

Incidence of four genera of fungi in organic and low-input farming conditions in/on the grain of bread wheat over a 13-year period in France

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Bernard Rolland, Denise Deffains, Patrick Delarue, Stephane Gilles, Antonin Le Campion, et al.. Incidence of four genera of fungi in organic and low-input farming conditions in/on the grain of bread wheat over a 13-year period in France. Organic Agriculture, 2023, Organic Agriculture, 3 (4), pp.503-511. 10.1007/s13165-023-00443-4. hal-04305725

HAL Id: hal-04305725 https://hal.science/hal-04305725

Submitted on 4 Jan 2024

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- 1 Title:
- 2 Incidence of four genera of fungi in organic and low-input farming conditions in/on the grain 2 of bread wheat over a 13-year period in France
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- 4
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Abstract Bread wheat can be infected and/or contaminated by several genera of mycotoxin-23 producing fungi. In the European Union, several mycotoxins are subject to defined limits for 24 25 human consumption. Speculation has arisen about the potential higher contamination level of mycotoxins produced by Fusarium species in organic compared to conventional production 26 systems. So far, many studies have focused on the level of mycotoxins, highlighting comparable 27 28 levels in organic and conventional farming. In this long-term study, we monitored, each year 29 over a 13-year period in several sites in France and for a set of bread wheat genotypes, the incidence of four fungal genera on bread wheat grains from both conventional low-input and 30 31 organic farming. During this long period of monitoring, we used the same morphological characterization of fungal colonies. The results obtained highlighted two general trends. First, 32 the incidence of the genus Fusarium in grains was generally lower than or equal to 1% in more 33 than 60% of 'year \times site' combinations, both in low-input and organic farming conditions. In 34 contrast, the genus Alternaria was dominant in the vast majority of combinations. Second, the 35 main explanatory factors of the incidence of fungus on grains, independently of the genus and 36 37 the farming conditions, were the year and the 'year \times site' interaction, suggesting the dominant influence of the environmental conditions of the year and the site on the relative development 38 of the fungal genera in/on grains. According to these results, organic or low-input production 39 of bread wheat does not represent a significant risk for the development of Fusarium head blight 40 41 in France. 42

- Keywords Fungal incidence . Morphological identification . *Fusarium . Alternaria . Epicoccum Microdochium*
- 45

46 Acknowledgements The authors acknowledge the teams from Institut national de la recherche

- 47 agronomique (INRA, currently INRAE) experimental units of Auzeville, Lusignan, Le Moulon,
- 48 Le Rheu, as well as the team of Agri-Obtentions for their involvement in these experiments.
- 49 Our work has been supported for 14 years by French Technical Institute for Organic farming
- 50 (Laurence Fontaine ITAB) and InterBio Bretagne as part of the State-Region (Brittany) Project

- 51 Contract (CPER). We would like to thank organic farmers who received INRA on-farm trials
- 52 in Brittany (GAEC La Mandardière), Poitou-Charentes (MM. Pinelli and Ingrandes) and Ile-
- de-France (F. Chevalier) regions for 13 years, and for sharing their knowledge and perspectives
- on agricultural practices. The publication is in memory of Maxime Trottet who developed the
- 55 procedure, initiated by Laurent Saur, of morphological characterization of fungal colonies in 56 INPA La Phan We thank the adjtor and the three anonymous reviewers who helped to improve
- 56 INRA Le Rheu. We thank the editor and the three anonymous reviewers who helped to improve 57 the manuscript
- 57 the manuscript.
- 58
- 59 **Competing interests** The authors declare no competing interests.
- 60

Author contribution BR conceive and design the study. Material preparation and data collection were performed by DD, PD, SG, ALC, AM, JYM, HN, AP and PW. Morphological characterization of fungal colonies were performed by DD, PD and JYM. Statistical analyses were performed by RP. The first draft of the manuscript was written by RP and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

- 67
- Data availability The datasets and metadata will be accessible on the publicly available Data
 INRAE repository at <u>https://entrepot.recherche.data.gouv.fr/dataverse/inrae</u>
- 70

71 Supplementary Information

72 Online Resource 1 Coordinates of the sampling locations in conventional low-input and 73 organic farming systems

- 74 Online Resource 2 List of bread wheat genotypes retained for the statistical analyses in
- 75 conventional low-input and organic farming systems

76 Introduction

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78 The agricultural intensive production model is currently questioned because of agronomic, environmental, public health and sometimes socio-economic issues (van der Werf 1996; Loyce

79 et al. 2012; Bourguet and Guillemaud 2016). In this context, reducing the reliance of agriculture 80

81 on external inputs constitutes a priority to achieve more sustainable models of production.

