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EFFECTS OF INFECTION TIME BY *FUSARIUM GRAMINEARUM* ON EAR BLIGHT, DEOXYNIVALENOL AND ZEARALENONE PRODUCTION IN WHEAT

ABSTRACT

Although it is generally agreed that most *Fusarium* head blight infection takes place during the wheat flowering period, very rainy conditions at the end of the plant growth, such as occurred in France in 2007, raise the question of the possibility of late infections on the development of the disease and the production of toxins. Such infections are not taken into account by predictive models. To explore the effect of infection time on disease severity, *Fusarium*-damaged kernels (FDK), desoxynivalenol (DON) and zearalenone (ZON), an experiment was set up in 2009 on two varieties. The experiment had a split-plot design. Plots were inoculated by spraying *Fusarium graminearum conidia* at different stage, from heading to ripening. Inoculation time had a significant effect on disease severity, FDK, DON and ZON levels (P<0.0001), as well as interaction between variety and inoculation time. The results of this trial have shown no significant difference between infection at heading, anthesis and post-anthesis until 15 days for disease severity, FDK and mycotoxins levels. Nevertheless, this trial has suggested different behaviour between the two varieties according to infection time and toxin accumulation. The period of high susceptibility to *Fusarium graminearum* has appeared unstable between varieties, ranging from closed to flowering to a large period from heading to several days after flowering.

Key words: wheat, *Fusarium graminearum*, infection time, desoxynivalenol, zearalenone

INTRODUCTION

Fusarium head blight (FHB) is a commonly disease on cereals due to a *Fusarium* and *Microdochium* species complex. Among these species, *F. graminearum* (teleomorph: *Gibberella zeae*) is the most common found in French wheat (BayerCropScience, unpublished data). In some years, FHB can considerably reduce grain yield and quality by producing mycotoxins in grain

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(Dexter *et al.*, 1997). Thus, this contamination can affect both profitability and human health.

One of the essential elements of cereal safety quality is the management of mycotoxin grain contamination. Because of its frequent occurrence and the consequences for human health, the European Community has set a legal limit on different mycotoxins for unprocessed cereals marketed for the food industry, notably 1,250µg/kg for deoxynivalenol (DON) and 100µg/kg for zearalenone (ZON) (EC 1881/2006). These mycotoxins, produced by *F.graminearum* and *F.culmorum* (Placinta *et al.*, 1999), are a crucial issue for cereal markets. ARVALIS-Institut du vegetal has therefore developed a DON predictive model including agronomic and weather data. The target of this decision making tool is to provide a DON risk before harvest to help grain merchants for the risk management. Thus, to make a pre-harvest prediction, the model use weather data up to 15 days after flowering, assuming that late infections are negligible (Lacey *et al.*, 1999). After a very rainy year (2007) from wheat flowering to the end of wheat growth, the severe attacks of FHB with high DON and ZON contents arose the question of late infections, not considering in the model. Although in the literature it is generally agreed that the most sensitive stage of wheat to *Fusarium graminearum* is flowering, the question of late infections is rarely considered. The propagation of the disease and the visual symptoms seem to have less impact with an infection by *F. graminearum* during grain ripening as an infection at the flowering stage. Nevertheless, concerning the toxin production during this late stage, this phenomenon is currently poorly understood (Osborne and Stein, 2007). Some studies have tested late infections by *Fusarium spp.* (Hart *et al.*, 1984 ; Lacey *et al.*, 1997 ; Gelisse *et al.*, 2006 ; Del ponte *et al.*, 2007 et Trottet, 2007). All suggested a decrease in DON contents with infection after anthesis stage (Hart *et al.*, 1984, Lacey *et al.*, 1997, del Ponte *et al.*, 2007). However, the experimental conditions were very variable from one trial to another and the results are not easily comparable.

Hence, the objective of the experiment led in France in 2009 was to check in field conditions, if the ear infection by *Fusarium graminearum* at different wheat growth stages, from heading to ripening, led to comparable disease severity, FDK, DON and ZON levels.

MATERIALS AND METHODS

The experiment was done on experimental microplots on the station of Boigneville (Essonne) in 2009. Two varieties were sown, Caphorn and Mercato. These varieties were chosen for having the same time of heading, thus facilitating inoculation, but different susceptibilities to DON accumulation: Caphorn is considered as susceptible whereas Mercato is moderately susceptible.

