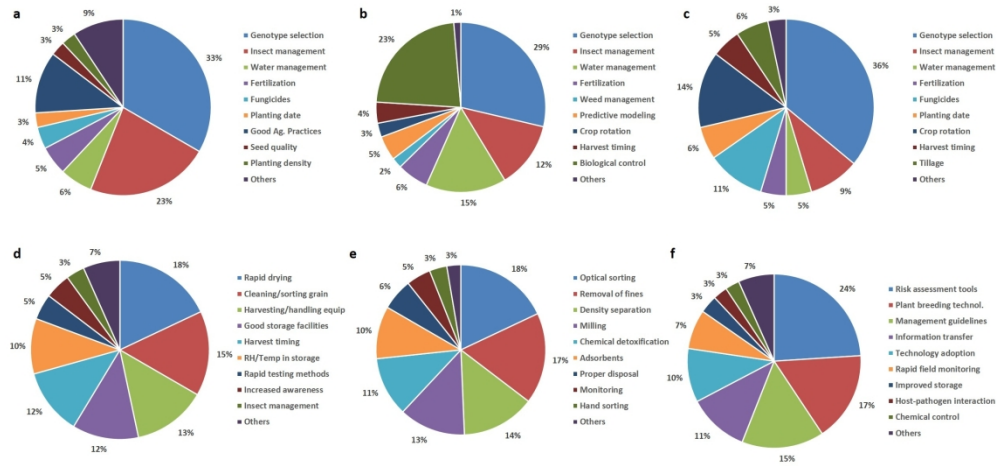


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Supplementary Table 1. Questions posed and responses recorded by MycoKey Maize Working group during its Roundtable Discussion on global maize mycotoxin issues, held in Bucharest, Romania, 6 June, 2018.

What strategies/measures are effective for minimizing fumonisin contamination in maize during crop growth (pre-harvest)?

Rank	Response	Votes
1	Host resistance/genotype	36
2	Insect control (ECB)	34
3	Use of proper hybrids	14
4	Limited knowledge of good agricultural practices	13
5	Water management	9
6	Fertilization	8
7	Fungicide application	6
8	Seedling density	4
9	Planting time	4
10	Seed quality	4
11	Crop stress management	4
12	Tillage/no tillage	3
13	Forecast	2
14	Prediction model	2
15	Weed management	2
16	Crop rotation	2
17	Crop residues management	2
18	Quality of chemical input	1
19	Incorrect use of input	0
20	Time of harvest	0

Which strategies/measures are effective for minimizing aflatoxin contamination in maize during crop growth (pre-harvest)?

Rank	Response	Votes
1	Biocontrol	34
2	Genotype selection	32
3	Water management/irrigation	23
4	Insect control (ECB)	19
5	Locally adapted varieties	11
6	Fertilization	9
7	Predictive model	7
8	Harvesting time	6
9	Crop rotation	4
10	Weed management	3
11	Planting time	2
12	Seeding density	0
13	Fungicide application	0
14	Intercropping	0
15	Soil management	0

Which strategies/measures are effective for minimizing deoxynivalenol and zearalenone contamination in maize during crop growth (pre-harvest)?

Rank	Response	Votes
1	Host resistance	40
2	Crop rotation	21
3	Fungicide application	16
4	Insect control	14
5	Locally adapted	10
6	Planting time	9
7	Harvest time	8
8	Fertilization	7
9	Irrigation	7
10	Crop residue management	6
11	Hybrid earliness	4
12	Tillage/no tillage	3
13	Weed control	2
14	Predictive model/risk maps	2
15	Plant density	1
16	Drainage	0
17	Seed dressing	0
18	Seed quality	0

Which strategies/measures are effective for minimizing mycotoxin contamination in maize post-harvest, from harvest through the storage period?

Rank	Response	Votes
1	Time from harvest to drying /logistic management from harvest to storage	23
2	Kind of storage	18
3	Harvest time	18
4	Differentiate harvesting	15
5	Cleaning grain	15
6	RH/temperature-moisture control of storage	12
7	Sorting contaminated lots	8
8	Rapid test method	7
9	Increase awareness	7
10	Contamination post-harvest management/separation of contaminated stocks	5
11	Control insect during storage	5
12	Drying methods	4
13	Temperature control during storage	3
14	Harvesting method/prevent mechanical damage	3
15	Checks for relevant mycotoxins	3
16	Grain handling	2
17	Farmer collaboration	2
18	Pre-harvest scouting/satellite imaging	0
19	Monitoring insect during storage	0
20	Methods of transportation	0
21	Chemical treatment post-harvest	0

Which processing steps or decontamination/detoxification actions have impact on mycotoxin content in maize products?

Rank	Response	Votes
1	Optical sorting	27
2	Specific weight sorting	21
3	Fine material removal	14
4	Sizing grains	12
5	Sorting after milling	10
6	Chemical detoxification	10
7	Adsorbents	9
8	Proper methods of disposal	9
9	Monitoring contamination	7
10	Nixtamalization	6
11	Dry milling	6
12	Sorting by hand	5
13	Use of physical adsorbents	3
14	Use of biological adsorbents	3
15	De-hulling grain	3
16	Blending/mixing	3
17	Grain brushing	1
18	Ozone treatment	1
19	Ammoniation	0
20	Control of DDG during bioethanol production	0
21	Aflatoxin degrading microorganisms	0

What information, should be generated or questions answered to facilitate mycotoxin mitigation in the maize chain considering the changing world?

Rank	Response	Votes
1	New technologies to improve breeding	20
2	Risk maps of mycotoxin occurrence	16
3	Standardized georeferenced guidelines	13
4	Information sharing	10
5	Hybrid susceptibility list	10
6	Availability of technologies	10
7	Tailored management strategies	10
8	Awareness creation	7
9	Develop very rapid diagnostic assays in field	6
10	Robust long term predictions	6
11	Real time monitoring	5
12	Improvement of storage methods for developing countries	5
13	Social aspects of technology adoption	5
14	Improved knowledge on resistance and toxin relationships	5
15	Prevalence data on mycotoxins	4
16	New technologies to improve knowledge on host-pathogen interactions	4
17	Development of new sustainable chemicals	4
18	Options for contaminated commodities	3
19	Improvement of registration process for maize varieties	3
20	Cost benefit analysis of mitigation strategies	2

21	Effects of climate change	1
22	Population dynamics of toxigenic fungi	1
23	Development of new RNAi technologies	0
24	Regulation enforcement	0
25	Effectiveness of actions	0
26	Environmental friendly decontamination methods	0



MycoKey Maize Working Group Round Table discussion session participants. Front row (L to R): George Mahuku, Antonio Logrieco, Paola Battilani, Alejandro Ortega-Beltran. Back row (L to R): Marco Camardo Leggieri, Oana Dumitru (IBA Bucharest), Alessandra Lanubile, Roberta Palumbo, Gary Munkvold, Adriana Torres, Akos Mesterhazy, Irina Smeu, Geert Haesaert, Nastasia Belc (IBA Bucharest)

1371x914mm (72 x 72 DPI)