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**Effect of agronomic programmes with different susceptibility to deoxynivalenol risk on emerging contamination in winter wheat**

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2 **Title: Effect of agronomic programmes with different**  
3 **susceptibility to deoxynivalenol risk on emerging**  
4 **contamination in winter wheat.**

5

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20

21 **Abstract**

22 Deoxynivalenol (DON) is the most prevalent mycotoxin in small cereal crops throughout  
23 the world, and its occurrence is closely linked to the presence of Fusarium Head Blight  
24 (FHB) disease.

25 In order to minimize the sanitary risk, wheat cropping systems are commonly designed to  
26 control DON contamination, as this represents the main target contaminant. However,  
27 several other mycotoxins and secondary metabolites produced by *Fusarium* and other  
28 fungal species have been detected in wheat. The objective of this study was to evaluate  
29 whether the application of agronomic programmes with different susceptibility to DON  
30 contamination could also affect the occurrence of emerging mycotoxins in wheat kernels.

31 Field experiments have been conducted in North Italy, under naturally-infected conditions,  
32 over a period of 3 growing seasons, by comparing 4 field programmes, which were  
33 constituted by the combination of wheat cultivars (a durum wheat variety that is  
34 susceptible to DON contamination and a common moderately resistant one) and 2  
35 fungicide applications at heading (untreated control compared to an azole application at  
36 heading).

37 Grain samples have been analyzed by means of a dilute-and-shoot multi-mycotoxin LC-  
38 MS/MS method, and 43 fungal metabolites were detected. In addition to DON, the most  
39 abundant compounds were aurofusarin, culmorin and deoxynivalenol-3-glucoside, which  
40 were detected in all the growing seasons and agronomic strategies. Other trichothecenes  
41 and zearalenone derivatives were also found, but in clearly lower concentrations.

42 Contamination by enniatins and moniliformin, produced by other *Fusarium* species e.g.  
43 *Fusarium avenaceum*, alternariol, alternariol methyl ether and tentoxin, produced by  
44 *Alternaria* species, has been observed for all the compared growing seasons. The  
45 presence of other mycotoxins and secondary metabolites was clearly affected by the

46 climatic conditions: fumonisin, beauvericin, bikaverin, fusaric acid and butenolid were  
47 detected in the warmer growing seasons, while chrysogine, infectopyrone, secalonic acid  
48 and ergot alkaloids (sum of 13 toxins) were only found in the more rainy and cool seasons.  
49 Equisetin, decalonectrin, toxin T-2 and HT-2 were only found in traces.

50 The application of the field programmes clearly affected DON contamination in each  
51 growing season: a significant increase in this toxin has been observed moving from the  
52 lowest risk agronomic strategy to the highest one. The application of the most favourable  
53 DON control field programme (a moderately resistant variety combined with fungicide  
54 application at heading) reduced the content of this mycotoxin by 89%, compared to the  
55 worst programme (untreated susceptible variety).

56 The application of the less risky agronomic strategy for DON contamination led to a  
57 significant reduction (>84%) of all the other mycotoxins produced by the DON producing  
58 fungal species. Moreover, although the considered agronomic factors (variety susceptibility  
59 and fungicide application) resulted in a control efficacy that varied in function of the  
60 environmental conditions and the type of mycotoxin, the results show a clear reduction  
61 trend, after the application of agronomic strategies that are able to minimize the DON  
62 content, for almost all the other *Fusarium*, *Alternaria*, *Claviceps* and *Penicillium*  
63 metabolites.

64 The results summarized in this work, which have been obtained under different  
65 environmental and agronomical conditions, allow a first assessment to be made of the  
66 agronomic strategies that could be applied to control emerging mycotoxins in wheat.

67

68 **KEYWORDS:** common wheat, durum wheat, fungicide, fusarium head blight, emerging  
69 mycotoxins.

70

71 **ABBREVIATIONS**

72 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; AME, alternariol  
73 methyl ether; AOH, alternariol; ANOVA, Analysis of variance; AUR, aurofusarin; BEA,  
74 beauvericin; BIK, bikaverin; BUT, butenolide; CHRY, chrysogine; CULM, culmorin; DEC,  
75 decalonectrin; DON, deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside; EC, European  
76 Commission; EFSA, European Food Safety Authority; ENNs, Enniatins A, A<sub>1</sub>, B, B<sub>1</sub>, B<sub>2</sub>;  
77 EQU, equisetin; EAs, ergot alkaloids; FA, fusaric acid; FB, fumonisins; FUS, fusaproliferin;  
78 GDD, Accumulated growing degree days; GS, Growth stage; INF, infectopyrone; LC-  
79 MS/MS, Liquid chromatography coupled with tandem mass spectrometry detection; LOD,  
80 limit of detection; LOQ, limit of quantification; MON, moniliformin; MR, moderately  
81 resistant; MS, mass spectrometry detection; NIV, nivalenol; S, susceptible; TENT,  
82 tentoxin; TW, test weight; SAD, secalonic acid; ZEA, zearalenone; ZEA-4-S, zearalenone-  
83 4-sulphate.

84

## 85 1. INTRODUCTION

86 Mycotoxins are secondary metabolites that are produced by several fungal species, which  
87 could have a range of toxic properties, including carcinogenicity and neurotoxicity, as well  
88 as developmental and reproductive toxicity for humans and reared animals and could  
89 result in illnesses and economic losses (Pestka and Smolinski, 2005).

90 Among the various agricultural commodities, cereals are most likely to be contaminated,  
91 and deoxynivalenol (DON), a type-B trichothecene produced by *Fusarium* spp., is the most  
92 prevalent toxin in small cereal crops throughout the world (Larsen et al., 2004). Regulatory  
93 limits have been set by the European Commission (EC) to protect humans from this  
94 mycotoxin exposure through cereal consumption (EC No. 1881/2006 and EC No.  
95 1126/2007, with a limit of 1250 and 1750  $\mu\text{g kg}^{-1}$  in unprocessed common and durum  
96 wheat, respectively). The occurrence of DON in wheat and other small cereals is closely  
97 linked to the presence of Fusarium Head Blight (FHB) disease, which causes total or  
98 partial premature ear senescence and consequent negative impacts on both crop yields  
99 and grain quality. Different *Fusarium* species are involved in promoting this disease,  
100 although *F. graminearum sensu stricto* and *F. culmorum*, are the most important FHB  
101 agents and the main causes of DON accumulation in grains in temperate areas (Yli-  
102 Mattila, 2010; Somma et al., 2014).

103 Although DON contamination in wheat grains depends on the meteorological conditions,  
104 particularly at flowering (van der Fels-Klerx *et al.*, 2013), an important role is played by  
105 agronomic factors, such as crop rotation, debris management, variety susceptibility and  
106 fungicide applications (Pirgozliev et al., 2003; Koch et al., 2006). At present, the most  
107 effective approaches adopted to minimize DON occurrence in wheat are the use of  
108 preventive agronomic practices to reduce the pathogen inocula in the field, the cultivation

109 of varieties that are less susceptible to FHB and the application of fungicides that are  
110 effective in controlling *Fusarium* spp, according to an integrated approach that addresses  
111 all of the possible risk factors (Blandino et al., 2012). Thus, in order to ensure low sanitary  
112 risks, wheat cropping systems in temperate areas are generally designed to control DON  
113 contamination, as this is the main target contaminant. However, to date, about 400  
114 different mycotoxins have been identified in different commodities, and several of these  
115 have been found in cereals (Berthiller et al., 2013). These other fungal metabolites, some  
116 of which have been referred to as “emerging” (Streit et al., 2013), have not yet received  
117 detailed scientific attention. The European Food Safety Authority (EFSA) is currently  
118 working on establishing a scientific opinion on the risks to public health related to the  
119 presence of emerging mycotoxins in feeds and food (EFSA, 2010; 2014). Nowadays, it is  
120 necessary to collect occurrence data on these mycotoxins in the most important cereal  
121 areas in the EU, in order to correctly consider the risk of exposure and to make risk  
122 assessments. In addition, there is also a greater interest in verifying the effect of the Good  
123 Agricultural Practices (GAP) that are normally applied to control FHB and DON, which is  
124 the reference mycotoxin for wheat in temperate areas, on the content of emerging  
125 mycotoxins in this crop. Since these compounds could be produced by other *Fusarium*  
126 species that are not directly involved in FHB disease, as well as by other fungal species  
127 belonging to the *Alternaria*, *Penicillium* and *Claviceps* families, more detailed knowledge  
128 on the environmental and agronomic conditions that promote their occurrence is essential  
129 in order to set up field programmes that will be able to minimize the overall sanitary risk for  
130 grain.

131 The aim of this study was to investigate the effect of agronomic strategies, with different  
132 susceptibility to DON, on the occurrence of emerging mycotoxins and fungal metabolites in  
133 wheat in different production situations.



## 134 **2. MATERIALS AND METHODS**

### 135 *2.1. Chemicals*

136 Methanol and acetonitrile (both LC gradient grade) were purchased from J.T. Baker  
137 (Deventer, The Netherlands); ammonium acetate (MS grade) and glacial acetic acid (p.a.)  
138 were obtained from Sigma–Aldrich (Vienna, Austria). Water was purified successively by  
139 means of reverse osmosis and a Milli-Q plus system from Millipore (Molsheim, France).

140 All the fungal metabolite standard solutions were stored at  $-20^{\circ}\text{C}$  and were brought to  
141 room temperature before use.

142

### 143 *2.2. Field Experimental Design and Samples*

144 The effect of agronomic strategies with different susceptibility to deoxynivalenol  
145 contamination on emerging mycotoxin occurrence in wheat was studied in North-West Italy  
146 in the 2010-11 growing season at Cigliano ( $45^{\circ} 18' \text{ N}$ ,  $8^{\circ} 01' \text{ E}$ ; altitude 237 m), in a sandy-  
147 loam soil (Typic Hapludalfs) and in the 2011-12 and 2012-13 growing seasons at  
148 Carmagnola ( $44^{\circ} 50' \text{ N}$ ,  $7^{\circ} 40' \text{ E}$ ; altitude 245 m) in a loam soil (Typic Udifluvents).

149 In each growing season, 4 field programmes, resulting from a factorial combination of 2  
150 wheat cultivars (cv), with different susceptibility to DON contamination, and 2 fungicide  
151 applications, were compared in naturally-infected field conditions:

- 152     ▪ a common wheat cv., classified as moderately resistant (MR) to FHB infection and  
153         DON contamination, combined with an azole fungicide application at heading  
154         [growth stage (GS) 59] (Zadoks et al., 1974),
- 155     ▪ an untreated common wheat MR cv.,
- 156     ▪ a durum wheat cv., classified as susceptible (S) to FHB infection and DON  
157         contamination, combined with an azole fungicide application at heading (GS59),
- 158     ▪ an untreated durum wheat S cv.

159 The common wheat MR cv. was Generale (Consorzio nazionale sementi, Conselice, RA,  
160 Italy), while the durum wheat S cv. was Saragolla (Produttori Sementi Bologna S.p.A.,  
161 Argelato, BO, Italy). In temperate areas, the durum wheat is characterized by a generally  
162 higher susceptibility to FHB than common one, limiting their potential cultivation in these  
163 areas.

