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Abstract

In this study, we investigated the effects of various wheat cropping systems on *Fusarium* head blight severity and mycotoxin (deoxynivalenol, nivalenol, zearalenone) levels. We investigated four wheat-cropping systems: a conventional system, an integrated system, a direct drilling system and an organic system, in fields with natural contamination. The study was carried out over three one-year periods: 1999-2000, 2000-2001 and 2001-2002. The severity of *Fusarium* head blight and toxin levels differed considerably between years, reflecting climatic effects. In a given year, the severity of *Fusarium* head blight also differed between cropping systems, with the most severe disease observed in the direct drilling and conventional system plots in 2000. The level of grain contamination by mycotoxins therefore depends on both climate and cropping system. Mycotoxin levels were highest in the year with the highest disease severity: 2000. Contamination levels were highest with the direct drilling system. We were unable to rank the organic and conventional systems, because neither was consistently more contaminated than the other. Moreover, no clear relationship was found between disease severity and levels of contamination with deoxynivalenol, nivalenol or zearalenone under conditions of natural contamination.

Key words

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

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

Since 1997, the four cropping systems (conventional, integrated, direct drilling and organic) were compared using "crop X – wheat – crop Y – wheat" crop rotations. We assigned two large plots (0.5 ha each) to each cropping system. Each plot was divided in half. On each half (subplot), we organised the rotation such that there was one wheat crop every year on each plot. The conventional system corresponded to intensive agriculture, in which the aim is to achieve the highest net income by maximising yield. The integrated system, in which input levels (and yields) were lower, aimed to maximise net income whilst preserving the environment. In the direct drilling system, crops had been planted without soil tillage and permanent vegetation cover (fescue) had been maintained since 1998-1999. We evaluated this system as an alternative to the integrated system, aiming to achieve the same objectives. No chemical inputs were used in the organic system. Each cropping system was defined in terms of a specific combination of technical choices, each closely related to the others. For example, the choice of cultivar depended on whether a fungicide was to be used. Thus, the cropping systems differed in several aspects: cultivar, tillage, chemical treatments, nitrogen fertilisation, time and density of sowing, and crop sequence. Furthermore, the techniques used within a given cropping system were prone to change from year to year, and depended on climate in particular. As part of a larger study, we compared the agronomic, environmental and economic aspects of the various systems (results not shown). We began to study *Fusarium* head blight in these experimental plots in 1999/2000. We set up additional treatments in each system to enable us to increase our understanding of the determinants of pathogen attack: the Charger cultivar (the cultivar used in the conventional system) was cropped without fungicide in a subplot of each system.

Over the three-year study period, the preceding crop and fungicide treatment changed each year for each system. In 1999-2000, the fungicide treatments used contained strobularins, whereas in 2000-2001 and 2001-2002, a mixture of strobularins and triazoles was used. Each system remained internally consistent from year to year and the technical details of each system are presented in Table 1. In 2000-2001, sowing conditions were extremely difficult for the direct drilling system due to large amounts of crop residue on the soil surface and high moisture levels in the seedbed (high rainfall during autumn). This led to poor emergence of the wheat crop in this system. We therefore took no samples from the direct drilling system in 2000-2001.

Evaluation of kernel infection by head blight

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.

Results

Disease severity

The severity of *Fusarium* head blight differed considerably from year to year (Table 2). In 2000, severe *Fusarium* head blight was observed, whereas no symptoms were detected in 2001. Disease severity differed significantly between the four cropping systems (Table 2-a) in 2000. No difference between the cropping systems was observed in 2001, and in 2002 slight differences were observed between cropping systems but the range of severity observed was limited. In 2000, the direct drilling system displayed the highest level of contamination, followed by the conventional and organic systems. In 2002, the conventional system was the most heavily contaminated. These rankings, in particular that for 2002, which was less clear-cut, were confirmed by analysis of variance: cropping system was found to have a significant effect on disease severity ($\alpha < 0.001$).

The pattern of disease severity observed in the subsystems was similar to that for the systems as a whole (Table 2-b): contamination was greatest in 2000, no symptoms were observed in 2001, and 2002 was intermediate. Analysis of the results for 2000 showed that in two systems (conventional and integrated), the subsystem (conventional cultivar used without fungicide) crop was more heavily infected that the adapted system (appropriate cultivar for the system) crop. In contrast, the subsystem and adapted plots were similar for the direct drilling and organic systems. The direct drilling subsystem displayed the highest level of disease severity. Thus, the factors responsible for this high level of disease were presumably not cultivar or fungicide strategy. In contrast, in the other three systems, disease severity seemed to depend on the cultivar and/or fungicide treatment used. In 2001 and 2002, the adapted cropping systems and the subsystems presented similar levels of disease. A plot effect was detected for the subsystem treatment, with plot I more heavily contaminated than plot II. This effect was significant ($\alpha \leq 0.001$) in 2000.

