COMPARISON OF FUSARIUM CULMORUM ISOLATES ASSOCIATED WITH VIRULENCE ON WHEAT

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Abstract

Fusarium culmorum is one of the most important causal agents of Fusarium head blight (FHB), results in yield and quality reductions and mycotoxin accumulation. It predominates on cereals in North-West of Turkey in cereal growing fields. In this research the aggressiveness among the F. culmorum isolates and reduction on yield was evaluated. Two wheat varieties (Golia and Ceyhan) and 16 isolates of the fungus were enclosed in the research. In addition, the mycotoxin content of zearalenone (ZEA) in the infected kernels was also compared. The wheat heads were inoculated by a syringe at mid-anthesis. Head blight severity was assessed 3 times and the data was used to calculate the area under disease progress curve (AUDPC). Significant variation in aggressiveness of isolates of F. culmorum was observed in both wheat varieties tested. The isolates of F. culmorum reduced significantly thousand kernel weights (TKW) at different levels in both varieties. No interaction was found between ZEA accumulation and isolates or wheat varieties. There was a positive correlation between head weight and TKW, but negative correlation between AUDPC and head weight or TKW. No correlation was found between ZEA and any other parameters assessed. We conclude that the enclosed isolates of F. culmorum differed in aggressiveness and reduced yield at different levels; ZEA accumulation was, however, not correlated with the disease severity. This result suggests that ZEA is not required for pathogenicity; therefore, selection in wheat for reduced visible disease symptoms does not reduce the risk of ZEA contamination.

Key words: *fusarium culmorum, wheat, audpc, head weight, thousand kernel weight, zea*

INTRODUCTION

One of the diseases that have gained importance among the head diseases of wheat in recent years is Fusarium Head Blight (FHB). It was stated that *Fusarium* species can reduce the yield up to 50 % (Cook 1968). Although it is described that *F. graminearum* is the predominant species causing FHB in the world, but it is known, that in the northern part of Europe F. culmorum causes mainly this disease (Snijders 1989).

FHB has a great economic impact worldwide because it is very destructive in wheat, barley and other small grain cereals. FHB epidemics are common in North America since the last 25 years. It has caused nearly $ 4 billion economic damage in the U.S and Canada in the 1990s (McMullen *et al*. 1997, Windels 2000). Recent FHB epidemics in Asia, Europe and South Africa are threatening the world's food supply (Goswami and Kistler 2004). Some *Fusarium* species synthesize secondary metabolites called mycotoxins (Nelson *et al*. 1993). These are species-specific, for example *F. graminearum and F. culmorum* produce mainly Deoxynivalenol an derivates of trichotehecenes, but also zearalenone and nivalenol, *F.verticillioides* produces fumonisin (Nelson *et al*. 1993).

ZEA's largest effect is seen in the reproductive system. In Australia it is reported that ZEA causes infertility in ruminants (Reed and Moore 2009). It has been known for many years that ZEA causes hyperestrogenism in pigs. In a study conducted with pregnant pigs fed with feed containing 25, 50, 100 ppm 95% purified ZEA, it was stated that premature birth, brucellosis, small and low body weight babies occurred (Chang *et al*. 1979).

Mycotoxin production of *F. culmorum* isolates has usually been investigated for DON. In a study of different wheat genotypes Snijders and Perkowski found that when inoculated with different

pathogenic isolates, virulence is in positive correlation with DON accumulation and yield reduction (Snijders and Perkowski 1990).

In the year 1995, when it was rainy during spring and summer in Bulgaria, ZEA, DON, 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), T-2 toxin and 4,15-diacetoxyscirpenol (DAS) presence were investigated in 140 samples collected after wheat harvest. DON and ZEA were the predominant toxins, with a contamination frequency of 67% and 69%, respectively. Maximum concentration analyzed, was 120 μg/kg for ZEA and 1800 μg/kg for DON (Vrabcheva *et al*. 1996).