164 The applied azole fungicide was prothioconazole [Proline®, Bayer, Italy, emulsifiable  
165 concentrate formulation (EC), applied at 0.250 kg of active ingredient (AI) ha<sup>-1</sup>] and it was  
166 sprayed at heading (GS59). The fungicides were applied at the manufacturer's  
167 recommended field rates using a 4 nozzle precision sprayer (T-Jeet 110/04) with a fine  
168 mist at a slow walk to ensure effective coverage. The delivery pressure at the nozzle head  
169 was 324 KPa. No other fungicide was applied to any other GS to control foliar diseases.

170 The commonly adopted agronomic growing area technique was applied. Briefly, the  
171 previous crop was maize, the field was ploughed each year, incorporating the debris in the  
172 soil, and this was followed by disk harrowing to prepare a proper seedbed. Planting was  
173 conducted in 12 cm wide rows at a seeding rate of 450 seeds m<sup>-2</sup> in the last decade of  
174 October. The weed control was conducted with isoproturon and diflufenican at wheat  
175 tillering (GS 23). A total of 170 kg N ha<sup>-1</sup> was applied as a granular ammonium nitrate  
176 fertilizer, split into 50 kg N ha<sup>-1</sup> at wheat tillering (GS 23), 80 kg N ha<sup>-1</sup> at stem elongation  
177 (GS 32) and 40 kg N ha<sup>-1</sup> at booting (GS 46). The sowing and harvest dates, and the date  
178 of fungicide application at heading are reported in Table 1 for each growing season.

179 Each field condition treatment was assigned to an experimental unit using a completely  
180 randomised block design with three replicates. The plot size was 7 x 2 m.

181 The grain yields were obtained by harvesting the whole plot using a Walter Wintersteiger  
182 cereal plot combine-harvester. A subsample was taken from each plot to determine the  
183 grain moisture and test weight (TW). The TW was determined using a Dickey-John

184 GAC2000 grain analysis meter, according to the supplied programme. The grain yield  
185 results were adjusted to a 13 % moisture content.

186 The harvested grains were mixed thoroughly and 2 kg grain samples were taken from  
187 each plot to analyze the mycotoxin content.

188

### 189 *2.3. FHB symptoms*

190 FHB incidence and severity were recorded for each plot by carrying out visual evaluations  
191 of the disease at the soft dough stage (GS 85). FHB head blight incidence was calculated  
192 as the percentage of 200 ears per plot with symptoms.

193 FHB severity was calculated as the percentage of kernels per ear with symptoms. A scale  
194 of 1 to 7 was used in which each numerical value corresponded to a percentage interval of  
195 surfaces exhibiting visible symptoms of the disease, according to the following schedule: 1  
196 = 0-5%, 2 = 5-15 %, 3 = 15-30%; 4 = 30-50 %, 5 = 50-75%, 6 = 75-90%, 7 = 90-100%  
197 (Parry et al., 1995). The FHB severity scores were converted into percentages of ears  
198 exhibiting symptoms, and each score was replaced with the mid-point of the interval.

199

### 200 *2.4. Multi-mycotoxin LC-MS/MS analysis*

201 A 2 kg representative sample of grain from each plot was ground using a ZM 200 Ultra  
202 Centrifugal Mill (Retsch GmbH, Haan, Germany) fitted with a 1 mm aperture sieve, and the  
203 resulting whole meal was used directly for the extraction. Five g representative sub-  
204 samples of the milled material were extracted using 20 mL of a mixture of  
205 acetonitrile/water/acetic acid 79:20:1 (v/v/v). After extraction, the samples were  
206 centrifuged, diluted 1:1 and injected, as described in detail by Sulyok et al. (2006).

207 Detection and quantification were performed using a QTrap 5500 LC–MS/MS System  
208 (Applied Biosystems, Foster City, CA), equipped with a TurbolonSpray electrospray

209 ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany).  
210 Chromatographic separation was performed at 25 °C in a Gemini® C18-column, 150×4.6  
211 mm i.d., 5 µm particle size, equipped with a C18 security guard cartridge, 4×3 mm i.d. (all  
212 from Phenomenex, Torrance, CA, US). The chromatographic and mass spectrometric  
213 parameters of the investigated analytes were described by Malachova et al. in 2014. The  
214 results of the mycotoxin concentrations were corrected on the basis of the recovery rate.

215

## 216 *2.5. Statistical analysis*

217 The normal distribution and homogeneity of variances were verified by performing the  
218 Kolmogorov–Smirnov normality test and the Levene test, respectively.

219 An analysis of variance (ANOVA) was conducted separately for each experiment, to verify  
220 the assumption of homogeneity, in order to evaluate the effect of the application of the  
221 agronomic strategy on grain yield, test weight, FHB incidence and severity, DON  
222 contamination and emerging mycotoxin occurrence using a completely randomized block  
223 design, in which the agronomic strategy was the independent variable. The incidence and  
224 the severity values of fungal ear rot incidence and severity were previously transformed  
225 using  $y' = \arcsin \sqrt{x} * 180 / \pi$  as percentage data derived from counting. The fungal metabolite  
226 concentrations were transformed using the  $y' = \ln(x+1)$  equation to normalize the residuals.  
227 Simple correlation coefficients were obtained for all the detected fungal metabolites,  
228 relative to each another, by joining the data sets that referred to the three growing  
229 seasons.

230 SPSS for Windows, Version 22.0 statistical package (SPSS Inc., Chicago), was used for  
231 the statistical analysis.

## 232 **3. RESULTS**

### 233 *3.1. Meteorological data*

234 The three growing seasons were subject to different meteorological trends, as far as both  
235 rainfall and temperature (expressed as growing degree days, GDDs) from the end of  
236 tillering to the harvest are concerned (Table 2). Frequent rainfall occurred in 2011, at the  
237 tillering stages (March) and at the end of ripening (June). Furthermore, the rainfall in this  
238 growing season at the Cigliano site was very low close to the anthesis stage (May) and the  
239 GDDs during this period were higher than in the other years. Instead, in 2012 and 2013,  
240 rainfall was frequent and regular from stem elongation (April) to the end of flowering. In  
241 2013, the month of May was characterized by a higher incidence of rainy days and lower  
242 GDDs than in the previous years.

243

### 244 *3.2. FHB symptoms*

245 The rapid canopy senescing process that occurred in the 2010-11 growing season  
246 prevented visual measurements of the disease at the dough stage.

247 In the 2011-12 and 2012-13 growing seasons, ANOVA showed a significant effect of the  
248 fungicide treatments on FHB symptoms (Table 3). The application of prothioconazole at  
249 heading led to an average reduction of 27% and 13% for FHB incidence and severity,  
250 respectively, compared to the untreated control. In the untreated conditions, significant  
251 differences were only observed between common and durum wheat for FHB incidence in  
252 the 2011-12 growing season.

253

254

255

### 256 3.3. *Yield parameters*

257 ANOVA only showed a significant effect of field programmes on grain yield in the 2011-12  
258 growing season (Table 3): the fungicide application significantly increased the durum  
259 wheat yield by 22%, compared to the untreated control, while no difference was observed  
260 for common wheat. In all the growing seasons, common wheat resulted in a significantly  
261 higher TW than durum wheat (+11%). Although the fungicide application did not lead to a  
262 significantly increase in TW, the application of this disease control strategy on average  
263 increased this parameter by 1.2 kg hl<sup>-1</sup>. Thus, an increasing trend of TW can be observed  
264 moving from the strategy with the highest risk of DON contamination (untreated durum  
265 wheat) to the one with the least risk (common wheat combined with a fungicide  
266 application).

267

### 268 3.4. *Mycotoxin occurrence*

269 DON was detected in all of the investigated production situations (environment X field  
270 programme) and the content of this mycotoxin was clearly related to the meteorological  
271 conditions, particularly close to anthesis, in each growing season. DON contamination was  
272 low and fell between 42 and 997 µg kg<sup>-1</sup> in the 2010-11 growing season, while it ranged  
273 between 119 and 4271 µg kg<sup>-1</sup> and 446 - 2774 µg kg<sup>-1</sup> in the 2011-12 and 2012-13  
274 growing seasons, respectively.

275 Moreover, considering the 3 growing seasons and the different agronomic strategies, 43  
276 other mycotoxins and secondary metabolites were detected: deoxynivalenol-3-glucoside  
277 (DON-3-G), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), culmorin (CULM),  
278 zearalenone (ZEA), zearalenone-4-sulphate (ZEA-4-S), butenolide (BUT), aurofusarin  
279 (AUR), enniatins (ENNs, sum of ENN A, ENN A<sub>1</sub>, ENN B, ENN B<sub>1</sub>, ENN B<sub>2</sub>), moniliformin  
280 (MON), chrysogine (CHRY), fumonisins (FB, sum of FB<sub>1</sub>, FB<sub>2</sub>, FB<sub>3</sub>), beauvericin (BEA),

281 bikaverin (BIK), fusaric acid (FA), decalonectrin (DEC), T-2 toxin, HT-2 toxin, equisetin  
282 (EQU), alternariol (AOH), alternariol methyl ether (AME), tentoxin (TENT), infectopyrone  
283 (INF), secalonic acid D (SAD) and ergot alkaloids (EAs, sum of ergometrine,  
284 ergometrinine, ergocristine, ergocristinine, ergocornine, ergocorninine, ergocryptine,  
285 ergocryptinine, ergosine, ergotamine, ergovaline, agroclavine and chanoclavine).

286 In addition to DON (average contamination of 1067  $\mu\text{g kg}^{-1}$ ), the most abundant  
287 metabolites were AUR, CULM and DON-3-G, which were detected in all of the samples,  
288 with an average contamination of 701  $\mu\text{g kg}^{-1}$ , 689  $\mu\text{g kg}^{-1}$  and 250  $\mu\text{g kg}^{-1}$ , respectively.  
289 Other mycotoxins produced by *F. graminearum* and *F. culmorum*, such as 3A-DON, NIV,  
290 ZEA, ZEA-4S and BUT, were found in generally lower concentrations ( $< 28 \mu\text{g kg}^{-1}$ ) and  
291 not in all of the compared environmental and agronomical situations.

292 Table 4 reports the correlation coefficients and the significance between all of the recorded  
293 mycotoxins and metabolites. Among the mycotoxins produced by *Fusarium graminearum*  
294 and *culmorum*, all the previously reported ones, with the exception of NIV ( $r=0.30$ ), result  
295 to be significantly correlated to DON: the closest relationship was found for CULM  
296 ( $r=0.94$ ), and this was followed by DON-3G ( $r=0.74$ ), 3A-DON ( $r=0.66$ ), ZEA ( $r=0.63$ ), BUT  
297 ( $r=0.48$ ) and ZEA-4S ( $r=0.44$ ).

