Influence of cropping system on Fusarium head blight and mycotoxin levels in winter wheat

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3   **Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter**

4   **wheat**

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14   **Abstract**

15   In this study, we investigated the effects of various wheat cropping systems on *Fusarium* head

16   blight severity and mycotoxin (deoxynivalenol, nivalenol, zearalenone) levels. We

17   investigated four wheat-cropping systems: a conventional system, an integrated system, a

18   direct drilling system and an organic system, in fields with natural contamination. The study

19   was carried out over three one-year periods: 1999-2000, 2000-2001 and 2001-2002. The

20   severity of *Fusarium* head blight and toxin levels differed considerably between years,

21   reflecting climatic effects. In a given year, the severity of *Fusarium* head blight also differed

22   between cropping systems, with the most severe disease observed in the direct drilling and

23   conventional system plots in 2000. The level of grain contamination by mycotoxins therefore

24   depends on both climate and cropping system. Mycotoxin levels were highest in the year with

25   the highest disease severity: 2000. Contamination levels were highest with the direct drilling

26   system. We were unable to rank the organic and conventional systems, because neither was

27   consistently more contaminated than the other. Moreover, no clear relationship was found

28   between disease severity and levels of contamination with deoxynivalenol, nivalenol or

29   zearalenone under conditions of natural contamination.

30

31   **Key words**

32   Cropping systems, mycotoxins, deoxynivalenol (DON), *Fusarium, Fusarium* head blight,

33   wheat

34

Introduction

In recent years, the food industry has been faced with several major health crises. This has led to an increase in public concern about food safety. Several mycotoxins cause major safety problems in cereal production (Le Boulc'h *et al.*, 2000). The ingestion of mycotoxins may result in vomiting, reproductive disturbances, leukoencephalomalacia, pulmonary oedema, impairment of the humoral and cellular immune responses, nervous disorders, myocardial hypertrophy and several cancers (Placinta *et al.*, 1999). Some of these toxins are produced before harvest (trichothecenes, fumonisins and zearalenone), whereas others are produced after harvest (ochratoxin A and aflatoxins). Several species of *Fusarium* are responsible for toxin production before harvest (Maurin and Chenet, 1993). These fungi, together with *Microdochium,* are responsible for *Fusarium* head blight, or scab (Cassini, 1970; Ban, 2000). As the deleterious effects of this disease include yield losses of up to 50% (Atanasoff, 1920; Parry *et al.*, 1995) and the production of various mycotoxins: zearalenone, deoxynivalenol and derivatives, nivalenol, T_2-toxin, HT_2-toxin and other toxins (Placinta *et al.*, 1999). Climate is thought to be a key determinant of *Fusarium* head blight (Langseth *et al.*, 1995; Lacey *et al.*, 1999). However, in a given year, neighbouring fields may display different levels of contamination: the cropping system may therefore affect the level of mycotoxin production by *Fusarium.* The use of fungicides during growth of the wheat crop does not guarantee the absence of toxins in the harvested grain. The entire cropping system must be considered. Several studies have demonstrated effects of soil tillage (Teich and Hamilton, 1985; Dill-Macky and Jones, 2000), preceding crop (Teich and Nelson, 1984; Dill-Macky and Jones, 2000) and wheat genotype (Mesterhazy, 1983; Teich and Hamilton, 1985; Snijders, 1990; Dill-Macky and Jones, 2000) on scab development and/or mycotoxin contamination. However, few studies have analysed both disease severity and mycotoxin contamination. There is also a lack of studies comparing the effects of entire cropping systems rather than aspects of crop management. The aim of this study was to investigate the effects of four cropping systems (conventional, integrated, direct drilling and organic systems) on *Fusarium* head blight severity and on levels of several mycotoxins in grains. We also tried to correlate both disease severity and levels of contamination with deoxynivalenol, nivalenol and zearalenone levels under conditions of natural contamination.