In a Turkish study conducted in the Marmara region in 1997-1998 (Tunalı *et al*. 2006), it was observed that *F. culmorum* and *F. graminearum* infection led to significant crop losses and degradation of quality due to high DON contents. Pathogenicity studies made with 39 domestic and foreign wheat varieties revealed that there were statistically significant differences in the susceptibility to *F. culmorum* and *F. graminearum* and a positive correlation between FHB and the production of DON was found (Tunalı *et al*. 2006). Mert-Turk et al. (2014), reported that *F. graminearum* is common in wheat fields in Marmara Region of Turkey, and they also reported that all isolates were DON-producers.

The aim of this study was to investigate the effect of different *F. culmorum* isolates on yield parameters in the two wheat varieties Golia and Ceyhan. The other purpose of this study was to determine disease progression of sixteen *F. culmorum* isolates in both wheat varieties and to quantify the amount of ZEA in diseased grains after harvest. It was also investigated whether there is a correlation between *F. culmorum* infection and ZEA content.

MATERIALS AND METHODS

Trial Materials and Inoculation

In this study the following two wheat varieties were used: Golia which is tolerant/resistant against *F. culmorum* and many other pathogens (F. Mert-Türk, personal observations) and Ceyhan which is susceptible but has a wide planting area in Turkey. Sixteen *F. culmorum* isolates were collected from different geographic origins; those of 15 from Turkey and 1 from the UK as it is known to be highly virulent. They were recovered from infected wheat crowns or heads as described by Mert-Turk and Gencer (2013) (Table 1). Infected host tissues and crown were surface sterilised with 1% sodium hypochlorite and rinsed several times with sterile distilled water and air dried. Then they were placed in potato dextrose agar (PDA) medium containing 100 μg/ml streptomycin sulphate. The plates were incubated at ±25°C for 5–7 days. Monoconidial isolates were obtained by streaking spore suspensions onto water agar plates and single conidium was transferred onto new plates.

Randomized plot design with three replications was established during the production season 2010-2011. Each plot consisted out of four rows with 0,21 m in between the rows and plot length was 7 m long. Thirty heads in each plot were selected for inoculation

Fungal cultures of 3 - 4 weeks developed on petri dishes containing potato dextrose agar (PDA) were used for artificial inoculation. The aerial mycelium were scraped gently from Petri dishes, homogenized with sterilised distilled water. Spore suspension was adjusted to 5 $x10^5$ conidia/ml and 1 drop of Tween 20 was added (Miedaner *et al*. 2003).

For each plot 30 heads were inoculated using a hypodermic syringe method at the mid-anthesis stage (Miedaner *et al*. 2003). Two spikelets (front and rear) in the middle of heads were injected with about 10 ml aliquots of the spore suspension and then heads were covered with transparent polyethylene bags for 48 hours.

Table 1. Code names, origin and plant tissues from which *Fusarium culmorum* isolates were recovered.

***F. culmorum* Isolate**	**Plant Tissue Isolated**	**Origin**
M-103a	CR	Şarköy, Tekirdağ, TR
L-201a	CR	Biga, Çanakkale, TR
D-807a	CR	Burhaniye, Balıkesir, TR
B-045	CR	Ezine, Çanakkale, TR
T-107	CR	Çorlu, Tekirdağ, TR
B-211	Head	Gelibolu, Çanakkale, TR
B-094	CR	Ezine, Çanakkale, TR
B-002	CR	Ezine, Çanakkale, TR
B-174	Head	Eceabat, Çanakkale, TR
L-606a	Head	Eceabat, Çanakkale, TR
B-044	CR	Ezine, Çanakkale, TR
N-2-D-5	Head	Çorlu, Tekirdağ, TR
K-502b	CR	Lapseki, Çanakkale, TR
B-083	CR	Ezine, Çanakkale, TR
N-905	CR	Gönen, Balıkesir, TR
Fg4	Head	UK

CR, crown rot; Head: infected heads

Monitoring of Disease Development

Disease development was assessed at 7, 14 and 21 days after inoculation. Disease development was evaluated by measuring the length of the visually diseased spikelets with a ruler and relating it to the whole length of the head (modified from Miedaner *et al*. 2003). The data obtained from three different observations for each isolate were used to calculate the area under the disease progress curve (AUDPC) as proposed by Shaner and Finney 1977.