298 As far as the modified mycotoxins are concerned, the ratio between 3-ADON, a phase I  
299 plant metabolite, and DON was always lower than 1% in all of the compared production  
300 situations. On the other hand, the DON-3G/DON ratio was 54% 31% and 8%, in the 2010-  
301 11, 2011-12 and 2012-13 growing seasons, respectively, while the ratio between the  
302 different field programmes was more consistent within the growing seasons. The ZEA-  
303 4S/ZEA ratio also clearly varied within the growing seasons and was 59%, 22% and 136%  
304 in 2010-11, 2011-12 and 2012-13, respectively. Furthermore, the occurrence of this phase  
305 II plant metabolite derived from sulfatation seem to be influenced above all by the

306 genotype, since the ZEA-4S/ZEA ratio on average was 134% and 11% for the common  
307 and durum wheat cv., respectively.

308 Although AUR could be produced mainly by *F. graminearum* and *culmorum* species, it was  
309 here found not to be significantly correlated to DON ( $r = 0.20$ ), while it was significantly  
310 related to ZEA-4S (0.70), ZEA (0.54), BUT (0.51), NIV (0.49) and CULM (0.45).  
311 Conversely, this metabolite, which was found in all of the compared production situations  
312 above the LOQ, showed a clear and more significant relationship with the other  
313 mycotoxins produced by *F. avenaceum* (a probable AUR producer), such as ENNs  
314 ( $r=0.85$ ), MON ( $r=0.71$ ) and BEA ( $r=0.68$ ). The highest average content of all these  
315 mycotoxins was found in the 2010-11 growing season, which was also characterized by  
316 the lowest DON occurrence.

317 ENNs ranging from 7 to 698  $\mu\text{g kg}^{-1}$  were found for all of the growing seasons and field  
318 programmes. The most abundant compounds were enniatin B (48% of the cases) and B<sub>1</sub>  
319 (41% of the cases), while the other forms (A, B<sub>2</sub>, B<sub>3</sub>) were only found in traces. The ratio  
320 between the different ENN forms was extremely stable within the different growing  
321 seasons and field programmes. Unlike the AUR and ENNs, MON was found to be  
322 correlated to the DON content ( $r=0.42$ ), although the average content of this toxin was  
323 higher in the 2010-11 growing season (80  $\mu\text{g kg}^{-1}$ ). CHRY, was only found in the 2012-13  
324 growing season (average content in the positive samples of 16  $\mu\text{g kg}^{-1}$ ).

325 Among the other *Fusarium* toxins and metabolites, FB, BEA, BIK and FA, which are mainly  
326 produced by the *Fusarium* species section *Liseola*, were found in traces in the 2010-11  
327 and 2011-12 growing seasons, with an average concentration in positive samples of 12,  
328 14, 5 and 44  $\mu\text{g kg}^{-1}$ , respectively.

329 A significant correlation was found between DON and DEC, a metabolite produced by both  
330 *F. graminearum* and *F. sporotrichioides*. This metabolite was found in all of the growing



331 seasons, but in half of the compared production situations, with an average concentration  
332 of 2  $\mu\text{g kg}^{-1}$ . Among the mycotoxins produced by other *Fusarium* species, T-2 and HT-2  
333 toxins (*F. sporotrichioides*, *F. langsaethiae*, *F. poae*) were infrequent and often at very low  
334 contamination levels in all of the compared production situations. Conversely, EQU,  
335 produced by *F. equiseti*, was found in traces in the 2011-12 and 2012-13 growing  
336 seasons, but reached up to a maximum of 74  $\mu\text{g kg}^{-1}$  in the 2010-11 season.

337 Among the toxins produced by the *Alternaria* species, AOH, AME and TENT were found in  
338 all the growing seasons. The occurrence of all of these toxins was higher in the 2010-11  
339 growing season, with average contamination levels of 9, 6 and 3  $\mu\text{g kg}^{-1}$ , respectively.  
340 AOH and AME did not result to be correlated to DON, while a significant relationship was  
341 observed for TENT, which, however, was not correlated to the other *Alternaria* toxins.

342 INF, produced by *A. infectoria*, was only found in samples collected in the 2012-13  
343 growing season (average content in the positive samples of 70  $\mu\text{g kg}^{-1}$ ). SAD (8  $\mu\text{g kg}^{-1}$ ),  
344 produced by *Penicillium* and *Aspergillum spp.* and *Claviceps purpurea*, and EAs (21  $\mu\text{g}$   
345  $\text{kg}^{-1}$ ), produced by *Claviceps purpurea*, were only found in this growing season. Although  
346 produced by fungal species from different genera, the occurrence of INF, SAD and EAs  
347 was here found to be significantly correlated to each other ( $r>0.70$ ). The main ergot  
348 alkaloids were ergometrinine (25%) and ergocristine (22%), followed by ergocornine  
349 (16%), ergocryptine (13%) and ergometrine (11%), while the other metabolites were only  
350 found in traces. Common wheat was characterized by a higher relative occurrence of  
351 ergocristine and ergocornine, while durum wheat was characterized more by  
352 erogometrinine and ergocryptine.

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### 356 3.5. *Effect of the agronomic strategies on mycotoxin contamination*

357 In all the trials, the DON contamination was significantly affected by the application of the  
358 tested field programmes (Figure 1): the efficacy of the fungicides in reducing DON was on  
359 average 58% and 45% for common and durum wheat, respectively. Moreover, a higher  
360 susceptibility to the accumulation of this mycotoxin was confirmed for the durum wheat (+  
361 6 times), compared to the common wheat.

362 With the exception of the comparison of the untreated common wheat and the durum  
363 wheat combined with a fungicide application in the 2011-12 and 2012-13 growing seasons,  
364 which resulted to be similar, a clear and significant increase in DON content was reported  
365 moving from the lowest risk strategy for this mycotoxin contamination to the highest one.  
366 Therefore, the most favourable strategy for DON contamination control (MR variety and  
367 fungicide application at heading) reduced this mycotoxin content by 94%, 95% and 78%,  
368 compared to the worst one (untreated S variety), in the 2010-11, 2011-12 and 2012-13  
369 growing seasons, respectively.

370 Moreover, ANOVA also showed a significant effect of the compared agronomic strategies  
371 in almost all of the growing seasons for DON-3G, 3A-DON, NIV, CULM, ZEA, ZEA-4S and  
372 BUT (Table 5). The application of the less risky agronomic strategy for DON contamination  
373 led to a clear reduction in all of these mycotoxins, compared to the worst case. The best  
374 DON contamination control strategy reduced the DON-3G, 3A-DON, NIV, CULM, ZEA,  
375 ZEA-4S and BUT contents by 89%, 95%, 89%, 98%, 84% and 98%, respectively,  
376 compared to the worst one.

377 A similar trend was also observed within the agronomic strategies for AUR and ENNs  
378 (Table 6), which were significantly reduced by the cultivation of the common MR wheat  
379 compared to the S durum one. The application of fungicide led to a reduction in the  
380 content of both these mycotoxins, which was significant in several cases, in particular for

381 AUR. The MON content was affected less clearly by the application of the field  
382 programmes, although the average content of this mycotoxin was also reduced, moving  
383 from the worst to the best strategy for DON control. The adoption of the most careful  
384 cropping system on average minimized the AUR, ENN and MON contents by 99%, 90%  
385 and 94%, respectively.

386 CHRY was not affected by variety susceptibility in the 2012-13 growing season, while it  
387 was significantly reduced, by 74%, in both cvs. for the fungicide application at heading.

388 The FB, BEA, BIK and FA occurrences were significantly higher in the durum than in the  
389 common wheat in the 2010-11 growing season, while no significant differences were  
390 observed for the fungicide application (Table 7). A significant effect was only observed for  
391 cv susceptibility for BEA in the 2011-12 period, while this mycotoxin was not found in the  
392 next year. As far as the other *Fusarium* toxins are concerned, the highest occurrences of  
393 DEC, HT-2 toxin and EQU were always found for the worst agronomic strategy for DON  
394 contamination (Table 8), which often resulted in a significantly higher content than for the  
395 other field programmes.

396 Moreover, the adoption of the agronomic strategies in all the growing seasons also  
397 affected the contamination by *Alternaria* toxins, in a similar way to that of DON: in all the  
398 growing seasons, with the exception of AME in 2011-12, the AOH, AME, TENT and INF  
399 contents were significantly higher in the durum S cv. than in the common MR one (Table  
400 9). In addition, the application of a fungicide significantly reduced the AME content in the  
401 durum wheat in the 2010-11 season and TENT in common wheat in the 2011-12 period.

402 The application of the more effective GAP for DON control led to average reductions of  
403 96%, 96%, 86% and 42% of AOH, AME, TENT and INF, respectively, compared to the  
404 riskiest strategy.

405 EAs and SAD were found only in the 2012-13 growing seasons. The EAs content in the  
406 common wheat was  $< 1 \mu\text{g kg}^{-1}$  for the wheat treated with fungicide and  $8 \mu\text{g kg}^{-1}$  in the  
407 untreated control (data not shown). The durum wheat content was significantly higher  
408 ( $P<0.01$ ) and reached  $43$  and  $33 \mu\text{g kg}^{-1}$  with and without the fungicide application,  
409 respectively. No significant differences were observed for SAD, although its occurrence  
410 was  $2, 7, 13$  and  $10 \mu\text{g kg}^{-1}$  in the treated common wheat, the untreated common wheat,  
411 the treated durum wheat and the untreated durum wheat, respectively. The most  
412 favourable strategy for DON control reduced the EA and SAD contents by  $98\%$  and  $84\%$ ,  
413 respectively, compared to the worst case.

414

#### 415 **4. DISCUSSION**

416 The data collected in 3 growing seasons with different climatic conditions give clear  
417 information on the presence of different fungal metabolites in wheat kernel at harvest, also  
418 considering the so called “emerging mycotoxins”, which till now have not received detailed  
419 scientific attention, particularly in raw materials.

420 Data confirm that DON is the most prevalent mycotoxin in winter wheat in temperate  
421 growing areas (Larsen et al., 2004), while CULM was present in similar concentrations as  
422 DON and is closely associated with this toxin (Ghebremeskel and Langseth, 2000).  
423 Considering the 3 growing seasons, the sum of DON and CULM on average represents  
424 more than 60% of the total mycotoxins that were encountered.

425 In addition to DON, the most abundant metabolites were AUR, CULM and DON-3-G, while  
426 other mycotoxins produced by *F. graminearum* and *F. culmorum*, such as 3A-DON, NIV,  
427 ZEA, ZEA-4S and BUT, were found in generally lower concentrations. These results are in  
428 agreement with the results of a another survey conducted on common and durum wheat in  
429 North Italy (Bertuzzi et al., 2014). **In literature, the co-presence in wheat of DON, other**  
430 **type B trichothecens and ZEA has been variable, since it depends to a great extent on the**  
431 **predominant species or chemotype strains.** The low rate of NIV and DON occurrence  
432 observed in the present work (average NIV/DON=0.5%), confirms that the *F. graminearum*  
433 DON chemotypes in Italy are predominate over the chemotype strains that are able to  
434 produce NIV (Somma et al., 2014). Conversely, in South America (Del Ponte et al., 2012)  
435 and Asia (Tanaka et al., 2010), the co-contamination of DON and NIV has been reported  
436 at a similar level. The correlation between DON and ZEA in wheat has been found to be  
437 very low in Poland (Chelkowsky et al., 2012), compared to the present experiment.