Materials and methods

Experimental design

69   Since 1997, the four cropping systems (conventional, integrated, direct drilling and organic)

70   were compared using "crop X – wheat – crop Y – wheat" crop rotations. We assigned two

71   large plots (0.5 ha each) to each cropping system. Each plot was divided in half. On each half

72   (subplot), we organised the rotation such that there was one wheat crop every year on each

73   plot. The conventional system corresponded to intensive agriculture, in which the aim is to

74   achieve the highest net income by maximising yield. The integrated system, in which input

75   levels (and yields) were lower, aimed to maximise net income whilst preserving the

76   environment. In the direct drilling system, crops had been planted without soil tillage and

77   permanent vegetation cover (fescue) had been maintained since 1998-1999. We evaluated this

78   system as an alternative to the integrated system, aiming to achieve the same objectives. No

79   chemical inputs were used in the organic system. Each cropping system was defined in terms

80   of a specific combination of technical choices, each closely related to the others. For example,

81   the choice of cultivar depended on whether a fungicide was to be used. Thus, the cropping

82   systems differed in several aspects: cultivar, tillage, chemical treatments, nitrogen

83   fertilisation, time and density of sowing, and crop sequence. Furthermore, the techniques used

84   within a given cropping system were prone to change from year to year, and depended on

85   climate in particular. As part of a larger study, we compared the agronomic, environmental

86   and economic aspects of the various systems (results not shown). We began to study

87   *Fusarium* head blight in these experimental plots in 1999/2000. We set up additional

88   treatments in each system to enable us to increase our understanding of the determinants of

89   pathogen attack: the Charger cultivar (the cultivar used in the conventional system) was

90   cropped without fungicide in a subplot of each system.

91

92   Over the three-year study period, the preceding crop and fungicide treatment changed each

93   year for each system. In 1999-2000, the fungicide treatments used contained strobularins,

94   whereas in 2000-2001 and 2001-2002, a mixture of strobularins and triazoles was used. Each

95   system remained internally consistent from year to year and the technical details of each

96   system are presented in Table 1. In 2000-2001, sowing conditions were extremely difficult for

97   the direct drilling system due to large amounts of crop residue on the soil surface and high

98   moisture levels in the seedbed (high rainfall during autumn). This led to poor emergence of

99   the wheat crop in this system. We therefore took no samples from the direct drilling system in

100   2000-2001.

101

102   ***Evaluation of kernel infection by head blight***

3

For each cropping system (and each subplot), we used four randomly selected samples of 25 heads to determine disease severity, every week between flowering and physiological maturity of the grain. Disease severity was estimated by determining the percentage of spikelets per head for which at least one third of the external surface of glumes presented symptoms of head blight.

Mycotoxin extraction and analysis

We determined the mycotoxin content of grains from plants sampled at harvest. Plant sampling was based on the 98/53/CE directive, which lays down the sampling procedure for official controls of aflatoxin level. We used this directive as there is no similar text dealing with *Fusarium* toxins. According to this directive, for plots with yields lower than 1 t, 10 samples of 100 g must be harvested and pooled to give a total sample of 1 kg. We collected 1 kg grain samples in this manner from each plot. Samples were dried (48h - 80°C) and sent to Qualtech (Vandoeuvre-les-Nancy, France) for testing. In the first two years, we determined the levels of the following mycotoxins: zearalenone, group A and B trichothecenes (diacetoxyscirpenol (DAS), T-2, HT-2, nivalenol (NIV), deoxynivalenol (DON) and derivatives (3-aDON and 15-aDON), fusarenone (FUX), and fumonisins B1 and B2. In the final year, we determined levels of zearalenone, group A and B trichothecenes (diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), T-2, HT-2, neosolaniol, T2-triol, nivalenol (NIV), deoxynivalenol (DON) and derivatives (3-aDON and 15-aDON), and fusarenone (FUX). Zearalenone levels were determined with an immunoaffinity high-performance liquid chromatography (HPLC) method, validated by the French norm NF V03-110. Fumonisines were determined by HPLC, and trichothecenes by gas chromatography-mass spectroscopy (GC-MS), validated by the French norm NF EN ISO/CEI 17025.