However, an alternative model such as organic farming has been questioned for several decades 82

about its ability to ensure food safety (Magkos et al. 2006; Lairon 2010; Garcia and Teixeira 83 2017). Currently, organic farming is characterized by an increasing consumer demand, which 84

requires paying attention to the sanitary quality of food production, including mycotoxin 85 contamination (Brodal et al. 2016). 86

- This is particularly the case for bread wheat for which grain can be contaminated by several 87 genera of mycotoxin-producing fungi (Brodal et al. 2016), especially by different species of 88 Fusarium causing head blight. This plant disease results from a complex of species belonging 89
- to the Fusarium genus (Parry et al. 1995). Fusarium head blight (FHB) leads to important 90 economic consequences worldwide, important yield losses and reduction in quality production 91
- leading to the destruction of contaminated grain lots, as well as potential contamination by a 92
- range of harmful mycotoxins to human and animal health (Reddy et al. 2010). These 93 94 mycotoxins, amongst others deoxynivalenol and zearalenone, are produced by several

Fusarium species, especially Fusarium graminearum (Reddy et al. 2010; Dean et al. 2012). 95

The mycotoxins are subject to government regulation in the EU, i.e., defined limits in cereals 96 97 for human consumption (Commission Regulation (EC) No 1881/2006).

Speculation has arisen about the potential higher contamination level of mycotoxins produced 98

by Fusarium species in organic compared to conventional production model, and a set of studies 99

100 have investigated this issue. However, in the current state of knowledge, organic farming seems usually able to maintain a low level of contamination by mycotoxins, comparable or lower than

101 in conventional farming (Brodal et al. 2016). The hypothesis that several agricultural practices 102

would be less favourable for the development of Fusarium species causing FHB in organic 103

- 104 farming has been proposed. In fact, a low level of nitrogen supply and a crop sequence including fewer cereals reduce the severity of FHB and the level of mycotoxins (Dill-Macky and Jones 105 2000; Lemmens et al. 2004; Heier et al. 2005; Bernhoft et al. 2012).
- 106

While many studies have focused on the level of mycotoxins in organic and conventional 107 farming (see e.g., Edwards 2009; Bernhoft et al. 2012; Brodal et al. 2016), only few have 108

- considered the incidence of different fungal genera in/on the grain of bread wheat. These latter 109
- studies have shown a low incidence of *Fusarium* species on organically produced grain, while 110
- the fungal community was dominated by other fungal genera (Lazzaro et al. 2015; Lenc 2015). 111
- However, these studies mainly relate to few years and a limited number of varieties, furthermore 112 not identifying the main explanatory factors of the Fusarium incidence in the different 113
- production models. 114
- In this study, we monitored, each year over a 13-year period in several sites in France and for 115

a set of bread wheat genotypes, the incidence of four fungal genera on grain of bread wheats 116 from both conventional and organic farming conditions. 117

- More specifically, we addressed the following questions: 118
- 119 (1) What was the incidence of the four genera of fungi on the grain of bread wheat over the period and between sites in both conventional and organic farming systems? 120
- (2) To what extent do year, site, genotype and their interactions explain differences in incidence 121
- of the genus Fusarium as well as other fungal genera in/on the grain of bread wheat in both 122
- conventional and organic farming systems? 123
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- 125

- 126 Material and methods
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128 Sample collection

Wheat samples were collected immediately after harvest from experimental field trials from 2007 to 2019 in three areas in France (Brittany, Ile-de-France and Poitou-Charentes) in both conventional and organic farming systems. In our study, conventional farming systems corresponded to low-input systems with no fungicide and growth regulator used, a lower sowing rate and a reduction of nitrogen application (based on N balance sheet minus 60 kgN/ha) but with herbicides used to suppress weeds (see Le Campion et al. 2014 for details).

For each site in low-input farming, when available, a site in organic farming located near the conventional farming site was chosen to limit variation due to large-scale environmental variables such as meteorological conditions (see Online Resource 1 for coordinates). However, no organic farming site was repeatedly available near the low-input site in Poitou-Charentes, so only two sites were monitored in organic farming conditions while three sites were monitored in low-input farming conditions. In each site and each year, four repetitions were carried out for each wheat genotype in plots of 8.5m² using a randomised block design.