For each of the two varieties, seven infection times were tested from heading to ripening. The inoculations were made at heading, around anthesis (+/- 3 days) and then every 7 days until 38 days after anthesis. Two uninoculated control microplots were also included. Each treatment was replicated four times in a split plot design**.** Concerning the infection dates, the time between heading and flowering for Mercato was very short, which would have led to a time lag of three days between the two varieties, which were nevertheless inoculated on the same dates.

The inoculation was done by spraying at the end of the day. The inoculated microplots were then covered with a plastic sheet so as to maintain a high relative humidity, and then misted for 48 h. These conditions were used to facilitate germination of the spores and infection.

The inoculations were made with a suspension of conidia of three strains of *Fusarium graminearum* with a concentration of 10^9 spores/ml. Among the three strains, two are characterised by their toxigenicity ; the first produces DON and ZON and is particularly aggressive ; the second only produces DON and is less aggressive but develops a lot of mycelium and spores. Finally, since the quantity of inoculum was insufficient, a third uncharacterised strain was added, isolated from wheat grain harvested in the 2008 season.

During growth, severity of the disease was scored 350dd after each inoculation. However for the latest inoculation, it was not possible to carry out this notation merged with the maturity of the ears. The notation was done on 50 ears on which the number of diseased spikelets was counted in relation to the total number of spikelets on the ear. In order to thoroughly describe the trial and to confirm the efficacy of the inoculation, a microbiological analysis was done on grains from the first four inoculation dates and on the controls. Once harvested, a sample of grains from each microplot were cleaned and ground. Grinding was carried out with a rotary mill fitted with 1 mm mesh sieve (ECMA/FAO) and from the flour, a sample of 500g was taken and sent to the laboratory for DON and ZON analysis by chromatographic method (LC-MSMS). On a subsample of grain, an analysis of the *Fusarium*-damaged kernel (FDK), expressed as a percentage, was done using image analysis. Statistical analysis was done using STATBOX®. Analysis of variance (ANOVA) were realised to test the effect of infection time, variety or split-plot design, on disease severity, FDK, DON and ZON. DON and ZON contents were log-transformed to normalise their distribution, as well as FDK which was arcsin-transformed. Correlations were also studied between the variables.

RESULTS AND DISCUSSION

Firstly, it is important to note that the effect of variety does not appear significant at the 5% threshold in the ANOVA done on disease severity, FDK and DON contents (Table 1). On the other hand, the infection time appears highly significant for these three variables, as do the interaction between variety and infection date. This first result suggests that the two studied varieties have different behaviours according to infection time.

Table 1

Results of the ANOVA on variety, infection time and interaction between both.

Effect	% disease severity Pr > F	%FDK Pr > F	DON Pr > F
Variety	0.24	0.15	0.08
Infection date	<0.0001	<0.0001	<0.0001
Variety*Date	0.0002	<.0001	0.0083

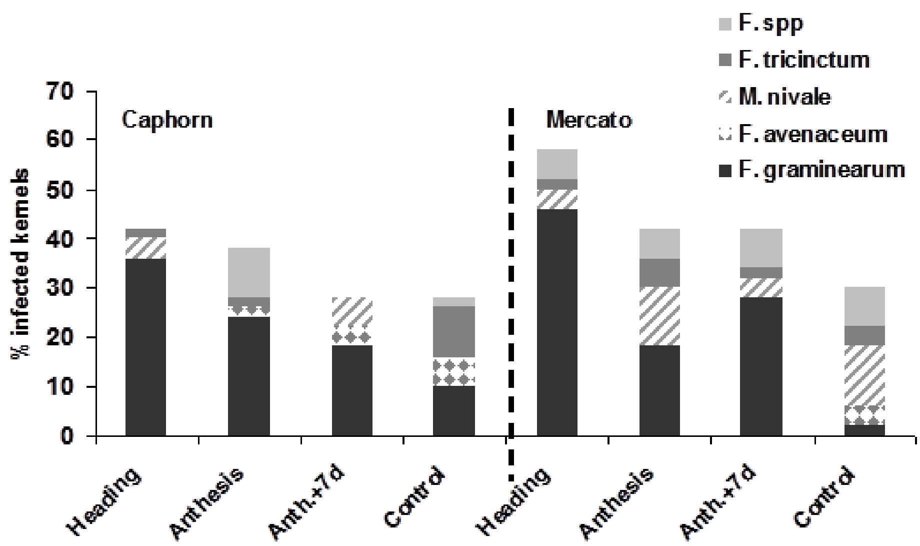

Fig. 1. Distribution of *Fusarium* and *Microdochium* species on grains from plots inoculated at heading, anthesis and 7 days after anthesis, in comparison with the controls.