Statistical methods

We used the classic ANOVA (one-way analysis of variance) program of the SAS software suite to compare the systems. If a significant F ratio was obtained ($\alpha < 0.05$), we analysed differences between treatments, using Bonferroni's multiple range test. In this study, there were interactions between year and climate and between year and changes in the cropping system. We therefore chose not to analyse the year effect directly. We focused instead on cropping system effects. ANOVA was carried out with all the individual measurements from each replicate.

138 **Results**

139 *Disease severity*

140 The severity of *Fusarium* head blight differed considerably from year to year (Table 2). In

141 2000, severe *Fusarium* head blight was observed, whereas no symptoms were detected in

142 2001. Disease severity differed significantly between the four cropping systems (Table 2-a) in

143 2000. No difference between the cropping systems was observed in 2001, and in 2002 slight

144 differences were observed between cropping systems but the range of severity observed was

145 limited. In 2000, the direct drilling system displayed the highest level of contamination,

146 followed by the conventional and organic systems. In 2002, the conventional system was the

147 most heavily contaminated. These rankings, in particular that for 2002, which was less clear-

148 cut, were confirmed by analysis of variance: cropping system was found to have a significant

149 effect on disease severity ($\alpha < 0.001$).

150

151 The pattern of disease severity observed in the subsystems was similar to that for the systems

152 as a whole (Table 2-b): contamination was greatest in 2000, no symptoms were observed in

153 2001, and 2002 was intermediate. Analysis of the results for 2000 showed that in two systems

154 (conventional and integrated), the subsystem (conventional cultivar used without fungicide)

155 crop was more heavily infected that the adapted system (appropriate cultivar for the system)

156 crop. In contrast, the subsystem and adapted plots were similar for the direct drilling and

157 organic systems. The direct drilling subsystem displayed the highest level of disease severity.

158 Thus, the factors responsible for this high level of disease were presumably not cultivar or

159 fungicide strategy. In contrast, in the other three systems, disease severity seemed to depend

160 on the cultivar and/or fungicide treatment used. In 2001 and 2002, the adapted cropping

161 systems and the subsystems presented similar levels of disease. A plot effect was detected for

162 the subsystem treatment, with plot I more heavily contaminated than plot II. This effect was

163 significant ($\alpha \leq 0.001$) in 2000.

164

165 *The accumulation of mycotoxins in grain*

166 Very little deoxynivalenol (DON) was accumulated in the harvested grain (Table 3-a). The

167 DON content was below the maximum levels (750 µg/kg) cited in provisional European

168 recommendations, for most treatments. The highest levels of accumulation of this toxin were

169 recorded in 2000. In addition to this year effect, a cropping system effect was observed: the

170 direct drilling system (in 2000 and 2002) was systematically and significantly the most

171   heavily contaminated with this toxin. In 2001, when no samples were available for the direct

172   drilling system, the organic system showed significantly higher levels of DON contamination

173   than the other systems.

174

175   The results obtained for the subsystems were very similar (Table 3-b), with the same year and

176   cropping system effects identified. However, the effects of not applying fungicide differed

177   according to the year. DON contamination of the conventional system plots was lower in the

178   absence than in the presence of fungicide in 2000 and 2001, and higher in 2002. Conversely,

179   the Charger cultivar systematically displayed higher levels of contamination than the cultivar

180   used in the organic system. These factors had different effects on mycotoxin contamination.

181   The integrated and direct drilling systems (for which fungicide protection and cultivar varied

182   simultaneously) displayed higher levels of mycotoxin contamination for the subsystems than

183   for the adapted system, with the exception of the direct drilling system in 2000. However, the

184   choice of cultivar and the decision not to treat had a relatively small effect in this system in

185   this year because DON levels consistently exceeded 4000 µg/kg (which is much higher than

186   the maximum level recommended). No significant plot effect was identified.