Calculating the Effects of *Fusarium culmorum* Isolates on Yield Parameters

The artificially inoculated heads with *F. culmorum* were harvested by hand after maturation GS 90 (Zadoks *et al*., 1974) and head weight was weighed. Head weight (HW) varied greatly between artifically infected and control plots. Therefore in order to interpret the data better, weights was subjected to analysis of variance after converted to the relative weight. The relative weight was calculated by dividing the weights obtained from diseased kernels to the weight in the control plot. The relative weight was also applied to a thousand kernel weight (TKW).

Analysis of ZEA content in Infected Wheat Samples

ZEA content was determined in grains obtained from diseased and control heads. All infected heads. The used method based on extraction of the analysis sample with acetonitrile/water, clean-up of ZEA with immuno-affinity column (IAK, R-Biopharm AG, Germany) that contains specific monoclonal

antibodies, ZEA's elution from the column with acetonitrile and determination with reverse-phase liquid chromatography with flourescense detector (De Saeger *et al*. 2003).

For standardization of HPLC, zearalenone standards (Sigma, USA) concentration was diluted with HPLC grade as 8-10 µg/ml. Maximum absorbance measurement that is observed around 274 nm in a spectrophotometer wavelength of prepared stock solution was made and concentration was calculated (Fazekas and Tar 2001).

To form a calibration chart, standard solutions prepared at 5 different concentrations were injected 3 times to HPLC. In the preparation of the calibration chart, standard ZEA that contains 25, 250, 500, 1000 and 1250 ng/ml (ppb) was used. At the end of the analysis with the calculation of the areas obtained after each injection, calibration chart was formed.

Statistical Analysis

The data were analyzed using SAS V8 statistical software package (SAS 1999). Analysis of variance was performed using the PROC GLM command and to compare the levels of found significant variance components analysis, least significant difference (LSD) test was utilized. In order to determine the relationship between the properties examined, Pearson correlation coefficients were determined by PROC CORR command.

RESULTS

All *F. culmorum* isolates from different origins and recovered from different plant tissues were pathogenic on both wheat varieties used in this study. Fungal isolates and wheat varieties had a significant effect on AUDPC, TKW, HW and ZEA concentration (Table 2).

Table 2. Significance of mean square values for AUDPC, head weight (HW),, thousand kernel weight (TKW) and ZEA concentration for each source of variation (P ≤ 0,001***).

		Mean Square Values			
Source of variance	**df**	**AUDPC**	**HW**	**TKW**	**ZEA**
Repeat	2	232,3	0,007	0,002	19,63
Isolate	15	3160,31***	0,0267***	0,0370***	1239,80***
Variety	1	49065,51***	0,2871***	3,0745***	1976,90***
Variety * isolate	15	509,02***	0,0035	0,0110***	1574,63***
Error	62	32,9	0,002	0,0003	7,44
Total	95				

Aggressiveness measured as AUDPC differed significantly among the sixteen *F. culmorum* isolates inoculated onto wheat spikes of the varieties Ceyhan and Golia. The mean value of AUDPC in Ceyhan was much higher than obtained from Golia. For example, when inoculated to Ceyhan wheat variety, D-807a isolate's AUDPC value was higher compared to most isolates whereas disease progress by L606a was the least. Also for Golia, it was also determined that AUDPC values varied among isolates. An isolate that caused fastest disease development was T-107 (AUDPC value was 109,5), whereas the slowest was B-211 (AUDPC value was 38,94) (Table 3).

Head weight was affected negatively by all isolates inoculated on both wheat varieties. Although isolate effect was not significant among isolates within a variety, in total Ceyhan (0,61) was more affected than Golia (0,72) (Table 3). As a result of the analysis of variance, variety, isolate and variety isolate were to have a significant influence on TKW. In consideration of the relative TKW, Golia was observed as less affected as Ceyhan (Table 3).