438 Moreover, the data reported on the occurrence of modified mycotoxins (Rychlik et al.,  
439 2014) confirm the results of previous studies (Berthiller et al., 2009; De Boevre et al.,  
440 2012; Scarpino et al., 2015) in which the relationship between DON and its phase II plant  
441 metabolite changed in relation to the environmental conditions. **The rate between DON-3-**  
442 **G/DON was lower in growing seasons with frequent rainfall and low temperature from**  
443 **stem elongation to the end of flowering, while this ration was higher in the drier and**  
444 **warmer season.**

445 Overall, the levels of ENNS, MON and BEA are comparable with those reported in wheat  
446 in the North (Lindblad et al., 2013, Uhlig et al., 2013) and South of Europe (Jestoi et al.,  
447 2004 and 2008, Scarpino et al., 2015). As far as the occurrence of AUR is concerned, the  
448 higher correlation found between this metabolite and ENNS, MON and BEA suggest that,  
449 in the compared conditions, the occurrence of AUR is related more to *F. avenaceum* (Uhlig  
450 et al., 2006). CHRY, another metabolite produced by *F. avenaceum* (Uhlig et al., 2006),  
451 was found only in the cooler year.

452 T-2 and HT-2 toxins (*F. sporotrichioides*, *F. langsaethiae*, *F. poae*) were infrequent and  
453 often at very low contamination levels in all of the compared production situations, thus  
454 highlighting a low incidence of these compounds in winter wheat compared to other small  
455 cereals, such as oats (van der Fels-Klerx and Stratakou, 2010). Otherwise, the occurrence  
456 of metabolite produced by other *Fusarium* species (*F. verticillioides*, *F. equiseti*) is more  
457 frequent, but only in traces.

458 The occurrence of *Alternaria* metabolites is lower than those reported in wheat cultivated  
459 in North Europe (Uhlig et al., 2013), but confirm previous findings for Southern European  
460 growing areas (Scarpino et al., 2015). **The occurrence of AOH and AME was higher in**  
461 **2010-11 growing season, with drier conditions before and during flowering, less favourable**  
462 **to *Fusarium* ear infection.** Otherwise INF, produced by *A. infectoria* (Larsen et al., 2003),

463 SAD produced by *Penicillium* and *Aspergillum spp.* and *Claviceps purpurea* (Wang and  
464 Polya, 1996), and EAs, produced by *Claviceps purpurea* (Malysheva et al., 2014), seem to  
465 be related to more rainy environmental conditions.

466 Overall, the results obtained in the current study point out the crucial role of the  
467 environmental conditions on the diffusion of different mycotoxins, both the common and  
468 emerging ones, in winter wheat (Doohan et al., 2003). Moreover, the collected data  
469 highlight the important role that cultural practices can play in determining their level of  
470 contamination in the grains. The hypothesis on the different susceptibilities of the  
471 compared agronomic field programmes to DON occurrence was confirmed for all the  
472 growing seasons. This finding is in agreement with previous findings pertaining to the  
473 same environments confirms previous data obtained in the same (Blandino et al., 2006;  
474 2009a; Blandino et al., 2012) and in other environments (Beyer et al., 2006; Koch et al.,  
475 2006). Above all, conditions such as preceding host crops, especially maize and sorghum,  
476 which leave high amount of infected residues in the field, and the cultivation of a  
477 susceptible variety contribute to heavy DON contamination of wheat crops (Blandino et al.,  
478 2012). In addition, our results confirm that the efficacy of fungicide in reducing DON  
479 content is related to the environmental and agronomic conditions (Blandino et al., 2011).  
480 Thus, mycotoxin control in wheat should first focus on the agronomic factors that influence  
481 the occurrence of inoculum (preceding crop and management of crop residues) and the  
482 variety susceptibility to fungal infection, according to a integrate multiple strategy approach  
483 (Beyer et al., 2006).

484 Moreover, the data collected allow a first holistic evaluation of the effect of the combination  
485 of crop practices in wheat on several fungal metabolites, produced by different species.  
486 Although the considered agronomic factors (variety susceptibility and fungicide application)  
487 resulted in a variable control efficacy, in function of the environmental conditions and type

488 of mycotoxin, the results show a clear and similar reduction trend after the application of  
489 agronomic strategies for DON control for almost all of the mycotoxins present at different  
490 contamination levels. This effect was observed for toxins produced by *Fusarium* species  
491 involved in FHB infection in temperate areas, but also for metabolites related to the  
492 development of other *Fusarium* species that are not involved directly in this disease, such  
493 as *F.* section *Liseola* or *F. equisetum*, or other species that can cause other diseases,  
494 such as *Claviceps* spp. or saprophytic fungi such as *Alternaria* spp.

495 ~~Thus, in the considered production situations, no evidence has emerged of an increase in~~  
496 ~~any mycotoxin as a consequence of a modification in the fungal community, related to the~~  
497 ~~different control capacities of the applied strategies on fungal species characterized by~~  
498 ~~different ecologies. In other words, Thus, in the considered production situations~~ the  
499 application of field programmes that are able to reduce the predominant FHB pathogens,  
500 which are responsible for most of the contamination of winter wheat (DON, CULM), did not  
501 lead to an increase in any other metabolite, as a consequence of the development of other  
502 species that occupy their ecological niche, and they even resulted to be reduced.

503 The change in the relative competition capacity among fungal species with the application  
504 of a control factor, which results in a reduction of certain mycotoxins and the simultaneous  
505 increase of others, has been named the “flora inversion” phenomenon and it has been  
506 observed for *Fusarium* toxins in maize by Folcher et al. (2010). A change in the relative  
507 competition capacity between FB and trichothecens producers was observed for this crop,  
508 in the same growing areas as those of the present study, as a consequence of the control  
509 of the insects that are responsible for *Fusarium* infection (Blandino et al., 2009b; Blandino  
510 et al., 2015). The co-occurrence of fungal species in maize seems to be more complex to  
511 manage in temperate areas than that of small cereals. The ecology of the maize infecting  
512 fungal species (*Fusarium* section *Liseola* and *Discolor*, *Aspergillus flavus* and *Penicillium*



513 spp.) is slightly different and their relative occurrence is clearly influenced by the climatic  
514 conditions during the crop cycle (Doohan et al., 2003). Moreover, the importance of  
515 pathway infection (seed, silk and through kernel damage caused by insects) changes  
516 according to the fungal species (Munkvold et al., 1997) and this could lead to a different  
517 control with the adoption of agronomic control factors, which would make it more  
518 complicated to minimize the overall mycotoxin contamination of this crop.

519 Conversely, floral infection seems to be the main pathway for all of the different fungal  
520 species in winter wheat (Xu and Nicholson, 2009), but there is a clear predominance of  
521 *Fusarium* species, which are responsible for FHB, compared to the other *Fusarium* or  
522 genus species. Thus, the negative interaction between different fungal species and  
523 mycotoxins in wheat, as a consequence of the application of control strategies, could be  
524 more infrequent than in maize, and could thus allow an easier setup of GAP for the overall  
525 control of mycotoxins.

526 However, the presence of fungal competitive interaction phenomenon on small cereals  
527 has also been documented in literature. In fact, it is well known that the application of  
528 fungicides (such as strobilurins) at heading is less effective against *F. graminearum* and  
529 *culmorum*, but is able to significantly reduce the non-toxigenic *Microdochium nivale*, and it  
530 could therefore increase DON contamination in wheat (Pirgozliev et al., 2003, Blandino et  
531 al., 2006). Both the active ingredients and the timing of fungicide application could impact  
532 on the composition of the Fusarium Head Blight disease complex (Audenaert et al., 2011).

533 Moreover, from a comparison of *Fusarium* and *Alternaria* species, it has emerged that the  
534 different fungicide classes show a different growth reduction capacity (Müllenborn et al.,  
535 2007). A field survey conducted in France on barley reported that DON contamination is  
536 negatively correlated to that of T-2 and HT-2 toxin (Orlando et al., 2010), thus suggesting  
537 the need for a different risk management for these mycotoxins in the considered

538 agronomic conditions. The main agronomic factors identified to increase the risk of T-2  
539 and HT-2 toxins on barley were late sowing times, small grain cereals as previous crop  
540 and minimum or no tillage (Orlando et al., 2010).

541 Thus, although, in the present study, the single and combined application of a genetic and  
542 a chemical control of DON never led to an increase in the contents of any other mycotoxin  
543 or fungal metabolite, it remains necessary to verify, whether the agronomic factors that  
544 have been identified to reduce the risk of DON in winter wheat are different from those that  
545 are able to control the other emerging mycotoxins in different production situations.

## 546 **5. CONCLUSION**

547 The results of these experiments, obtained under naturally-infected field conditions and  
548 conducted over three different growing seasons, underline that the agronomic strategies  
549 generally applied in temperate areas to control DON contamination also contribute to  
550 minimizing the risk of contamination of the other mycotoxins that could occur in winter  
551 wheat grains. Thus, wheat protection programmes, based on the combination of MR  
552 varieties and a fungicide application at heading, in order to control FHB, could clearly  
553 contribute to improving the global sanity of this crop, by reducing the mycotoxins produced  
554 by different *Fusarium* section species, but also other fungal genera, such as *Alternaria*,  
555 *Claviceps* and *Penicillium*.

556 These results, which need to be confirmed in other environments and considering other  
557 important risk factors, such as crop rotation and soil tillage or the application of other  
558 fungicide active substances with different mechanisms of action, suggest that the “flora  
559 inversion” phenomenon rarely occurs in winter wheat, thus making it easier to find and  
560 apply integrated management strategies for the overall control of mycotoxins in this crop.

561

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571 **6. REFERENCES**

- 572 Audenaert, K., Landschoot, S., Vanheule, A., Waegemam, W., De Baets, B., Haesaert, G.,  
573 2011. Impact of fungicide timing on the composition of the Fusarium Head blight  
574 disease complex and the presence of deoxynivalenol (DON) in wheat. Fungicide –  
575 beneficial and harmful aspects, Dr. Nooruddin Thajuddin (Ed.). ISBN: 978-953-307-  
576 451-1, InTech, Available from: [http://www.intechopen.com/books/fungicides-  
577 beneficial-and-harmful-aspects/impact-of-fungicide-timing-on-the-composition-of-the-  
578 fusarium-head-blight-disease-complex-and-the-pr](http://www.intechopen.com/books/fungicides-beneficial-and-harmful-aspects/impact-of-fungicide-timing-on-the-composition-of-the-fusarium-head-blight-disease-complex-and-the-pr)
- 579 Berthiller, F., Crews, C., Dall’Asta, C., De Saeger, S., Haesaert, G., Karlovsky, P., Oswald,  
580 I.P., Walburga, S., Gerrit, S., Stroka, J., 2013. Masked mycotoxins: a review. Mol.  
581 Nutr. Food Res. 57(1), 165-186. DOI: 10.1002/mnfr.201100764.
- 582 Berthiller, F., Dall’Asta, C., Corradini, R., Marchelli, R., Sulyok, M., Krska, R., Adam, G.,  
583 Schuhmacher, R., 2009. Occurrence of deoxynivalenol and its 3-b-D-glucoside in  
584 wheat and maize. Food Addit. Contamin. Part A 26, 507-511. DOI:  
585 10.1080/02652030802555668.
- 586 Bertuzzi, T., Leggieri, M.C., Battilani, P., Pietri, A., 2014. Co-occurrence of type A and B  
587 trichothecens and zearalenone in wheat grown in northern Italy over the years 2009-  
588 2011. Food Addit. Contamin. Part B 7(4), 273-281. DOI:  
589 10.1080/19393210.2014.926397.
- 590 Beyer, M., Klix, M.B., Klink, H., Verreet J.-A., 2006. Quantifying the effect of previous crop,  
591 tillage, cultivar and triazole fungicides on the deoxynivalenol content of wheat grain –  
592 a review. J. Plant Dis. Prot. 113, 241-246.
- 593 Blandino, M., Haidukowski, M., Pascale, M., Plizzari, L., Scudellari, D., Reyneri, A., 2012.  
594 Integrated strategies for the control of Fusarium head blight and deoxynivalenol

595 contamination in winter wheat. *Field Crop. Res.* 133, 139-149. DOI:  
596 10.1016/j.fcr.2012.04.004.