The accumulation of mycotoxins in grain

Very little deoxynivalenol (DON) was accumulated in the harvested grain (Table 3-a). The DON content was below the maximum levels (750 µg/kg) cited in provisional European recommendations, for most treatments. The highest levels of accumulation of this toxin were recorded in 2000. In addition to this year effect, a cropping system effect was observed: the direct drilling system (in 2000 and 2002) was systematically and significantly the most

heavily contaminated with this toxin. In 2001, when no samples were available for the direct drilling system, the organic system showed significantly higher levels of DON contamination than the other systems.

The results obtained for the subsystems were very similar (Table 3-b), with the same year and cropping system effects identified. However, the effects of not applying fungicide differed according to the year. DON contamination of the conventional system plots was lower in the absence than in the presence of fungicide in 2000 and 2001, and higher in 2002. Conversely, the Charger cultivar systematically displayed higher levels of contamination than the cultivar used in the organic system. These factors had different effects on mycotoxin contamination. The integrated and direct drilling systems (for which fungicide protection and cultivar varied simultaneously) displayed higher levels of mycotoxin contamination for the subsystems than for the adapted system, with the exception of the direct drilling system in 2000. However, the choice of cultivar and the decision not to treat had a relatively small effect in this system in this year because DON levels consistently exceeded 4000 µg/kg (which is much higher than the maximum level recommended). No significant plot effect was identified.

The fields were also contaminated with nivalenol and zearalenone. Nivalenol contamination differed significantly ($\alpha \leq 0.05$) between cropping systems in 2001. In all the years studied, nivalenol was detected only in the organic and direct drilling systems. Zearalenone contamination differed considerably from year to year. Zearalenone levels were highest in 2000, with the direct drilling system displaying significantly higher levels of contamination than the other systems ($\alpha \leq 0.05$) in that year. In 2001, only one field (the organic subsystem) had zearalenone contamination. Zearalenone was not detected in 2002. We detected no other mycotoxins.

The relationship between disease severity and deoxynivalenol accumulation

No relationship between disease severity and mycotoxin levels was observed, between or within years (Figure 1). For example, disease severity was scored at 0% despite DON levels of 0-600 µg/kg (years 2001 and 2002). Furthermore, DON levels were low (350 µg/kg) on plots in which disease severity score was higher (22%).

The lack of a relationship between *Fusarium* head blight severity and mycotoxin content was even more evident for the subsystems. No disease was observed despite DON levels of 0-

1120 µg/kg (years 2001 and 2002) whereas moderate DON contamination (530 µg/kg) was recorded for plots with high levels of disease severity (63%). We also found that there was no relationship between disease severity and mycotoxin levels for nivalenol and zearalenone.

Discussion

Our results demonstrate that *Fusarium* head blight severity depends mainly on climatic effects. Similar results have been obtained in previous studies (Andersen, 1948; Langseth *et al.*, 1995). However, in years with average or high levels of disease, cropping system affects disease severity. The direct drilling system resulted in the highest disease severity in both years for which results were available. In 2000, the direct drilling system wheat crop followed a maize crop whereas the wheat crop of the integrated system followed oilseed rape. In 2002, the preceding crop was pea in both cases. However, the major difference between the direct drilling and integrated systems was the absence of tillage in the direct drilling system: comparison of these two systems, in 2002, can therefore be used to determine the effect of ploughing in residues. Our results seem to support previous conclusions concerning the effects of land preparation (Teich and Nelson, 1984; Teich and Hamilton, 1985; Dill-Macky and Jones, 2000).

An analysis of agronomic traits (head density, yield, grain protein content, *Septoria* leaf spot severity, eyespot severity) revealed no clear reason for the plot effect observed for disease severity in 2000. Follow-up of scab severity between flowering and harvest provided a potential clue: disease severity rapidly increased between June 27 and July 3 in plot I, but not in plot II. This increase in disease severity was probably due to heavy rainfall (21 mm) on July 2nd, after 20 days without precipitation. The experimental field was surrounded by trees and plot II was more protected from rain than plot I, which may have resulted in an edge effect.