Samples were made in conditions of natural contamination on a substantial list of genotypes 143 144 representative of the winter bread wheat varieties sown in low-input and organic farming over the period considered, including susceptible to tolerant wheat genotypes to FHB. Fusarium 145 graminearum was the main species of Fusarium in France during the period studied. Given the 146 147 duration of the period studied, the genotypes monitored have been replaced over the years, representing 34 wheat genotypes under low-input conditions and 14 wheat genotypes under 148 organic conditions, for a total of 289 wheat samples collected under low-input and 155 wheat 149 samples collected under organic conditions. Only wheat genotypes monitored for at least two 150 years were retained in this study. The kernels were harvested at maturity with 14% of moisture 151 (0.5-1kg per sample), and sub-samples of 100 kernels were chosen randomly and stored in paper 152 bags at room temperature with 13-15% humidity until analysed for about 6 months. Visible 153 FHB contamination on spikes was not observed in the field during any of the years concerned 154

- in this study.
- 156

157 Morphological characterization of fungal colonies

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To determine the incidence of the different fungal genera, one hundred kernels were randomly 159 chosen from each sub-sample, surface disinfected with 1% NaClO solution for 2 min, rinsed 4 160 times with running water, and dried for 12h under laminar flow hood. The use of running water 161 cannot exclude a contamination introducing fungal spores and/or bacteria on the grains. Ten 162 kernels were evenly placed into 10 separate Petri dishes (Ø 55 mm) containing potato dextrose 163 agar, and incubated for 5 days at room temperature under conventional lighting without day-164 night cycle. Each colony was identified on cultural and morphological criteria to genus level 165 among four genera, i.e. Alternaria, Epicoccum, Fusarium and Microdochium based on 166 pigmentation of aerial mycelium and growth rate of mycelium, especially following the 167 descriptions proposed by Pitt and Hocking (1997). The incidence was considered as the 168 169 proportion of kernels infected by fungi of these four genera. During this long-term monitoring, we used the same morphological characterization of fungal colonies. However, we point out 170 that the results of this study are relative to the method used. In particular, it is possible that some 171 fungal species can be overgrown by colonies of other fungal species growing more rapidly, 172 even if the incubation time was intended to limit this type of bias. 173

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175 Incidence of different fungal genera on wheat grains in low-input and organic farming 176 conditions

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The average incidence of each genus, or the absence of aerial mycelium development, is 178 graphically represented per year and site, considering all bread wheat genotypes combined. This 179 180 graphical representation constitutes a synthesis of the useful data for low-input and organic farming, i.e., 13 years × 3 sites × 5-16 genotypes per year for low-input farming conditions and 181 13 years \times 2 sites \times 5-8 genotypes per year in organic farming conditions. This graphical 182 representation has not been subjected to statistical analysis due to the unbalanced design of the 183 data, i.e., the lack of data in certain years on certain sites. The statistical analyses described 184 hereafter were conducted on a suitable sub-dataset. 185

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Sources of variability of the incidence of fungi on wheat grain per genus in low-input andorganic farming conditions

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190 For each fungal genus and farming condition separately, we assessed the effects of year, site,

191 genotype and their interactions on the incidence of fungi on wheat grains using linear models.

First, some data were removed before applying statistical analyses, i.e., the genotypes tested in

a single or two sites for low-input and a single site for organic farming conditions. The datasets
focused on 7 years × 3 sites × 26 genotypes in low-input and 11 years × 2 sites × 13 genotypes
in organic farming conditions (see Online Resource 2 for the list of genotypes).

Second, we implemented a full additive linear model including year, site, genotype and all 196 second-order interactions, and we compared this full model with all other simpler additive 197 models, including only a subset of the environmental variables. The comparison was based on 198 199 the second-order Akaike information criterion (AIC_c; Sugiura 1978), and considering all the explanatory variables as factors. We checked for homogeneity of variances (Bartlett test, visual 200 inspection), normality of the residuals (Shapiro test, visual inspection) and influential points 201 (Cook's distance). Sum to zero contrasts were applied prior to fitting models. We applied a 202 203 transformation (log₁₀+1, Box-Cox+1, Box and Cox 1964) or not on the response variable due to asymmetry in the data distribution depending on the fungal genus. Assumptions were met 204 205 after transformation or not for the genera Fusarium, Epicoccum and Alternaria.