597 Blandino, M., Minelli, L., Reyneri, A., 2006. Strategies for the chemical control of *Fusarium*  
598 head blight: effect on yield, alveographic parameters and deoxynivalenol  
599 contamination in winter wheat grain. *Eur. J. Agron.* 25, 193-201. DOI:  
600 10.1016/j.eja.2006.05.001.

601 Blandino M., Pilati A., Reynari A., 2009a. Effect of foliar treatments to durum wheat on flag  
602 leaf senescence, grain yield, quality and DON contamination in North Italy. *Field*  
603 *Crop. Res.* 114, 214-222. DOI: 10.1016/j.fcr.2009.08.008.

604 Blandino, M., Scarpino, V., Vanara, F., Sulyok, M., Krska, R., Reyneri, A., 2015. The Role  
605 of the European Corn Borer (*Ostrinia Nubilalis*) on contamination of maize with  
606 thirteen *Fusarium* mycotoxins. *Food Addit. Contamin. Part A* 32(4), 533-43, DOI:  
607 10.1080/19440049.2014.966158.

608 Blandino M, Reyneri A, Vanara F, Tamietti G, Pietri A. 2009b. Influence of agricultural  
609 practices on *Fusarium* infection, fumonisin and deoxynivalenol contamination of  
610 maize kernels. *World Mycotoxin J.* 2, 409–418. DOI: 10.3920/WMJ2008.1098

611 Chełkowski, J., Gromadzka, K., Stępień, Ł., Lenc, L., Kostecki, M., Berthiller F., 2012.  
612 *Fusarium* species, zearalenone and deoxynivalenol content in preharvest scabby  
613 wheat heads from Poland. *World Mycotoxin J.* 5(2), 133-141. DOI:  
614 <http://dx.doi.org/10.3920/WMJ2011.1304>.

615 De Boevre, M., Diana Di Mavungu, J., Landschoot, S., Audenaert, K., Eeckhout, M.,  
616 Maene, P., Haesaert, G., De Saeger, S., 2012. Natural occurrence of mycotoxins and  
617 their masked forms in food and feed products. *World Mycotoxin J.* 5, 207-219. DOI:  
618 0.3920/WMJ2012.1410

619 Del Ponte, E., Garda-Buffon, J., Badiale-Furlong, E., 2012. Deoxynivalenol and nivalenol  
620 in commercial wheat grain related to *Fusarium* head blight epidemics in southern  
621 Brazil. *Food Chem.* 132, 1087-1091. DOI :10.1016/j.foodchem.2011.10.108

622 Doohan, F. M., Brennan, J., Cooke, B. M., 2003. Influence of climatic factors on *Fusarium*  
623 species pathogenic to cereals. *Eur. J. Plant Pathol.* 109, 755-768. DOI:  
624 10.1023/A:1026090626994.

625 EFSA, 2010. Request for a scientific opinion on the risks for public health related to the  
626 presence of moniliformin in feed and food, Mandate M-2010-0312, Reception Date  
627 21-07-2010, Acceptation Date 13-04-2016.

628 EFSA, 2014. Scientific Opinion on the risks to human and animal health related to the  
629 presence of beauvericin and enniatins in food and feed. *EFSA Journal* 12(8), 3802.

630 European Commission, 2006. Commission regulation No. 1881/2006, of 10 December  
631 2006 setting maximum levels for certain contaminants in food stuff. *Official Journal of*  
632 *the European Union* L 364, pp. 5-24.

633 European Commission, 2007. Commission regulation No. 1126/2007, of 28 September  
634 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain  
635 contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products  
636 L 255, pp. 14-17.

637 Folcher L, Delos M, Marengue E, Jarry M, Weissenberger A, Eychenne N, Regnault-Roger  
638 C. 2010. Lower mycotoxin levels in Bt maize grain. *Agron. Sustain. Dev.* 30, 711–  
639 719. DOI: 10.1051/agro/2010005

640 Ghebremeskel, M., Langseth, W., 2000. The occurrence of culmorin and hydroxy-  
641 culmorins in cereals. *Mycopathologia* 152(2), 103-108.

642 Jestoi, M. 2008. Emerging Fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and  
643 moniliformin—A review. *Crit. Rev. Food Sci. Nutr.* 48, 21–49. DOI:  
644 10.1080/10408390601062021.

645 Jestoi, M., Somma, M.C. , Kouva, M., Veijalainen, P. , Rizzo, A., Ritieni, A., Peltonen,  
646 K.,2004. Levels of mycotoxins and sample cytotoxicity of selected organic and  
647 conventional grain-based products purchased from Finnish and Italian markets. *Mol.*  
648 *Nutr. Food Res.* 48(4), 299-307. DOI: 10.1002/mnfr.200400026.

649 Koch, H.-J., Pringas, C., Maerlaender, B., 2006. Evaluation of environmental and  
650 management effects on Fusarium head blight infection and deoxynivalenol  
651 concentration in the grain of winter wheat. *Eur. J. Agron.* 24, 357-366. DOI:  
652 10.1016/j.eja.2006.01.006.

653 Larsen, J.C., Hunt, J., Perrin, I., Ruckenbauer P., 2004. Workshop on trichothecenes with  
654 a focus on DON: summary report. *Toxicol. Lett.* 153 (1), 1–22.  
655 doi:10.1016/j.toxlet.2004.04.020.

656 Larsen T.O., Perry, N.B., Andersen, B., 2003. Infectopyrone, a potential mycotoxin from  
657 *Alternaria infectoria*. *Tetrahedron Lett.* 44(24), 4511-4513. doi:10.1016/S0040-  
658 4039(03)01018-9.

659 Lindblad, M., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olsen, M., Fredlund, E.,  
660 2013. Deoxynivalenol and other selected Fusarium toxins in Swedish wheat -  
661 occurrence and correlation to specific *Fusarium* species. *Int. J. Food Microbiol.*  
662 167(2), 284-291. DOI: 10.1016/j.ijfoodmicro.2013.07.002.

663 Malachova, A., Sulyok, M., Beltran, E.; Berthiller, F., Krska, R., 2014. Optimization and  
664 validation of a quantitative liquid chromatography - tandem mass spectrometric  
665 method covering 295 bacterial and fungal metabolites including all relevant



666 mycotoxins in four model food matrices. J. Chromatogr. A 1362, 145-156. DOI:  
667 10.1016/j.chroma.2014.08.037.

668 Malysheva, S.V., Larionova, D.A., Di Mavungu, J.D., De Saeger, S., 2014. Pattern and  
669 distribution of ergot alkaloids in cereals and cereal products from European  
670 countries. World Mycotoxin J. 7, 217–230. DOI:  
671 <http://dx.doi.org/10.3920/WMJ2013.1642>.

672 Müllenborn, C., Steiner, U., Ludwig, M., Oerke, E.-C., 2007. Effect of fungicides on the  
673 complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. Eur.  
674 J. Plant Pathol. 120(2), 157-166. DOI: 10.1007/s10658-007-9204-y.

675 Munkvold, G.P., McGee, D.C., Carlton, W.M., 1997. Importance of different pathways for  
676 maize kernel infection by *Fusarium moniliforme*. Phytopathology 87, 209-217. DOI:  
677 10.1094/PHYTO.1997.87.2.209.

678 Orlando, B., Barrier-Guillot, B., Gourdain, E., Mourmené, C. 2010. Identification of  
679 agronomic factors that influence the levels of T-2 and HT-2 toxins in barley grown in  
680 France. World Mycotoxin J. 3,169-174. DOI.org/10.3920/WMJ2009.1191.

681 Parry, D.W., Jenkinson, P., McLeod, L., 1995. *Fusarium* ear blight (scab) in small grain  
682 cereal – a review. Plant Pathol. 44, 207-238. DOI: 10.1111/j.1365-  
683 3059.1995.tb02773.x.

684 Pestka, J.J., Smolinski, A.T., 2005. Deoxynivalenol: toxicity and potential effects on  
685 humans. Critical Reviews. J. Toxicol. Env. Heal. B 8, 39-69. DOI: 10.2478/v10102-  
686 010-0019-x.

687 Pirgozliev, S.R., Edwards, S.G., Hare, M.C., Jenkinson, P., 2003. Strategies for the control  
688 of *Fusarium* head blight in cereals. Eur. J. Plant Pathol. 109, 731-742. DOI:  
689 10.1023/A:1026034509247.

690 Rychlik, M., Humpf, H.U., Marko, D., Dänicke, S., Mally, A., Berthiller, F., Klaffke H.,  
691 Lorenz N., 2014. Proposal of a comprehensive definition of modified and other forms  
692 of mycotoxins including "masked" mycotoxins. *Mycotoxin Res.* 30(4), 197-205. doi:  
693 10.1007/s12550-014-0203-5.

694 Scarpino, V., Sulyok, M., Krska, R., Reyneri, A., Blandino, M., 2015. Effect of fungicide  
695 application to control *Fusarium* head blight and 20 *Fusarium* and *Alternaria*  
696 mycotoxins in winter wheat (*Triticum aestivum*). *World Mycotoxin J.* 8(4), 499-510.  
697 DOI: 10.392/WMJ2014.1814.

698 Somma, S., Petruzzella, A.L., Logrieco, A.F., Meca, G., Cacciola, O.S., Moretti A., 2014.  
699 Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and  
700 characterisation of their molecular and chemical chemotypes. *Crop Pasture Sci.* 65,  
701 52-60. DOI:10.1080/19440049.2014.984244.

702 Streit, E., Schwab, C., Sulyok, M., Naehrer, K., Krska, R., Schatzmayr, G., 2013. Multi-  
703 mycotoxin screening reveals the occurrence of 139 different secondary metabolites  
704 in feed and feed ingredients. *Toxins* 5(3), 504-523. DOI: 10.3390/toxins5030504

705 Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R., 2006. Development and validation  
706 of a liquid chromatography/tandem mass spectrometric method for the determination  
707 of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* 20, 2649–  
708 2659. DOI: 10.1002/rcm.2640.