187

188   The fields were also contaminated with nivalenol and zearalenone. Nivalenol contamination

189   differed significantly ($\alpha \leq 0.05$) between cropping systems in 2001. In all the years studied,

190   nivalenol was detected only in the organic and direct drilling systems. Zearalenone

191   contamination differed considerably from year to year. Zearalenone levels were highest in

192   2000, with the direct drilling system displaying significantly higher levels of contamination

193   than the other systems ($\alpha \leq 0.05$) in that year. In 2001, only one field (the organic subsystem)

194   had zearalenone contamination. Zearalenone was not detected in 2002. We detected no other

195   mycotoxins.

196

197   ***The relationship between disease severity and deoxynivalenol accumulation***

198   No relationship between disease severity and mycotoxin levels was observed, between or

199   within years (Figure 1). For example, disease severity was scored at 0% despite DON levels

200   of 0-600 µg/kg (years 2001 and 2002). Furthermore, DON levels were low (350 µg/kg) on

201   plots in which disease severity score was higher (22%).

202

203   The lack of a relationship between *Fusarium* head blight severity and mycotoxin content was

204   even more evident for the subsystems. No disease was observed despite DON levels of 0-

205   1120 µg/kg (years 2001 and 2002) whereas moderate DON contamination (530 µg/kg) was

206   recorded for plots with high levels of disease severity (63%). We also found that there was no

207   relationship between disease severity and mycotoxin levels for nivalenol and zearalenone.

208

209   **Discussion**

210   Our results demonstrate that *Fusarium* head blight severity depends mainly on climatic

211   effects. Similar results have been obtained in previous studies (Andersen, 1948; Langseth *et*

212   *al.*, 1995). However, in years with average or high levels of disease, cropping system affects

213   disease severity. The direct drilling system resulted in the highest disease severity in both

214   years for which results were available. In 2000, the direct drilling system wheat crop followed

215   a maize crop whereas the wheat crop of the integrated system followed oilseed rape. In 2002,

216   the preceding crop was pea in both cases. However, the major difference between the direct

217   drilling and integrated systems was the absence of tillage in the direct drilling system:

218   comparison of these two systems, in 2002, can therefore be used to determine the effect of

219   ploughing in residues. Our results seem to support previous conclusions concerning the

220   effects of land preparation (Teich and Nelson, 1984; Teich and Hamilton, 1985; Dill-Macky

221   and Jones, 2000).

222

223   An analysis of agronomic traits (head density, yield, grain protein content, *Septoria* leaf spot

224   severity, eyespot severity) revealed no clear reason for the plot effect observed for disease

225   severity in 2000. Follow-up of scab severity between flowering and harvest provided a

226   potential clue: disease severity rapidly increased between June 27 and July 3 in plot I, but not

227   in plot II. This increase in disease severity was probably due to heavy rainfall (21 mm) on

228   July 2nd, after 20 days without precipitation. The experimental field was surrounded by trees

229   and plot II was more protected from rain than plot I, which may have resulted in an edge

230   effect.

231

232   This work shows that the *Fusarium* mycotoxin (deoxynivalenol, nivalenol and zearalenone)

233   content of wheat crops depends on both year and cropping system. These results are

234   consistent with those of previous studies (Teich and Hamilton, 1985; Dill-Macky and Jones,

235   2000): the direct drilling system generally results in the highest levels of contamination,

236   particularly in years with high levels of disease, such as 2000. Moreover, in 2000, the

237   presence of maize immediately preceding the wheat crop in the rotation of the direct drilling

238   system seemed to increase the production of mycotoxins (Teich and Hamilton, 1985; Dill-

7

239 Macky and Jones, 2000). It was not possible to rank the other systems because no consistent

240 pattern emerged. In 2000 and 2002, the conventional system was more contaminated than the

241 organic system, whereas the reverse was true for 2001. Higher levels of contamination in the

242 conventional system may result from disease severity being high in that year (which was the

243 case in 2000) or may be due to the fungicide used. Indeed in 2000, we used a mixture of

244 strobularins whereas in 2001 and 2002, we used a mixture of strobularins and triazoles.