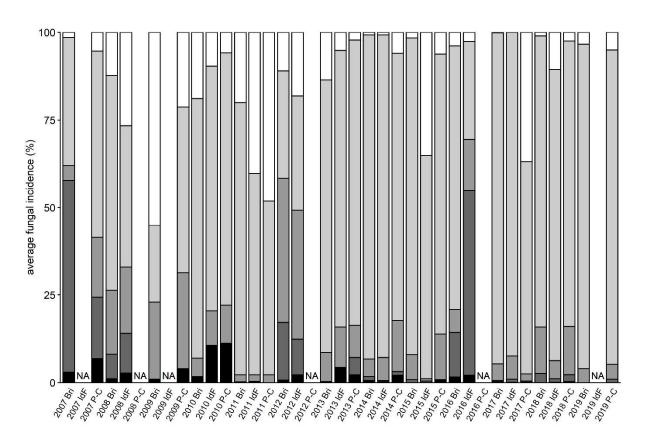
Third, the best model was retained, that corresponds to the most parsimonious model with the lowest AICc (AIC_{best}). The best model was compared with the intercept-only model using AIC_c values, i.e., $\Delta AIC_c = AIC_0 - AIC_{best}$ in order to ensure the relevance of the best model (a value lower than 2 indicating that the best model was equivalent to the intercept-only model, Burnham and Anderson 2004). The goodness of fit of the models was estimated using an adjusted R² (R²_{adj}). We performed a type III ANOVA on the best model.

Concerning the genus Microdochium, no transformation was relevant to validate the 212 assumptions. This result is due to the very strong asymmetry in the data distribution due to the 213 absence of Microdochium observed in most cases (70% in low-input conditions, 52% in organic 214 conditions), making the application of a linear model unsuitable. We have chosen not to present 215 the results for the genus Microdochium in this study. First, this asymmetry requires the use of 216 another type of model that would not be directly comparable with the results of the linear 217 218 models (e.g. quasi-binomial generalized linear model). Then, there was non-independence of the four fungal genera in our study, the development of the aerial mycelium of a given fungal 219 genus excluding the development of another genus (except in very rare exceptions), so showing 220 221 the results of the four genera would lead to overinterpreting the data. Statistical analyses were performed using R 4.0.1 (R Development Core Team 2022), its 222

222 Statistical analyses were performed using R 4.0.1 (R Development Core Team 2022), its
 223 packages 'ggplot2' for graphical presentation, 'forecast' for Box-Cox transformation,
 224 'AICcmodavg' for model selection based on AIC_c and 'car' for type III ANOVA.

225 **Results**

- 226
- Incidence of different fungal genera on wheat grain in low-input and organic farming conditions
- 229 Data is based on 289 wheat samples collected under low-input and 155 wheat samples collected
- under organic farming conditions. Several main trends can be drawn from these long-term time
- monitoring. As stated above, it should be noted that the results are relative to the method used
- to characterize the fungal colonies.
- First, the incidence of the genus *Fusarium* in bread wheat grains was generally low to nil, with
- a few rare exceptions (Fig. 1, Fig. 2). Indeed, the incidence of *Fusarium* was higher than 10%
- only in 2008 in organic conditions and in 2010 in low-input conditions, while lower than or
 equal to 1% in 61% of 'year × site' combinations in organic conditions and 64% of 'year × site'
- 237 combinations in low-input conditions.
- Second, the absence of aerial mycelium on bread wheat grain was not the most frequently
- observed case. Indeed, we observed a single combination with more than 50% of grains without
 fungal development in low-input conditions and no combination in organic farming conditions
- (Fig. 1, Fig. 2). On average, 89% of wheat grains were characterized by a fungal development
- in both low-input and organic conditions, with significant variability between 'year \times site'
- 243 combinations.
- 244 Third, in the vast majority of combinations considered, the genus *Alternaria* was dominant on
- wheat grains, i.e., in 89% of combinations in low-input conditions and 92% of combinations in
- organic conditions. The other genera showed a high incidence only in certain years and in
- 247 certain sites. For instance, the genus Microdochium showed substantial development in Ile-de-
- France in 2016, while the genus *Epicoccum* was characterized by a higher incidence in 2012
- 249 (Fig. 1, Fig. 2).
- 250