709 Tanaka, H., Sugita-Konishi, Y., Takino, M., Tanaka, T., Toriba, A., Hayakawa, K., 2010. A  
710 survey of the occurrence of *Fusarium* mycotoxins in biscuits in Japan by using  
711 LC/MS. *Journal of Health Science* 56, 188-194. DOI: 10.1248/jhs.56.188.

712 Uhlig, S., Eriksen, G.S., Hofgaard, I. S., Krska, R., Beltrán, E., Sulyok, M., 2013. Faces of  
713 a changing climate: semi-quantitative multi-mycotoxin analysis of grain grown in

714 exceptional climatic conditions in Norway. *Toxins* 5(10), 1682-97. DOI:  
715 10.3390/toxins5101682

716 Uhlig, S., Jestoi, M., Knutsen, A.K., Heier, B.T., 2006. Multiple regression analysis as a  
717 tool for the identification of relations between semi-quantitative LC-MS data and  
718 cytotoxicity of extracts of the fungus *Fusarium avenaceum* (syn. *F. arthrosporioides*).  
719 *Toxicon*. 48, 567–579. DOI: 10.1016/j.toxicon.2006.07.007.

720 van der Fels-Klerx, H.J., Stratakou, I., 2010. T-2 toxin and HT-2 toxin in grain and grain-  
721 based commodities in Europe: occurrence, factors affecting occurrence, co-  
722 occurrence and toxicological effects. *World Mycotoxin J.* 3, 349-367. DOI:  
723 <http://dx.doi.org/10.3920/WMJ2010.1237>.

724 van der Fels-Klerx, H.J., van Asselt, E.D., Madsen, M.S., Olesen, J.E., 2013. Impact of  
725 climate change effects on contamination of cereal grains with deoxynivalenol. *PloS*  
726 *One* 8(9), 1-10. DOI:10.1371/journal.pone.0073602

727 Wang, B.H., Polya, G.M., 1996. The fungal teratogen secalonic acid D is an inhibitor of  
728 protein kinase C and of cyclic AMP-dependent protein kinase. *Planta Medica* 62(2),  
729 111-114. DOI: 10.1055/s-2006-957829.

730 Xu, X., Nicholson, P., 2009. Community ecology of fungal pathogens causing wheat head  
731 blight. *Annu. Rev. Phytopathol.* 47, 83-103. DOI: 10.1146/annurev-phyto-080508-  
732 081737

733 Yli-Mattila, T., 2010. Ecology and evolution of toxigenic *Fusarium* species in cereal in  
734 northern Europe and Asia. *Journal of Plant Pathology* 92, 7-18. DOI:  
735 10.4454/jpp.v92i1.10.

736 Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of  
737 cereals. *Weed Res.* 14, 415-421. DOI: 10.1111/j.1365-3180.1974.tb01084.x.

738

739 **Table. 1**

740 Main trial information on the field experiments conducted on winter wheat in North West Italy in the 2010 - 2013 period.

<b>Growing season</b>	<b>Site</b>	<b>Sowing date</b>	<b>Fungicide application</b>	<b>Harvest date</b>
2010 - 2011	Cigliano	30 October 2010	13 May 2011	01 July 2011
2011 - 2012	Carmagnola	21 October 2011	23 May 2012	11 July 2012
2012 - 2013	Carmagnola	24 October 2012	21 May 2013	12 July 2013

741

742

743 **Table. 2**

744 Total rainfall, rainy days, relative humidity and growing degree days (GDDs) from March to June 2010-2013 in the research sites.

Year	Site	Month	Rainfall (mm)	Rainy days	GDDs <sup>a</sup> (°C d <sup>-1</sup> )
2011	Cigliano	March	169	9	268
		April	59	5	474
		May	30	5	578
		June	198	14	627
		July	54	8	670
2012	Carmagnola	March	20	1	347
		April	148	13	365
		May	147	6	539
		June	19	2	674
		July	37	5	725
2013	Carmagnola	March	96	10	241
		April	144	11	406
		May	147	11	499
		June	35	3	623
		July	137	7	740

745 <sup>a</sup>Accumulated growing degree days for each month using a 0°C base.

746

747 **Table. 3**

748 Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on Fusarium head blight (FHB) incidence  
 749 and severity, grain yield and test weight of winter wheat; field experiments conducted in North West Italy in the 2010 - 2013 period.

Growing season	Agronomic strategies <sup>a</sup>	FHB incidence <sup>b</sup>		FHB severity <sup>c</sup>		Grain yield t ha <sup>-1</sup>	Test weight kg hl <sup>-1</sup>
		T	N (%)	T	N (%)		
2010 - 2011	common wheat - fungicide	nd <sup>d</sup>		nd		5.4 a	79.1 a
	common wheat - untreated	nd		nd		5.2 a	78.4 a
	durum wheat - fungicide	nd		nd		4.7 a	66.4 b
	durum wheat - untreated	nd		nd		4.1 a	66.9 b
	<i>P</i> (F) <sup>e</sup> sem <sup>f</sup>					0.109 1.0	< 0.001 1.9
2011- 2012	common wheat - fungicide	37 C	37	8 c	2	6.2 b	83.6 a
	common wheat - untreated	62 Ab	78	28 a	11	6.0 b	82.9 a
	durum wheat - fungicide	52 B	62	13 bc	12	6.7 a	78.0 b
	durum wheat - untreated	65 A	81	19 b	23	5.5 c	74.3 b
	<i>P</i> (F) Sem	0.001 8.1		0.001 6.3		< 0.001 0.3	0.001 3.0
2012- 2013	common wheat - fungicide	38 C	50	10 b	10	6.6 a	83.8 a
	common wheat - untreated	62 B	77	30 a	26	6.4 a	82.7 a
	durum wheat - fungicide	45 C	75	18 b	19	7.5 a	79.9 b
	durum wheat - untreated	78 A	95	35 a	33	6.6 a	78.7 b
	<i>P</i> (F) Sem	< 0.001 10.5		< 0.001 7.7		0.413 1.3	0.001 1.6

750 <sup>a</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and a high susceptibility to FHB, respectively. The  
 751 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).

752 <sup>b</sup> FHB incidence was calculated as the percentage of ears with FHB damage, considering 100 ears per sample.

753 <sup>c</sup> FHB severity was calculated as the percentage of kernels per ear with FHB damage, considering 100 ears per sample.

754 The reported FHB incidence and severity means are transformed ( $T; y' = \arcsin \sqrt{x} * 180 / \pi$ ) and not transformed (N) values.

755 <sup>d</sup> nd: not detected, the rapid canopy senescing process that occurred in the 20120-11 growing season did not allow visual measurements to be carried out.

756 <sup>e</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
757 replications.

758 <sup>f</sup> sem: standard error of the means.

759

760 **Table. 4**

761 Correlation matrix for mycotoxin contamination in winter wheat kernel.

	DON	DON3G	3ADON	NIV	CULM	ZEA	ZEA-4S	BUT	AUR	ENNs	MON	CHRY	FB	BEA	BIK	FA	DEC	T-2	HT-2	EQU	AOH	AME	TENT	INF	SAD
<b>DON3G</b>	0.74**																								
<b>3ADON</b>	0.66**	0.22																							
<b>NIV</b>	0.30	0.44**	0.10																						
<b>CULM</b>	0.94**	0.86**	0.51**	0.43**																					
<b>ZEA</b>	0.63**	0.33*	0.32	0.34*	0.60**																				
<b>ZEA-4S</b>	0.44**	0.09	0.27	0.27	0.50**	0.766**																			
<b>BUT</b>	0.48**	0.89**	-0.03	0.57**	0.69**	0.26	0.09																		
<b>AUR</b>	0.20	0.32	-0.02	0.49**	0.45**	0.54**	0.70**	0.51**																	
<b>ENNs</b>	0.14	0.54**	-0.20	0.45**	0.45**	0.22	0.34*	0.76**	0.85**																
<b>MON</b>	0.42*	0.78**	-0.05	0.50**	0.68**	0.35*	0.31	0.86**	0.71**	0.89**															
<b>CHRY</b>	0.34*	-0.26	0.50**	0.00	0.17	0.49**	0.64**	-0.36*	0.10	-0.28	-0.27														
<b>FBs</b>	-0.02	0.29	-0.05	0.44**	0.10	-0.04	-0.15	0.50**	0.20	0.32	0.28	-0.35*													
<b>BEA</b>	-0.14	0.20	-0.26	0.34*	0.11	0.06	0.19	0.48**	0.68**	0.72**	0.61**	-0.20	0.25												
<b>BIK</b>	-0.16	0.21	-0.30	0.64**	0.13	0.09	0.29	0.51**	0.78**	0.81**	0.63**	-0.22	0.32	0.70**											
<b>FA</b>	-0.12	0.25	-0.31	0.27	0.16	0.07	0.30	0.46**	0.78**	0.82**	0.70**	-0.24	0.24	0.79**	0.83**										
<b>DEC</b>	0.76**	0.51**	0.41*	0.44**	0.71**	0.60**	0.49**	0.31	0.26	0.08	0.32	0.36*	-0.14	-0.07	0.03	-0.06									
<b>T-2</b>	0.45**	0.67**	0.30	0.18	0.47**	-0.04	-0.19	0.51**	0.06	0.25	0.40*	-0.32	0.16	0.04	0.04	0.04	0.33*								
<b>HT-2</b>	0.24	0.44**	-0.16	-0.04	0.36*	0.14	0.21	0.35*	0.33	0.49**	0.58**	-0.20	0.10	0.14	0.18	0.51**	0.06	0.22							
<b>EQU</b>	-0.15	0.19	-0.25	0.81**	0.09	0.11	0.21	0.47**	0.64**	0.67**	0.56**	-0.18	0.36*	0.645**	0.89**	0.59**	0.08	0.03	0.06						
<b>AOH</b>	0.15	0.21	-0.03	0.34*	0.37*	0.48**	0.72**	0.33*	0.90**	0.74**	0.61**	0.07	0.13	0.48**	0.68**	0.75**	0.15	-0.02	0.55**	0.51**					
<b>AME</b>	0.04	0.32	-0.28	0.18	0.30	0.16	0.42*	0.44**	0.71**	0.79**	0.71**	-0.22	0.20	0.49**	0.63**	0.83**	-0.01	0.06	0.82**	0.44**	0.84**				
<b>TENT</b>	0.82**	0.86**	0.29	0.25	0.81**	0.46**	0.14	0.64**	0.13	0.25	0.56**	-0.08	0.21	-0.08	-0.10	0.03	0.64**	0.49**	0.50**	-0.11	0.13	0.22			
<b>INF</b>	0.24	-0.29	0.44**	-0.05	0.12	0.21	0.370*	-0.41*	-0.05	-0.34*	-0.33*	0.48**	-0.40*	-0.23	-0.25	-0.28	0.25	-0.36*	-0.23	-0.21	0.05	-0.20	-0.09		
<b>SAD</b>	0.23	-0.21	0.36*	0.26	0.16	0.34*	0.44**	-0.25	0.13	-0.17	-0.17	0.44**	-0.23	-0.13	0.00	-0.18	0.34*	-0.30	-0.22	0.13	0.20	-0.12	-0.08	0.71**	
<b>EAs</b>	0.29	-0.20	0.46**	0.01	0.19	0.35*	0.42*	-0.32	0.06	-0.25	-0.21	0.40*	-0.31	-0.17	-0.19	-0.21	0.27	-0.28	-0.18	-0.16	0.20	-0.14	-0.01	0.85**	0.86**

762 (\*) = correlation significant at  $P \leq 0.05$ ; (\*\*) correlation significant at  $P \leq 0.01$ . The data reported in the table are Pearson product-moment correlation coefficients.