The microbiological analysis on the grains at harvest shows that the main species found on the inoculated grains was indeed *Fusarium graminearum* (Fig. 1). It also underlines that Mercato seems more affected than Caphorn. We can note that the controls were also affected with about 30% of the grains infected but a wider diversity of fungi, illustrating the presence of several species naturally present in the environment of the microplots. It also illustrates that the fungi profile is quite different between Caphorn and

Mercato which suggests a different selection pressure according to the variety.

Table 2

Disease severity, % of FDK and mean of DON and ZON (µg/kg) according to infection date and variety.

Inoculation	Caphorn growth stage	Disease sever-	FDK %	DON µg/kg	ZEA µg/kg
22/05	Heading	16 *(ab)*	15 *(ab)*	7095 *(a)*	164 *(ab)*
28/05	Flowering	25 *(a)*	25 *(a)*	10327 *(a)*	246 *(a)*
04/06	Flowering + 7 days	11 *(bc)*	23 *(a)*	9945 *(a)*	257 *(a)*
11/06	Flowering + 14 days	11 *(bc)*	19 *(a)*	9123 *(a)*	228 *(a)*
18/06	Flowering + 21 days	5 *(c)*	7 *(bc)*	2378 *(b)*	44 *(b)*
25/06	Flowering + 28 days	4 *(c)*	4 *(c)*	1527 *(b)*	30 *(b)*
02/07	Flowering + 35 days		5 *(c)*	1542 *(b)*	55 *(b)*
-	Control	4 *(c)*	5 *(c)*	1617 *(b)*	56 *(b)*
Inoculation dates	Mercato growth stage	Disease severity %	FDK %	DON µg/kg	ZEA µg/kg
22/05	Heading	19 *(a)*	17 *(a)*	7777 *(a)*	526 *(a)*
28/05	Flowering + 3 days	8 *(b)*	12 *(ab)*	4970 *(ab)*	292 *(ab)*
04/06	Flowering + 10 days	6 *(b)*	9 *(ab)*	2815 *(abc)*	134 *(bc)*
11/06	Flowering + 17 days	8 *(b)*	7 *(b)*	2735 *(abc)*	130 *(bc)*
18/06	Flowering + 24 days	7 *(b)*	6 *(b)*	1210 *(cd)*	103 *(cd)*
25/06	Flowering + 31 days	4 *(b)*	6 *(b)*	865 *(d)*	67 *(cd)*
02/07	Flowering + 38 days		5 *(b)*	689 *(d)*	70 *(cd)*
-	Control	4 *(b)*	5 *(b)*	723 *(d)*	33 *(d)*

Results of the Tukey test at 10% are represented by a letter.

Table 2 shows that the infection, expressed as disease severity and FDK, and production of toxins are possible from heading up to beyond the anthesis stage. In fact, for both varieties, the plots inoculated during the 17 days after flowering present symptoms and mycotoxin contents which are significantly higher than those of the controls, unlike at the later stages (from milky grain to ripening) for which the differences were not significantly different compared with the controls. Two hypotheses could be expressed. First of all, the conditions are no longer favourable to *Fusarium graminearum* to develop correctly in the grain and to produce toxins. Secondly, the physical barrier formed by the presence of teguments on the grain at late stage stops the fungi spreading. The behaviour of Caphorn, with a sudden decrease of disease severity, FDK, DON and ZON, seems to support this hypothesis, but it is never mentioned in the literature and it has yet to be verified. Nevertheless, the behaviour of Mercato supports more the first hypothesis (Fig. 2).