There were also significant differences between wheat varieties and fungal isolates for ZEA accumulation. Mean ZEA amount in Ceyhan wheat variety over all *F. culmorum* isolates was about 21.7 µg/kg, in Golia this amount was only 12.6 µg/kg. For Golia however three of the tested *F. culmorum* isolates did not accumulate any detectable amount of ZEA.

The highest amount of ZEA was accumulated by the isolate B-002 in Ceyhan and isolate Fg4 in Golia. In our study isolates accumulating high level of ZEA in one wheat variety not necessarily producing similar amounts in the other variety.

When compared the ZEA amount that *F. culmorum* isolates produced in two different varieties, it was determined that no matter what the varieties are some isolates accumulate the same amount of ZEA, but some of them have differences. These differences were generally found high in Ceyhan (B-211, B-002 and N-905), only Fg4 isolate's production of ZEA was found to be higher in Golia (Table 3).

Table 3. Comparison of AUDPC, thousand kernel weight (TKW), head weight (HW) and quantification of the amount of ZEA after the artificial inoculation of two different wheat varieties with16 different *Fusarium culmorum* isolates.

***F. culmorum* Isolates**	**AUDPC**		**TKW**		**HW**		**ZEA**	
	Ceyhan	**Golia**	**Ceyhan**	**Golia**	**Ceyhan**	**Golia**	**Ceyhan**	**Golia**
Kontrol							0,00 Ha*	0,00 Fa
M-103a	121,67 D-	65,43 C-Eb	0,23 EFb**	0,61 Fa	0,6	0,77	13,49 EFa	0,00 Fb
L-201a	111,29 FGa	58,62 EFb	0,26 Eb	0,69 Da	0,63	0,74	11,44 E-Ga	0,00 Fb
D-807a	149,51 Aa	81,30 Bb	0,18 Hb	0,60 Fa	0,5	0,63	7,07 Ga	9,43 B-Ea
B-045	130,69 CDa	86,85 Bb	0,20 GHb	0,65 Ea	0,56	0,71	7,26 Ga	12,39 Ba
T-107	137,34 BCa	109,50 Ab	0,22 FGb	0,59 Fa	0,53	0,62	13,40 EFa	10,63 B-Da
B-211	67,21 IJa	38,94 Gb	0,44 Ab	0,71 CDa	0,75	0,82	49,21 Ba	0,00 Fb
B-094	127,93 C-Ea	71,49 Cb	0,20 Gb	0,50 Ha	0,53	0,7	6,95 Ga	8,08 DEa
B-002	87,88 Ha	60,27 DEb	0,35 Db	0,54 Ga	0,67	0,73	89,60 Aa	7,99 DEb
B-174	67,97 Ia	45,34 Gb	0,44 ABb	0,74 BCa	0,63	0,79	19,04 Da	11,59 BCa
L-606a	56,83 Ja	42,00 Gb	0,41 Cb	0,79 Aa	0,75	0,79	14,3 DEa	6,69 Eb
B-044	142,24 ABa	66,99 C-Db	0,23 Fb	0,61 Fa	0,52	0,65	28,64 Ca	6,93 Eb
N-2-D-5	119,51 EFa	58,3 EFb	0,37 Db	0,77 ABa	0,67	0,72	8,06 Ga	11,02 B-Da
K-502b	114,09 FGa	60,04 D-Fb	0,23 EFb	0,64 Ea	0,62	0,74	8,78 FGa	8,07 DEa
B-083	129,43 C-Ea	69,93 Cb	0,22 FGb	0,60 Fa	0,54	0,68	7,47 Ga	11,54 BCa
N-905	103,64 Ga	52,75 Fb	0,22 FGb	0,69 Da	0,59	0,74	47,82 Ba	8,80 C-Eb
Fg4	68,97 Ia	45,01 Gb	0,42 BCb	0,61 Fa	0,73	0,75	14,52 DEb	88,66 Aa
Mean	**108,51** a	**63,3** b	**0,29** b	**0,65** a	**0,61** b	**0,72** a	**21,69** a	**12,61** b

*Uppercase letters show the difference between isolates in one variety, **lowercase letters show the difference between the two varieties for one isolate. Different letters indicate that there is a statistical difference (p =0,05).