252 Fig. 1 Average incidence of the four different fungal genera on bread wheat grain in low-input farming between

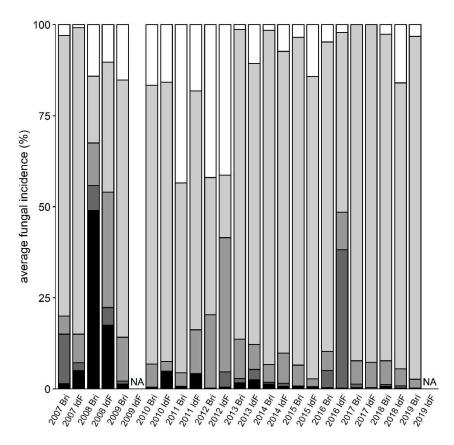
253 2007 and 2019 at three sites are represented by the following shades of gray from bottom to top: Fusarium in

254 black, Microdochium in dark gray, Epicoccum in gray, Alternaria in light gray, and absence of aerial mycelium

255 observed in white. All bread wheat genotypes per year and site have been used to compute the average incidence,

256 i.e., between 5 and 16 genotypes depending on the year considered. NA indicates an absence of data monitored 257 concerning the particular 'year \times site' combination. Bri = Brittany, IdF = Ile-de-France, P-C = Poitou-Charentes

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259 260

Fig. 2 Average incidence of the four different fungal genera on bread wheat grain in organic farming between 261 2007 and 2019 at two sites are represented by the following shades of gray from bottom to top: *Fusarium* in black, 262 Microdochium in dark gray, Epicoccum in gray, Alternaria in light gray, and absence of aerial mycelium observed 263 in white. All bread wheat genotypes per year and site have been used to compute the average incidence, i.e., 264 between 5 and 8 genotypes depending on the year considered. NA indicates an absence of data monitored 265 concerning the particular 'year \times site' combination. Bri = Brittany, IdF = Ile-de-France

Sources of variability of the incidence of fungi on wheat grain per genus in low-input and 267 organic farming conditions 268

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Considering the relevant sub-datasets and after confirmation that the prerequisites were met, a 270 best model was identified for each case studied. This best model was always much more 271 relevant than the intercept-only model, confirming that at least some explanatory variables are 272 significantly explanatory for each case (Table 1). Moreover, R²_{adj} was between 0.62 and 0.92, 273 274 highlighting medium to high accuracies, depending on the model (Table 1). The statistical analyses carried out underlined that, for all the models tested, regardless of the fungal genus 275 and the farming conditions, the year and the 'year \times site' interaction had a large effect on the 276 incidence of fungi on wheat grain. In contrast, the site effect was often non-significant or weakly 277 significant, especially under organic conditions, and a low genotype effect was observed only 278 for Alternaria in organic conditions (Table 1). It should be noted that a bias of the statistical 279 280 analysis was related to the replacement of genotypes studied over time. Indeed, the long-term 281 monitoring did not always characterize the same genotypes, this effect being then partially 282 confused with the year effect, potentially explaining why the year effect is usually the only one 283 significant in the analysis.

283 significant in the anal

285

286 **Table 1** Results of the best additive linear models indicating the effect of year, site, genotype and year \times site 287 second-order interaction on the incidence of the genera Fusarium, Epicoccum and Alternaria in both low-input 288 and organic farming conditions considered separately. Box-Cox+1 and log₁₀+1 refer to the Box-Cox 289 transformation (transformation parameter $\lambda = -1$) and the base 10 logarithm after adding a constant (+1) to the raw 290 values, respectively. AIC₀ - AIC_{best} corresponds to the difference of AIC_c between the best model and the intercept-291 only model. The goodness of fit of the models was estimated using an adjusted R² (R²_{adj}). P-value estimation is 292 based on F-test, *P < 0.05, ***P < 0.001, n.s., not significant. / indicates that the explanatory variable was not 293 included in the best model. Degrees of freedom (df) vary between models applied on datasets in low-input and 294 organic farming conditions