763 DON = deoxynivalenol, DON-3-G = deoxynivalenol-3-glucoside, 3-ADON = 3-acetyldeoxynivalenol, NIV = nivalenol, CULM = culmorin, ZEA = zearalenone, ZEA-4-S =

764 zearalenone-4-sulphate, BUT = butenolide, AUR = aurofusarin, ENNs = Enniatins A, A<sub>1</sub>, B, B<sub>1</sub>, B<sub>2</sub>, MON = moniliformin, CHRY = chrysogine, FB = fumonisins, BEA = beauvericin,



765 BIK = bikaverin, FA = fusaric acid, DEC = decalonectrin, T-2 toxin, HT-2 toxin, EQU = equisetin, AOH = alternariol, AME = alternariol methyl ether, TENT = tentoxin, INF =  
766 infectopyrone, SAD = secalonic acid, EAs = ergot alkaloids, sum of ergometrine, ergometrinine, ergocristine, ergocristinine, ergocornine, ergocorninine, ergocryptine,  
767 ergocryptinine, ergosine, ergotamine, ergovaline, agroclavine and chanoclavine.

768

769

**Table. 5**

770

Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on the occurrence<sup>a</sup> of other fungal

771

metabolites mainly produced by *F. graminearum* and *culmorum*; field experiments conducted in North West Italy in the 2010 - 2013

772

period.

Growin g season	Agronomic strategies <sup>b</sup>	DON-3G		3A-DON		NIV		CULM		ZEA		ZEA-4S		BUT	
		T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>	T	N µg kg <sup>-1</sup>
2010 - 11	common wheat - fungicide	3.0 c	19	< LOQ	< LOQ	< LOQ b	< LOQ	4.0 d	54	< LOQ c	< LOQ	< LOQ b	< LOQ	1.0 b	2
	common wheat - untreated	3.8 b	46	< LOQ	< LOQ	0.4 b	1	4.4 c	84	0.8 b	1	0.2 b	0	1.2 b	3
	durum wheat - fungicide	6.0 a	386	< LOQ	< LOQ	1.1 b	3	6.6 b	749	2.2 a	8	0.7 a	1	4.1 a	62
	durum wheat - untreated	6.0 a	395	< LOQ	< LOQ	2.9 a	21	6.9 a	1038	2.2 a	8	1.0 a	2	3.9 a	50
	<i>P</i> (F) <sup>c</sup> sem <sup>d</sup>	< 0.001 0.13				0.003 1.0		< 0.001 0.23		< 0.001 0.43		< 0.001 0.19		< 0.001 0.94	
2011- 12	common wheat - fungicide	4.0 d	52	< LOQ a	< LOQ	< LOQ b	< LOQ	4.6 c	99	< LOQ b	< LOQ	< LOQ a	< LOQ	0.4 b	1
	common wheat - untreated	5.5 c	264	1.6 a	7	0.5 ab	1	6.3 b	579	0.8 b	2	< LOQ a	< LOQ	1.2 b	5
	durum wheat - fungicide	6.1 b	440	1.4 a	5	0.9 ab	4	6.7 b	791	1.8 ab	8	< LOQ a	< LOQ	3.8 a	45
	durum wheat - untreated	6.9 a	1003	1.0 a	6	2.4 a	11	7.6 a	1988	2.6 a	14	0.5 a	1	4.4 a	84
	<i>P</i> (F) Sem	< 0.001 0.31		0.468 2.0		0.020 1.2		< 0.001 0.42		0.014 1.22		0.058 0.31		< 0.001 1.15	
2012- 13	common wheat - fungicide	3.6 c	38	0.2 b	0	0.6 b	1	5.6 c	275	0.2 c	0	< LOQ b	< LOQ		< LOQ
	common wheat - untreated	4.3 b	75	2.1 a	8	1.2 ab	2	6.3 b	587	0.5 c	1	0.6 b	1		< LOQ
	durum wheat - fungicide	4.8 a	121	2.3 a	9	1.5 a	4	6.7 b	818	1.7 b	5	0.6 b	1		< LOQ
	durum wheat - untreated	5.1 a	158	2.3 a	10	2.0 a	6	7.1 a	1203	3.3 a	28	1.3 a	3		< LOQ
	<i>P</i> (F) Sem	< 0.001 0.31		< 0.001 0.60		0.009 0.6		< 0.001 0.27		< 0.001 0.40		< 0.001 0.20			

773 <sup>a</sup> The reported mycotoxin contamination means are transformed [ $T; y' = \ln(x + 1)$ ] and not transformed (N) values.  
774 DON-3-G = deoxynivalenol-3-glucoside (LOQ = limit of quantification =  $0.2 \mu\text{g kg}^{-1}$ ), 3-ADON = 3-acetyldeoxynivalenol (LOQ =  $0.2 \mu\text{g kg}^{-1}$ ), NIV = nivalenol (LOQ =  $0.2 \mu\text{g kg}^{-1}$ ),  
775 ZEA = zearalenone (LOQ =  $0.2 \mu\text{g kg}^{-1}$ ), ZEA-4-S = zearalenone-4-sulphate (LOQ =  $0.1 \mu\text{g kg}^{-1}$ ), BUT = butenolide (LOQ =  $1.10 \mu\text{g kg}^{-1}$ ).  
776 15-ADON = 15-acetyldeoxynivalenol was also detected, but always under the LOQ ( $0.4 \mu\text{g kg}^{-1}$ ).  
777 <sup>b</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and a high susceptibility to FHB, respectively. The  
778 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC,  $0.250 \text{ kg active ingredient ha}^{-1}$ ).  
779 <sup>c</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
780 replications.  
781 <sup>d</sup> sem: standard error of the means.  
782

783 **Table. 6**

784 Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on the occurrence<sup>a</sup> of aurofusarin (AUR),  
 785 enniatins (ENNs), moniliformin (MON) and chrysogine (CHRY); field experiments conducted in North West Italy in the 2010 - 2013  
 786 period.

Growing Season	Agronomic strategies <sup>b</sup>	AUR		ENNs		MON		CHRY	
		T	N (ppb)	T	N (ppb)	T	N (ppb)	T	N (ppb)
2010 – 2011	common wheat - fungicide	3.6 c	36	2.9 c	18	<LOQ b	<LOQ		<LOQ
	common wheat - untreated	4.4 b	86	4.3 b	72	1.7 b	6		<LOQ
	durum wheat - fungicide	7.8 a	2488	6.2 a	501	4.8 a	117		<LOQ
	durum wheat - untreated	7.9 a	2701	6.4 a	620	4.8 a	117		<LOQ
	<i>P</i> (F) <sup>c</sup> sem <sup>d</sup>	< 0.001 0.47		< 0.001 0.49		< 0.001 0.8			
2011- 2012	common wheat - fungicide	1.8 d	5	3.6 b	41	2.4 a	19		<LOQ
	common wheat - untreated	3.6 c	39	4.3 ab	97	3.1 a	31		<LOQ
	durum wheat - fungicide	5.6 b	270	4.9 ab	147	4.0 a	63		<LOQ
	durum wheat - untreated	6.4 a	616	5.6 a	265	4.7 a	112		<LOQ
	<i>P</i> (F) Sem	< 0.001 0.52		0.019 1.0		0.093 1.6			
2012- 2013	common wheat - fungicide	2.5 c	11	2.4 c	10	<LOQ b	<LOQ	2.2 b	9
	common wheat - untreated	3.0 c	22	3.2 bc	27	1.5 ab	9	3.0 a	22
	durum wheat - fungicide	5.9 b	380	3.6 ab	38	2.9 a	18	1.5 b	4
	durum wheat - untreated	7.4 a	1757	4.5 a	87	3.4 a	31	3.3 a	28
	<i>P</i> (F) Sem	< 0.001 0.51		< 0.001 0.65		0.007 1.3		0.001 0.55	

787

788 <sup>a</sup> The mycotoxin contamination means reported are transformed [ T;  $y' = \ln(x + 1)$ ] and not transformed (N) values.

789 AUR = aurofusarin (LOQ = 0.1 µg kg<sup>-1</sup>), ENNs = Enniatins A, A<sub>1</sub>, B, B<sub>1</sub>, B<sub>2</sub>, (LOQ = 0.01 µg kg<sup>-1</sup>), MON = moniliformin (LOQ = 0.3 µg kg<sup>-1</sup>), CHRY = chrysogine (LOQ = 0.09 µg kg<sup>-1</sup>).  
 790

791 <sup>b</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and a high susceptibility to FHB, respectively. The  
792 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).

793 <sup>c</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
794 replications.

795 <sup>d</sup> sem: standard error of the means.

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798 **Table. 7**799 Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on the occurrence of mycotoxins<sup>a</sup> mainly  
800 produced by *Fusarium* section Liseola; field experiments conducted in North West Italy in the 2010 - 2013 period.

Growing Season	Agronomic strategies <sup>b</sup>	FB		BEA		BIK		FA	
		T	N (ppb)	T	N (ppb)	T	N (ppb)	T	N (ppb)
2010 - 2011	common wheat - fungicide	2.2 bc	8	<LOQ b	<LOQ	0.37 b	0.48	<LOQ b	<LOQ
	common wheat - untreated	1.9 c	6	<LOQ b	<LOQ	0.48 b	0.63	<LOQ b	<LOQ
	durum wheat – fungicide	2.9 a	19	3.7 a	39.8	3.21 a	27.58	4.5 a	100
	durum wheat – untreated	2.6 ab	13	3.3 a	26.4	2.48 a	11.00	4.6 a	97
	<i>P</i> (F) <sup>c</sup> sem <sup>d</sup>	0.002 0.4		< 0.001 0.6		< 0.001 0.4		< 0.001 0.5	
2011- 2012	common wheat - fungicide	2.3 a	9	<LOQ c	<LOQ	<LOQ a	<LOQ	<LOQ a	<LOQ
	common wheat - untreated	1.8 a	6	0.1 bc	0.1	0.11 a	0.11	1.1 a	5
	durum wheat – fungicide	2.8 a	27	0.5 a	0.8	0.54 a	0.77	1.5 a	14
	durum wheat – untreated	2.2 a	9	0.5 ab	0.7	0.50 a	0.68	1.0 a	3
	<i>P</i> (F) Sem	0.46 1.16		0.018 0.3		0.441 0.2		0.768 2.22	
2012- 2013	common wheat - fungicide		<LOQ		<LOQ		<LOQ		<LOQ
	common wheat - untreated		<LOQ		<LOQ		<LOQ		<LOQ
	durum wheat – fungicide		<LOQ		<LOQ		<LOQ		<LOQ
	durum wheat – untreated		<LOQ		<LOQ		<LOQ		<LOQ
	<i>P</i> (F) Sem								

801 <sup>a</sup> The reported mycotoxin contamination means are transformed [ T;  $y' = \ln(x + 1)$ ] and not transformed (N) values.802 FB = fumonisins (LOQ = limit of quantification = 0.70 µg kg<sup>-1</sup>), BEA = beauvericin (LOQ = 0.006 µg kg<sup>-1</sup>), BIK = bikaverin (LOQ = 0.06 µg kg<sup>-1</sup>), FA = fusaric acid (LOQ = 0.70 µg kg<sup>-1</sup>).  
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804 <sup>b</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and a high susceptibility to FHB, respectively. The  
805 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).