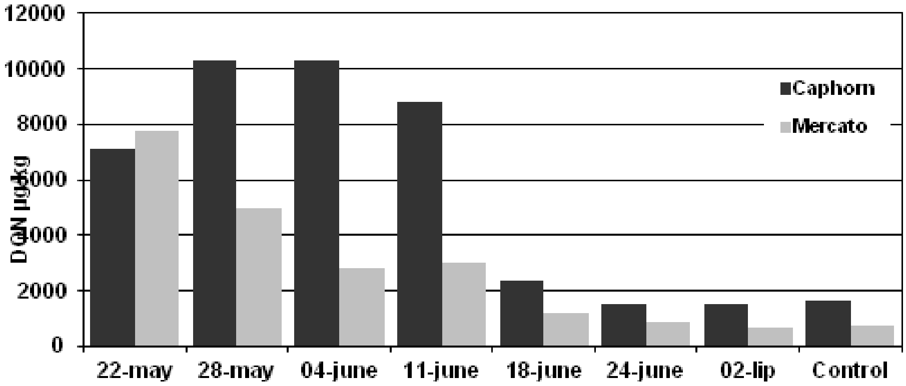

Fig. 2. Mean of DON (µg/kg) for each date of inoculation

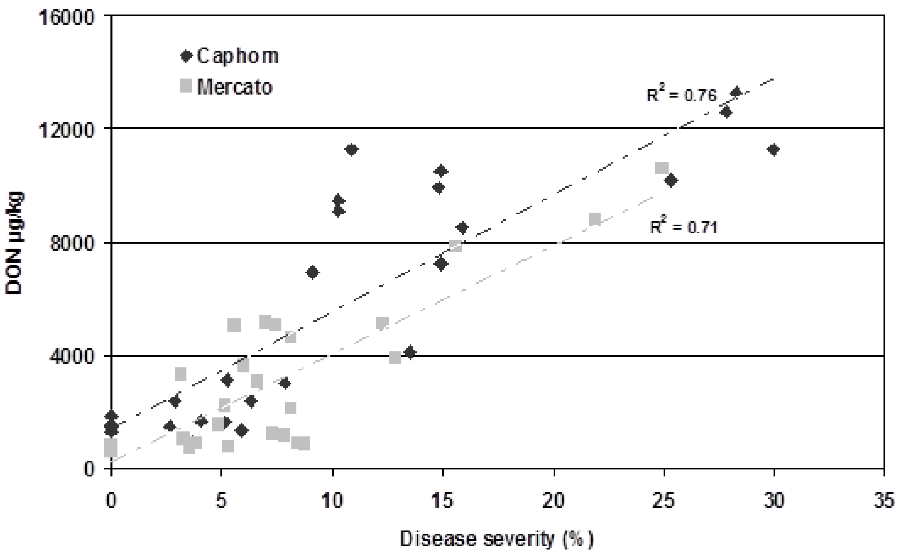

Fig. 3. Correlation between DON contents (µg/kg) and disease severity for each variety.

Throughout this experiment, it confirms that Caphorn is more susceptible to the accumulation of DON than Mercato, with contents going up to 10,000 µg/kg. Moreover, it seems that the heading stage is just as susceptible as the anthesis stage, a fact which is not clear from the literature. However, we have to note that the quantities inoculated are well above those normally used (10^6 spores/ml).

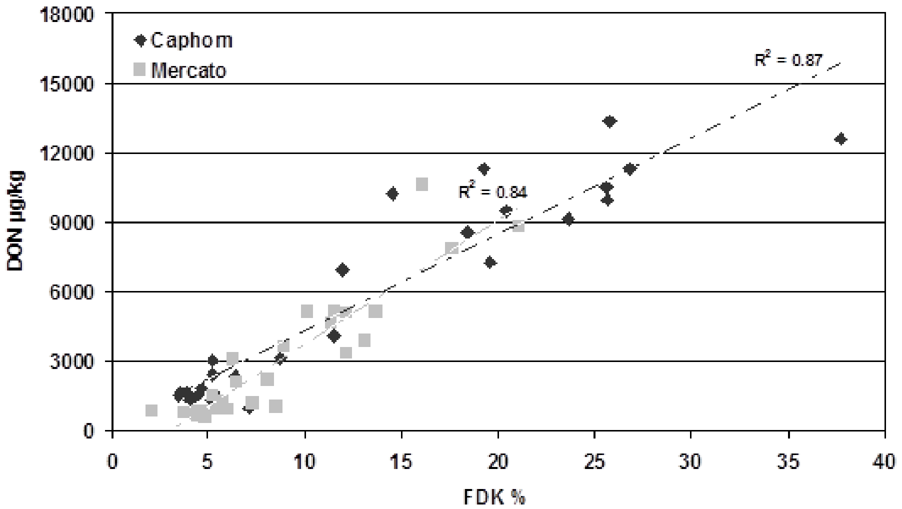

Fig. 4. Correlation between DON contents (µg/kg) and FDK for each variety.