245 Strobularins belong to a family of fungicide molecules that are particularly effective against

246 *Microdochium* strains but some *Fusarium* strains are limited effectively only by triazole

247 molecules (Maumené *et al.*, 2000). As *Microdochium* produces no toxins, the application of

248 strobularins does not limit mycotoxin levels. Conversely, it might favour the development of

249 pathogenic strains that produce toxins. Thus, the fungicide treatment used in 2000 might have

250 favoured an increase in mycotoxin levels in the adapted conventional system whereas

251 competition between *Fusarium* and *Microdochium* strains may have limited the production of

252 mycotoxins in the organic systems and in the conventional subsystem. In 2000, fungicide

253 application might have resulted in an increase in disease severity in the adapted conventional

254 system, whereas saprophytes might have limited disease development in the adapted organic

255 system. In contrast, in 2002, the absence of fungicide treatment resulted in higher levels of

256 disease severity. In this case, the molecule used was probably more suitable for the infecting

257 pathogenic strains.

258

259 The "contaminating complex" effect, operating under natural contamination conditions is

260 probably responsible of the absence of a relationship between disease severity and DON

261 levels. Our results, which are consistent with those of other authors (Liu *et al.*, 1997), are

262 especially important as they relate to moderate or low disease levels, which may conceal

263 significant contamination by mycotoxins. Indeed, in contrast to studies in which crops were

264 artificially contaminated with a single pathogenic strain of *Fusarium culmorum,* in which

265 such a relationship was found (Teich and Hamilton, 1985; Snijders and Perkowski, 1990),

266 several species of *Fusarium* and *Microdochium* are present in the field in natural

267 contamination conditions. Hence, DON levels, disease severity and the relationship between

268 these variables probably depend on the traits of the species present -capacity to produce

269 disease, capacity to produce mycotoxins (deoxynivalenol, nivalenol, zearalenone), capacity to

270 produce symptoms and mycotoxins- together with the time at which contamination occurs,

271 the climatic conditions between the time of contamination and harvest, and the nutritive

272   resources that the plant can provide to the pathogenic strains: in other words, the whole

273   cropping system.

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335

336 Table 1a: Principal traits of the four adapted cropping systems over the three years. Mixture 1:

337 Somme, Malacca, Renan, Camp Remy; Mixture 2: Somme, Malacca, Virtuose, Apache;

338 Mixture 3: Charger, Virtuose, Renan, Paindor.

339

340 The organic system received no nitrogen fertilisation in 1999-2000 and 2000-2001.

341 In the 2000-2001 study period, the wheat crop in the organic system was sown in January

342 2001. We planned to sow this crop in November, but incessant rain between October 2000

343 and January 2001 rendered this impossible.

344

345 Table 1b: Principal traits of the four cropping subsystems over the three years. No fungicide

346 treatment, cultivar Charger.

347

348 The organic system received no nitrogen fertilisation in 1999-2000 and 2000-2001.

349 In the 2000-2001 study period, the wheat crop in the organic system was sown in January

350 2001. We planned to sow this crop in November, but incessant rain between October 2000

351 and January 2001 rendered this impossible.

352

353 Table 2: Comparison of disease severity in the four cropping systems during three years.

354 Charger is the cultivar used in the adapted conventional system.

355

356 Table 3: Comparison of deoxynivalenol accumulation (µg/kg) for four cropping systems over

357 three years. nd: not detected (30 µg/kg is the detection limit).

358

359 Figure 1: Relationship between disease severity and deoxynivalenol accumulation in four

360 cropping systems over three years. C conventional, I integrated, DD direct drilling, O organic.