806 <sup>c</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
807 replications.

808 <sup>d</sup> sem: standard error of the means.

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811 **Table. 8**

812 Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on the occurrence<sup>a</sup> of decalonectrin (DEC),  
 813 T-2 and HT-2 toxins and equisetin (EQU); field experiments conducted in North West Italy in the 2010 - 2013 period.

Growing season	Agronomic strategies <sup>b</sup>	DEC		T-2 toxin		HT-2 toxin		EQU	
		T	N (ppb)	T	N (ppb)	T	N (ppb)	T	N (ppb)
2010 - 2011	common wheat – fungicide	<LOQ b	<LOQ	<LOQ	<LOQ	<LOQ b	<LOQ	<LOQ b	<LOQ
	common wheat - untreated	<LOQ b	<LOQ	<LOQ	<LOQ	<LOQ b	<LOQ	0.5 b	1.1
	durum wheat – fungicide	<LOQ b	<LOQ	<LOQ	<LOQ	<LOQ b	<LOQ	4.1 a	68.3
	durum wheat – untreated	0.9 a	1.8	<LOQ	<LOQ	1.8 a	8.3	3.2 a	22.8
	<i>P</i> (F) <sup>c</sup> sem <sup>d</sup>	0.053 1.3				0.042 1.1		< 0.001 0.8	
2011- 2012	common wheat – fungicide	<LOQ b	<LOQ	<LOQ a	<LOQ	<LOQ a	<LOQ	0.1 a	0.1
	common wheat - untreated	<LOQ b	<LOQ	<LOQ a	<LOQ	<LOQ a	<LOQ	0.5 a	0.7
	durum wheat – fungicide	0.6 b	1.0	<LOQ a	<LOQ	0.8 a	3.2	0.3 a	0.4
	durum wheat – untreated	1.6 a	4.2	0.3 a	0.3	1.6 a	4.2	0.4 a	0.5
	<i>P</i> (F) Sem	0.001 1.4		0.441 0.24		0.077 1.1		0.406 0.5	
2012- 2013	common wheat – fungicide	0.3 b	0.4		<LOQ		<LOQ	0.1 b	0.1
	common wheat - untreated	0.8 b	1.2		<LOQ		<LOQ	0.2 b	0.2
	durum wheat – fungicide	0.7 b	1.1		<LOQ		<LOQ	0.2 b	0.2
	durum wheat – untreated	1.7 a	4.6		<LOQ		<LOQ	0.4 a	0.5
	<i>P</i> (F) Sem	0.011 2.0						0.040 0.2	

814 <sup>a</sup> The reported mycotoxin contamination means are transformed [ $T; y' = \ln(x + 1)$ ] and not transformed (N) values.

815 DEC = decalonectrin (LOQ, limit of quantification = 0.06 µg/kg), T-2 toxin (LOQ = 0.2 µg/kg); HT-2 toxin (LOQ = 0.2 µg/kg), EQU = equisetin (LOQ = 0.09 µg/kg).

816 <sup>b</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and an high susceptibility to FHB, respectively. The  
 817 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).

818 <sup>c</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
 819 replications.



820 <sup>d</sup> sem: standard error of the means.

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823 **Table. 9**

824 Effect of agronomic strategies with different susceptibility to deoxynivalenol contamination on the occurrence<sup>a</sup> of mycotoxins produced  
 825 by *Alternaria* species; field experiments conducted in North West Italy in the 2010 - 2013 period.

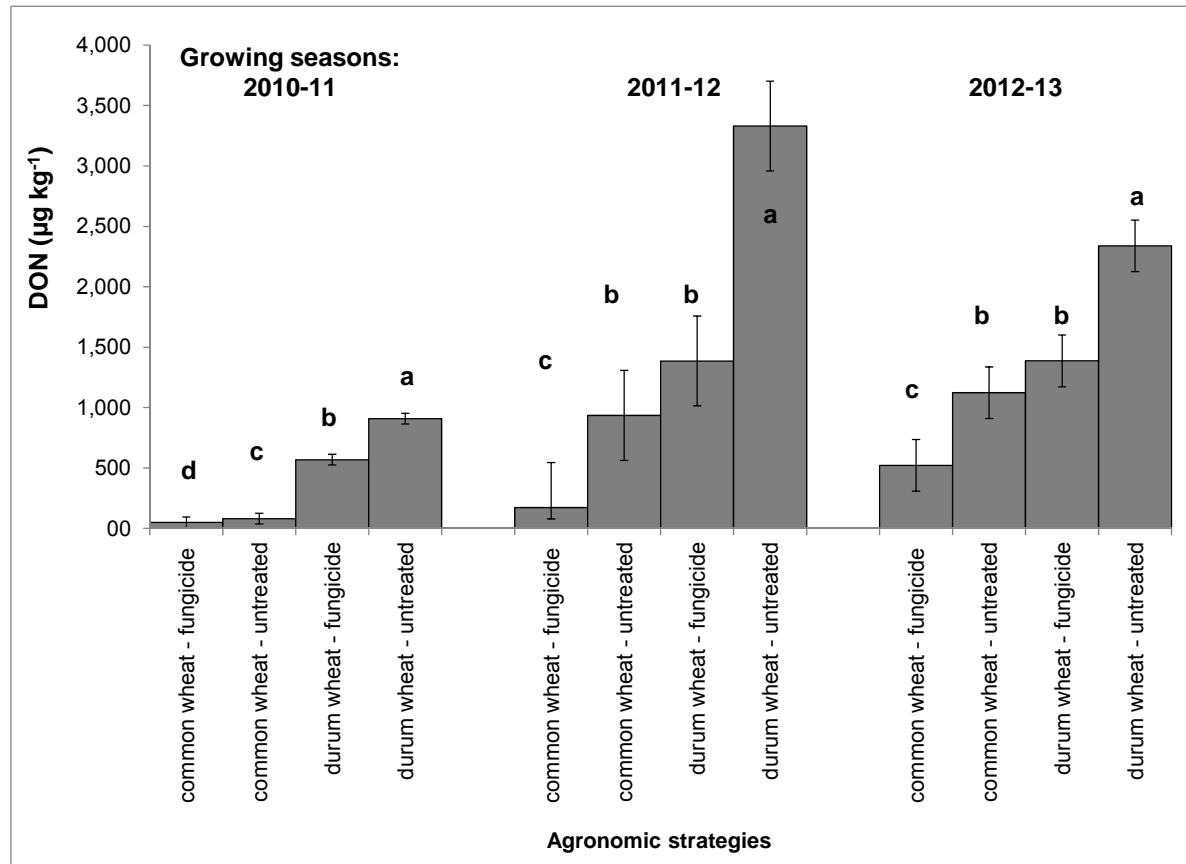
Growing season	Agronomic strategies <sup>b</sup>	AOH		AME		TENT		INF	
		T	N (ppb)	T	N (ppb)	T	N (ppb)	T	N (ppb)
2010 - 2011	common wheat - fungicide	0.9 b	1.5	0.1 c	0.1	0.7 c	0.9		<LOQ
	common wheat - untreated	1.3 b	2.7	0.4 c	0.5	1.1 bc	2.1		<LOQ
	durum wheat - fungicide	2.5 a	11.8	2.1 b	7.2	1.3 ab	2.7		<LOQ
	durum wheat - untreated	3.0 a	19.9	2.9 a	17.5	1.7 a	4.9		<LOQ
	<i>P</i> (F) <sup>c</sup> sem <sup>d</sup>	< 0.001 0.4		< 0.001 0.4		0.004 0.4			
2011- 2012	common wheat - fungicide	<LOQ b	<LOQ	<LOQ a	<LOQ	0.9 c	1.5		<LOQ
	common wheat - untreated	0.2 b	0.3	<LOQ a	<LOQ	1.5 b	3.5		<LOQ
	durum wheat - fungicide	0.9 ab	2.1	0.9 a	3.5	2.4 a	10.2		<LOQ
	durum wheat - untreated	1.4 a	2.9	1.3 a	3.1	2.8 a	15.0		<LOQ
	<i>P</i> (F) sem	0.026 0.7		0.139 1.1		< 0.001 0.4			
2012- 2013	common wheat - fungicide	<LOQ b	<LOQ	<LOQ b	<LOQ	0.6 b	1.0	3.6 b	42
	common wheat - untreated	<LOQ b	<LOQ	<LOQ b	<LOQ	1.0 b	1.8	3.7 b	39
	durum wheat - fungicide	2.0 a	6.3	0.9 a	1.6	1.6 a	3.8	4.9 a	127
	durum wheat - untreated	2.3 a	10.4	0.8 a	1.2	2.0 a	6.6	4.3 ab	73
	<i>P</i> (F) sem	< 0.001 0.4		< 0.001 0.2		< 0.001 0.4		0.011 0.60	

826 <sup>a</sup> The reported mycotoxin contamination means are transformed [ $T; y' = \ln(x + 1)$ ] and not transformed (N) values.

827 AOH = alternariol (LOQ = limit of quantification = 0.10 µg kg<sup>-1</sup>), AME = alternariol methyl ether (LOQ = 0.10 µg kg<sup>-1</sup>), TENT = tentoxin (LOQ = 0.10 µg kg<sup>-1</sup>), INF = infectopyrone  
 828 (LOQ = 0.10 µg kg<sup>-1</sup>).

829 <sup>b</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and an high susceptibility to FHB, respectively. The  
830 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).  
831 <sup>c</sup> Means followed by different letters within each growing season are significantly different (the level of significance is shown in the table). The reported values are based on 3  
832 replications.  
833 <sup>d</sup> sem: standard error of the means.  
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835 **Figure 1.** Effect of agronomic strategies<sup>a</sup> on deoxynivalenol (DON) contamination; field experiments conducted in North West Italy in  
 836 the 2010 - 2013 period.



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 838 Means followed by different letters within each growing season are significantly different ( $P < 0.05$ ). The reported values are based on 3 replications.  
 839 The bars report the standard error of the means for each growing season.

840 <sup>a</sup> The used cultivars were Generale (common wheat) and Saragolla (durum wheat), which are characterized by a medium and a high susceptibility to FHB, respectively. The  
 841 fungicide treatment at heading was carried out using the prothioconazole active ingredient (formulation: EC, 0.250 kg active ingredient ha<sup>-1</sup>).

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