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2
3 **Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter**
4 **wheat**

5
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13
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

35 **Introduction**

36 In recent years, the food industry has been faced with several major health crises. This has led
37 to an increase in public concern about food safety. Several mycotoxins cause major safety
38 problems in cereal production (Le Boulc'h *et al.*, 2000). The ingestion of mycotoxins may
39 result in vomiting, reproductive disturbances, leukoencephalomalacia, pulmonary oedema,
40 impairment of the humoral and cellular immune responses, nervous disorders, myocardial
41 hypertrophy and several cancers (Placinta *et al.*, 1999). Some of these toxins are produced
42 before harvest (trichothecenes, fumonisins and zearalenone), whereas others are produced
43 after harvest (ochratoxin A and aflatoxins). Several species of *Fusarium* are responsible for
44 toxin production before harvest (Maurin and Chenet, 1993). These fungi, together with
45 *Microdochium*, are responsible for *Fusarium* head blight, or scab (Cassini, 1970; Ban, 2000).
46 As the deleterious effects of this disease include yield losses of up to 50% (Atanasoff, 1920;
47 Parry *et al.*, 1995) and the production of various mycotoxins: zearalenone, deoxynivalenol
48 and derivatives, nivalenol, T₂-toxin, HT₂-toxin and other toxins (Placinta *et al.*, 1999).
49 Climate is thought to be a key determinant of *Fusarium* head blight (Langseth *et al.*, 1995;
50 Lacey *et al.*, 1999). However, in a given year, neighbouring fields may display different
51 levels of contamination: the cropping system may therefore affect the level of mycotoxin
52 production by *Fusarium*. The use of fungicides during growth of the wheat crop does not
53 guarantee the absence of toxins in the harvested grain. The entire cropping system must be
54 considered. Several studies have demonstrated effects of soil tillage (Teich and Hamilton,
55 1985; Dill-Macky and Jones, 2000), preceding crop (Teich and Nelson, 1984; Dill-Macky and
56 Jones, 2000) and wheat genotype (Mesterhazy, 1983; Teich and Hamilton, 1985; Snijders,
57 1990; Dill-Macky and Jones, 2000) on scab development and/or mycotoxin contamination.
58 However, few studies have analysed both disease severity and mycotoxin contamination.
59 There is also a lack of studies comparing the effects of entire cropping systems rather than
60 aspects of crop management. The aim of this study was to investigate the effects of four
61 cropping systems (conventional, integrated, direct drilling and organic systems) on *Fusarium*
62 head blight severity and on levels of several mycotoxins in grains. We also tried to correlate
63 both disease severity and levels of contamination with deoxynivalenol, nivalenol and
64 zearalenone levels under conditions of natural contamination.

65

66

67 **Materials and methods**

68 *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***

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

108

109 *Mycotoxin extraction and analysis*

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

127

128 *Statistical methods*

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

136

137

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 than 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 $\mu\text{g}/\text{kg}$) 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-

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.

274

275

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281

282

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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, Painsdor.

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 ($\mu\text{g}/\text{kg}$) for four cropping systems over
357 three years. nd: not detected ($30 \mu\text{g}/\text{kg}$ 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

363

364

365

366 Table 1(a)

Adapted system	Sowing time	Flowering time	Harvest time	Nitrogen (kg/ha)	Seed rate (seeds/m ²)	Fungicide treatment	Cultivar	Previous crop
Conventional	11/10/99	23/05/00		247	260		Charger	Oilseed
Integrated				197	165			Rape
Direct drilling	27/10/99	25/05/00	03/08/00	197	220	Strobularins (azoxystrobin)	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			
Direct drilling	25/10/00	01/06/01		167	220	Strobularins (azoxystrobin), cyprodinil	Mixture 1	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			
Direct drilling	26/10/01	25/05/02	30/07/02	141	220	Mixture of strobularins (azoxystrobin), triazoles (epoxinazole)	Mixture 2	
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/m ²)	Fungicide treatment	Cultivar	Previous crop
Conventional	11/10/99	23/05/00		247	260			Oilseed
Integrated				197	165			Rape
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
Integrated	24/10/00	01/06/01	30/07/01	201	165	None	Charger	Rape
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			

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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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