361 00 2000, 01 2001, 02 2002 (♦) Adapted cropping system, (□) Subsystem: cultivar Charger

362 without fungicide

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364

365

Table 1(a)

Adapted system	Sowing time	Flowering time	Harvest time	Nitrogen (kg/ha)	Seed rate (seeds/m²)	Fungicide treatment	Cultivar	Previous crop
Conventional	11/10/99	23/05/00		247	260		Charger	Oilseed Rape
Integrated				197	165	Strobularins (azoxystrobin)		
Direct drilling	27/10/99	25/05/00	03/08/00	197	220		Mixture 1	Maize with fescue
Organic	10/11/99	30/05/00		0	450	None		Oilseed Rape
Conventional	04/10/00	28/05/01		249	260	Mixture of strobularins (azoxystrobin), triazoles (tebuconazole), prochloraze, cyprodinil	Charger	Oilseed Rape
Integrated	24/10/00		30/07/01	201	165	Strobularins (azoxystrobin), cyprodinil	Mixture 1	
Direct drilling	25/10/00	01/06/01		167	220			Maize with fescue
Organic	17/01/01	27/06/01		0	450	None	Mixture 3	Spring lupin
Conventional	05/10/01	06/06/02	29/07/02	208	260	Mixture of strobularins (azoxystrobin), triazoles (fluquinconazole, epoxinazole), prochloraze	Charger	Pea
Integrated	17/10/01	23/05/02		131	165	Mixture of strobularins (azoxystrobin), triazoles (epoxinazole)	Mixture 2	
Direct drilling	26/10/01	25/05/02	30/07/02	141	220			
Organic	31/10/01	23/05/02		54	450	None	Apache	

367

368 Table 1(b)

Subsystem	Sowing time	Flowering time	Harvest time	Nitrogen (kg/ha)	Seed rate (seeds/m²)	Fungicide treatment	Cultivar	Previous crop
Conventional	11/10/99	23/05/00		247	260			Oilseed Rape
Integrated				197	165			
Direct drilling	27/10/99	25/05/00	03/08/00	197	220	None	Charger	Maize with fescue
Organic	10/11/99	30/05/00		0	450			Oilseed Rape
Conventional	04/10/00	28/05/01		249	260			Oilseed Rape
Integrated	24/10/00	01/06/01	30/07/01	201	165	None	Charger	
Organic	17/01/01	27/06/01		0	450			Spring lupin
Conventional	05/10/01	06/06/02	29/07/02	208	260			
Integrated	17/10/01	06/06/02		131	165			
Direct drilling	26/10/01	06/06/02	30/07/02	141	220	None	Charger	Pea
Organic	31/10/01	06/06/02		54	450			

369

370

371 Table 2

Cropping systems	Plots	(a) Adapted cropping system			(b) Subsystem: Charger without fungicide		
		2000	2001	2002	2000	2001	2002
Conventional	I	24%	0%	3%	63%	0%	4%
	II	23%	0%	1%	22%	0%	1%
Integrated	I	6%	0%	0%	28%	0%	2%
	II	8%	0%	0%	17%	0%	2%
Direct Drilling	I	34%	.	1%	47%	.	5%
	II	38%	.	0%	23%	.	3%
Organic	I	5%	0%	0%	2%	0%	5%
	II	22%	0%	0%	1%	0%	3%

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373   Table 3

Cropping systems	Plots	(a) Adapted cropping system			(b) Subsystem: Charger without fungicide		
		2000	2001	2002	2000	2001	2002
Conventional	I	1230	120	150	490	nd	310
	II	860	nd	380	530	60	340
Integrated	I	430	nd	nd	530	nd	310
	II	270	nd	280	320	160	340
Direct Drilling	I	7420	nd	400	4230	.	800
	II	9100	nd	600	8220	.	1100
Organic	I	230	430	100	370	1120	600
	II	350	370	110	670	1020	600

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375   Figure 1
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