By comparing the results obtained on this trial with those in the literature for comparable studies, all point towards a decrease in DON contents according to the infection time. Nevertheless, this reduction is often steady and consistent between varieties, whereas our trial showed difference between varieties and for the most susceptible, a discontinuity in toxin levels around the milky grain stage, rarely seen in other publications (Lacey *et al.*, 1997 ; del Ponte *et al.*, 2007 ; Hart *et al.*, 1984).

The trial results show also good correlations between DON contents and disease severity (R^2=0.7) and DON contents and FDK (R^2=0.8) for each variety (Figs.3 and 4). Good correlations between symptoms and toxin contents demonstrate, for these conditions, the strong relationship between disease severity and grain toxin contents.

The analysis of the correlation between DON and ZON (Fig. 5) underlines a good correlation between the two toxins (R^2=0.8) but although the correlation coefficient is similar between the two varieties, the slope differs. It is difficult to understand the cause, considering that 3 strains were inoculated and that, at least, one of them is known to produce ZON. This result therefore raises the question of a variable toxin production by the fungal strains present according to variety. Hence, it turns out that in this trial, Mercato accumulated more ZON toxins than Caphorn, which accumulated more DON. This result merits confirmation in later trials to check the hypothesis of an affinity exercised by the variety on the strain.

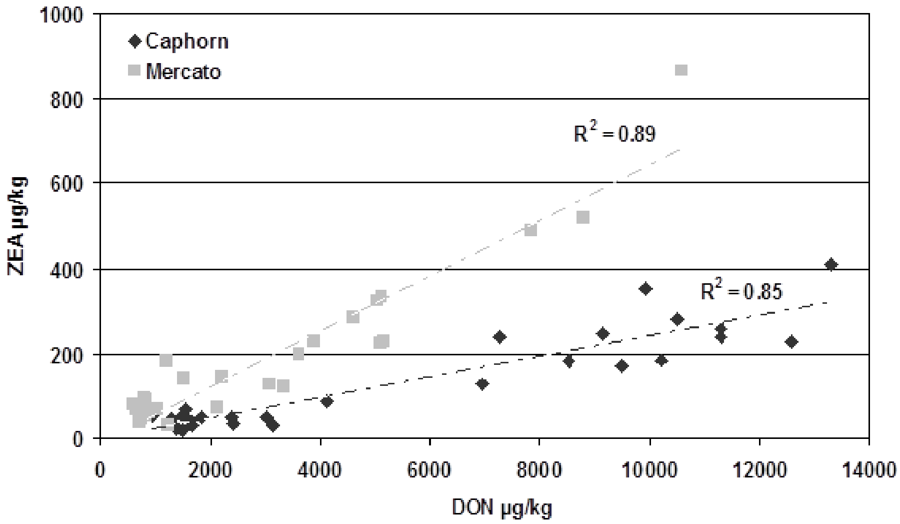

Fig. 5. Correlation between DON and ZON (µg/kg) for each inoculation date and each variety

CONCLUSIONS

This experiment, done in the field by inoculation of spores of *Fusarium graminearum* at different growth stages of wheat, has shown that post-anthesis infection is possible, but only up to a specific stage. It has underlined that the possible infection period seems to be different between varieties and longer than the only anthesis stage. Regarding to the toxin contents, Caphorn has the same level of DON and ZON for heading until 14 days before anthesis infection times whereas Mercato has a decrease level of DON. Thus, this trial has shown differences in FHB susceptibility between varieties according to wheat growth stage, but also differences in accumulation of the two toxins studied. This first result has to be verified in a specific trial with a wide range of varieties but it is confirmed, it could lead to the possibility of a fungicide protection adapted to the variety. Nevertheless, before considering this specific treatment, we have to now if in natural conditions, the ejection of ascospores is possible during the late stages of wheat. On this point, Caron *et al.*, in 2006, showed a decrease in the liberation of spores from heading to ripening stage. Nevertheless, spores could be liberated several days after flowering, and in that case, a late fungicide application on variety such as Caphorn could be a useful way of improving efficiency of FHB treatment. Moreover, experiments carried out in recent years on durum wheat in the region Centre support this view.

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