This work shows that the *Fusarium* mycotoxin (deoxynivalenol, nivalenol and zearalenone) content of wheat crops depends on both year and cropping system. These results are consistent with those of previous studies (Teich and Hamilton, 1985; Dill-Macky and Jones, 2000): the direct drilling system generally results in the highest levels of contamination, particularly in years with high levels of disease, such as 2000. Moreover, in 2000, the presence of maize immediately preceding the wheat crop in the rotation of the direct drilling system seemed to increase the production of mycotoxins (Teich and Hamilton, 1985; Dill-

Macky and Jones, 2000). It was not possible to rank the other systems because no consistent pattern emerged. In 2000 and 2002, the conventional system was more contaminated than the organic system, whereas the reverse was true for 2001. Higher levels of contamination in the conventional system may result from disease severity being high in that year (which was the case in 2000) or may be due to the fungicide used. Indeed in 2000, we used a mixture of strobularins whereas in 2001 and 2002, we used a mixture of strobularins and triazoles. Strobularins belong to a family of fungicide molecules that are particularly effective against *Microdochium* strains but some *Fusarium* strains are limited effectively only by triazole molecules (Maumené *et al.*, 2000). As *Microdochium* produces no toxins, the application of strobularins does not limit mycotoxin levels. Conversely, it might favour the development of pathogenic strains that produce toxins. Thus, the fungicide treatment used in 2000 might have favoured an increase in mycotoxin levels in the adapted conventional system whereas competition between *Fusarium* and *Microdochium* strains may have limited the production of mycotoxins in the organic systems and in the conventional subsystem. In 2000, fungicide application might have resulted in an increase in disease severity in the adapted conventional system, whereas saprophytes might have limited disease development in the adapted organic system. In contrast, in 2002, the absence of fungicide treatment resulted in higher levels of disease severity. In this case, the molecule used was probably more suitable for the infecting pathogenic strains.

The “contaminating complex” effect, operating under natural contamination conditions is probably responsible of the absence of a relationship between disease severity and DON levels. Our results, which are consistent with those of other authors (Liu *et al.*, 1997), are especially important as they relate to moderate or low disease levels, which may conceal significant contamination by mycotoxins. Indeed, in contrast to studies in which crops were artificially contaminated with a single pathogenic strain of *Fusarium culmorum*, in which such a relationship was found (Teich and Hamilton, 1985; Snijders and Perkowski, 1990), several species of *Fusarium* and *Microdochium* are present in the field in natural contamination conditions. Hence, DON levels, disease severity and the relationship between these variables probably depend on the traits of the species present -capacity to produce disease, capacity to produce mycotoxins (deoxynivalenol, nivalenol, zearalenone), capacity to produce symptoms and mycotoxins- together with the time at which contamination occurs, the climatic conditions between the time of contamination and harvest, and the nutritive

resources that the plant can provide to the pathogenic strains: in other words, the whole cropping system.

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Table 1a: Principal traits of the four adapted cropping systems over the three years. Mixture 1: Somme, Malacca, Renan, Camp Remy; Mixture 2: Somme, Malacca, Virtuose, Apache; Mixture 3: Charger, Virtuose, Renan, Paindor.

The organic system received no nitrogen fertilisation in 1999-2000 and 2000-2001.
In the 2000-2001 study period, the wheat crop in the organic system was sown in January 2001. We planned to sow this crop in November, but incessant rain between October 2000 and January 2001 rendered this impossible.

Table 1b: Principal traits of the four cropping subsystems over the three years. No fungicide treatment, cultivar Charger.

The organic system received no nitrogen fertilisation in 1999-2000 and 2000-2001.
In the 2000-2001 study period, the wheat crop in the organic system was sown in January 2001. We planned to sow this crop in November, but incessant rain between October 2000 and January 2001 rendered this impossible.

Table 2: Comparison of disease severity in the four cropping systems during three years. Charger is the cultivar used in the adapted conventional system.

Table 3: Comparison of deoxynivalenol accumulation (µg/kg) for four cropping systems over three years. nd: not detected (30 µg/kg is the detection limit).