Eamaina	Fungal genus	F-test (P-value)							
Farming conditions		Transformation	AIC ₀ - AIC _{best}	R ² adj	Intercept	Year	Site	Genotype	Year × Site
						df = 6	df = 2		df = 12
Low-input	Fusarium	Box-Cox+1	140.03	0.66	216.46***	22.67***	22.15***	/	4.39***
	Epicoccum	$log_{10}+1$	129.40	0.64	371.00***	16.68***	8.30***	/	8.92***
	Alternaria	none	120.74	0.62	854.01***	15.62***	0.80n.s.	/	6.63***
						df = 10	df = 1	df = 12	df = 10
Organic	Fusarium	log10+1	167.79	0.77	48.06***	18.78***	6.65*	/	5.71***
	Epicoccum	log ₁₀ +1	101.37	0.62	95.68***	19.03***	1.74n.s.	/	6.36***
	Alternaria	none	279.47	0.92	519.28***	66.46***	2.54n.s.	2.94**	14.66***

295 Discussion

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This long-term study focusing on the incidence of four fungal genera on bread wheat grain over a 13-year period in several sites on hundred samples collected in both conventional low-input and organic farming conditions led to conclude two general trends.

First, the incidence of the genus Fusarium in bread wheat grains was generally low to nil, both 300 in low-input and organic farming conditions, as observed in previous studies (Lazzaro et al. 301 2015; Lenc 2015). In the vast majority of situations studied, the genus Alternaria was dominant 302 on wheat grain, while the other genera studied were generally few or not observed, except in 303 atypical years, sometimes only in one site. In our case, these particular results could be 304 explained by meteorological conditions favourable to the development of fungal genera 305 associated with maize as preceding crop (van der Velde et al. 2020), as also observed in other 306 307 studies during part of the period considered here (Giraud et al. 2010; Chandelier et al. 2011). It should be noted that in 2008 in low-input conditions in Brittany, i.e., 1km away from the organic 308 trial characterized by a incidence near 50% of the genus Fusarium, considering a similar sowing 309 period but faba bean as preceding crop, the incidence of Fusarium was under 5%. 310

Second, the main explanatory factors of the incidence of fungi on wheat grain, independently of the genus and the farming conditions, were the year and the 'year × site' interaction, while the site and the genotype were less explanatory. These results appear consistent with the trends observed on the graphical representations, suggesting the dominant influence of the environmental conditions of the year and the site on the relative development of the fungal

genera on wheat grains. If certain conditions are known to favour the genus *Fusarium*, their 316 combination have been rarely observed during our study period, except in 2008 in Brittany. 317 Indeed, in organic farming but also in low-input conditions, both the low level of nitrogen 318 supply and a crop sequence including fewer cereals with maize rarely as the preceding crop, 319 reduce the risk of FHBdevelopment (Dill-Macky and Jones 2000; Lemmens et al. 2004; Heier 320 321 et al. 2005; Osborne and Stein 2007; Bernhoft et al. 2012). Moreover, the main conducive weather conditions to FHB development are known, i.e., usually warm and moist conditions 322 near and during the flowering period of winter wheat (Osborne and Stein 2007). However, the 323 best predictors and window frames identified are partially different among studies (Kriss et al. 324 325 2010; Bernhoft et al. 2012; Landschoot et al. 2012; Shah et al. 2013). In the absence of these conditions, the genus Alternaria predominated very frequently in our conditions. 326

327 328

329 Conclusions

330 331 Even though it should be noted that the results of this study are relative to the particular method used to characterize the fungal colonies, these results confirm the low to nil incidence of the 332 genus Fusarium in most years in organic farming, just as in low-input farming conditions. These 333 results are in accordance with previously published studies highlighting the low contamination 334 level of mycotoxins, comparable or lower in organic than in conventional farming (Brodal et 335 al. 2016). Moreover, from a breeder's point of view, this low incidence of FHB largely 336 dependent on environmental conditions and agronomic choices (Osborne and Stein 2007), 337 makes phenotypic selection of more resistant genotypes in fields both more complex and less 338 effective than for other diseases in northwestern Europe. Indeed, resistance testing appears to 339 340 be more efficient under uniform and intermediate to high disease pressure, as usually achieved using artificial inoculation, and marker-assisted selection is widely put forward for FHB 341 resistance (Buerstmayr et al. 2020). However, some additional morphological characteristics 342 may also be considered in a breeding scheme, with taller plants characterized by anther 343 extrusion being frequently more resistant to FHB (Buerstmayr et al. 2020). The results of the 344 survey support that organic and low-input production of bread wheat does not represent a 345 significant risk for the development of FHB in France. To go further in the characterization of 346 Fusarium graminearum for future studies aimed at improving resistance to FHB, we strongly 347 recommend the use of other methods (Horevaj et al. 2011; Kumar et al. 2015). 348

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