Figure 1: Relationship between disease severity and deoxynivalenol accumulation in four cropping systems over three years. C conventional, I integrated, DD direct drilling, O organic. 00 2000, 01 2001, 02 2002 (♦) Adapted cropping system, (□) Subsystem: cultivar Charger without fungicide

366 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	03/08/00	247	260	Strobularins (azoxystrobin)	Charger	Oilseed Rape
Integrated	27/10/99	25/05/00		197	165		Mixture 1	Maize with fescue
Direct drilling				197	220			
Organic	10/11/99	30/05/00		0	450	None		Oilseed Rape
Conventional	04/10/00	28/05/01	30/07/01	249	260	Mixture of strobularins (azoxystrobin), triazoles (tebuconazole), prochloraze, cyprodinil	Charger	Oilseed Rape
Integrated	24/10/00	01/06/01		201	165	Strobularins (azoxystrobin), cyprodinil	Mixture 1	Maize with fescue
Direct drilling	25/10/00			167	220			
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	30/07/02	131	165	Mixture of strobularins (azoxystrobin), triazoles (epoxinazole)	Mixture 2	
Direct drilling	26/10/01	25/05/02		141	220			
Organic	31/10/01	23/05/02		54	450	None	Apache	

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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	03/08/00	247	260	None	Charger	Oilseed Rape
Integrated	27/10/99	25/05/00		197	165			
Direct drilling				197	220			Maize with fescue
Organic	10/11/99	30/05/00		0	450			Oilseed Rape
Conventional	04/10/00	28/05/01	30/07/01	249	260	None	Charger	Oilseed Rape
Integrated	24/10/00	01/06/01		201	165			
Organic	17/01/01	27/06/01		0	450			Spring lupin
Conventional	05/10/01	06/06/02	29/07/02	208	260	None	Charger	Pea
Integrated	17/10/01	06/06/02	30/07/02	131	165			
Direct drilling	26/10/01	06/06/02		141	220			
Organic	31/10/01	06/06/02		54	450			

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371 Table 2

		(a) Adapted cropping system			(b) Subsystem: Charger without fungicide		
Cropping systems	Plots	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

		(a) Adapted cropping system			(b) Subsystem: Charger without fungicide		
Cropping systems	Plots	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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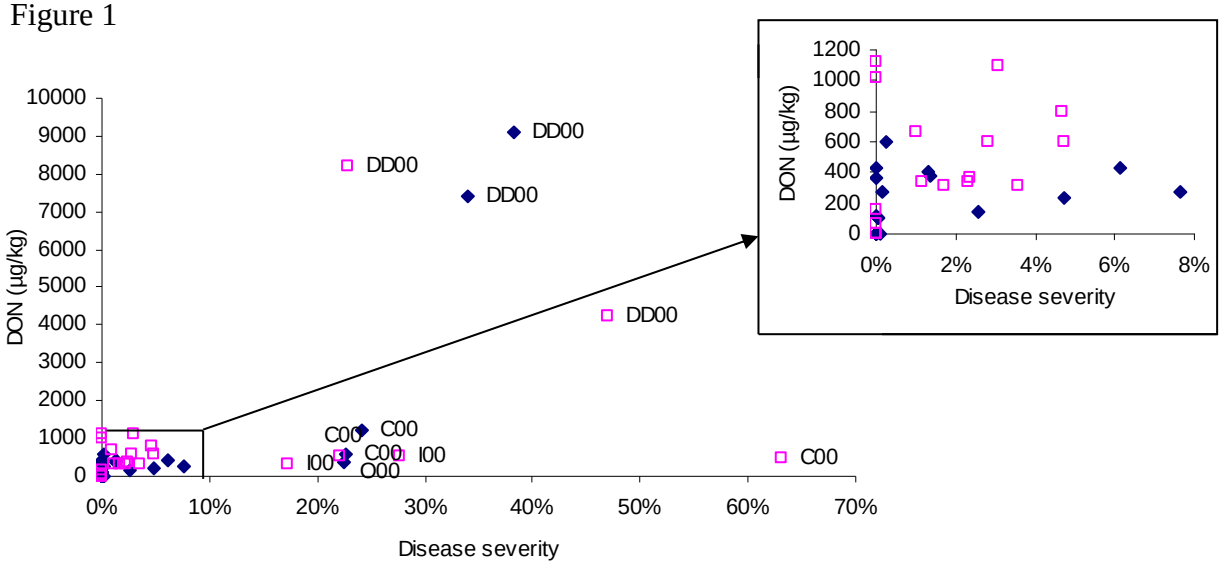
Figure 1


DON (µg/kg)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0%
10%
20%
30%
40%
50%
60%
70%
Disease severity
DD00
DD00
DD00
DD00
C00
C00
C00
I00
I00
O00
C00
DON (µg/kg)
1200
1000
800
600
400
200
0
0%
2%
4%
6%
8%
Disease severity