Correlation between all parameters observed revealed that there was a positive correlation between the HW and TKW but a negative relationship between the HW and AUDPC, and between TKW and AUDPC. In addition, it was determined that there was no correlation between the ZEA content and HW, TKW or AUDPC (Table 4).

DISCUSSION

Despite the development of the disease was taken 7, 14 and 21 days after inoculation in the present study, it is seen that in some other studies the first observation was taken only14 days after inoculation, and the following measurements after 21 and 28 days after inoculation (Chrpová *et al*. 2007). In some studies 1-9 scale was used to determine the progress of the FHB (Chrpová *et al*. 2007, Perkowski *et al*. 1995). In some other studies, the observations were made with the proportion in the total number of diseased spikelets to the number of all spikelets in the head (Miedaner *et al*. 2003). In this study we measured the diseased area of the head with a ruler and calculated the proportion to the total head length. With all three methods mentioned numerical data are gained that assess the disease spreads in relation to the whole head.

AUDPC value provides a single value for the development of a disease and is a method that has been reported in many studies (Jeger and Viljanen-Rollinson 2001, Steiner *et al*. 2004). In this study, AUDPC allowed to make better interpretations of the observations that were made in three different times. Generally lower AUDPC values were seen in Golia and general average was 63.3, in contrast to Ceyhan where the average AUDPC value was 108.51 (Table 3). In terms of AUDPC, it is seen that *F. culmorum* isolates in general were more virulent to Ceyhan than to Golia. Different AUDPC values obtained revealed the differences in aggressiveness among the isolates. It is seen that some isolates causes high disease severity (for example D-807a in Ceyhan, T-107 in Golia), whereas some causes low disease severity (for example L-606a in Ceyhan, B-211 in Golia). It was previously reported that disease severity by an isolate can differ in aggressiveness in different wheat varieties (Ittu *et al*. 2000). But on the contrary in another study, it was reported that there is no interaction between the virulence of the isolates and also there is no relationship between the isolate and the variety (Gilbert *et al*. 2001).

Table 4. Correlation coefficient and severity levels related to the parameters examined in the field trial (P≤0,001***)

	HW	TKW	AUDPC
TKW	0.75199***		
AUDPC	-0.85136***	-0.85006***	
ZEA	0.03211	-0.13771	-0.06465

HW and TKW were affected negatively by inoculation of any isolates of *F. culmorum.* It was observed that isolates that caused high AUDPC value reduced the thousand kernel weight of the inoculated spikes. It was determined that there is a negative correlation between the two parameters. The ability of an isolate to cause high disease severity was expressed by a high AUDPC, therefore any reduction in HW and TKW due to high AUDPC values was expected because of less nutrient supply. In the present study a positive correlation between TKW and HW was revealed in both varieties.. Tunali et al., found TKWs between 9,36-33,00 g after *F. culmorum and F. graminearum* inoculation (Tunalı *et al*. 2006). In another study Snijders and Perkowski, showed that losses in thousand kernel weights are significant in blighted kernels (Snijders and Perkowski 1990).

HPLC method which is one of the most preferred method to determine ZEA was used in this study (Omurtag and Beyoğlu 2003). ZEA quantities that *F. culmorum* isolates accumulated varied between 0 - 88 µg/kg in Golia and 7 - 89 µg/kg in Ceyhan (Table 3). However, when viewed overall samples, the average was about 21.7 µg/kg ZEA content in Ceyhan and 12.6 µg/kg in Golia. Varga et al. and Ohlinger et al. have found ZEA amounts between 3,5 – 18 µg/kg, 20-240 µg/kg respectively and these results are similar to those obtained in this study (Varga *et al*. 2004, Ohlinger *et al*. 2004). Therefore, wheat variety with high tolerance to the disease could be preferred over susceptible varieties when ZEA content and yield loss are concerned.

It was seen that all of the isolates tested in Ceyhan wheat variety produce detectable levels of ZEA. The highest amount was obtained from the kernels that were inoculated with B-002 isolate. AUDPC value of B-002 isolates was about 87 and this value is below the general average (108.5). The isolate that had the highest AUDPC was D-807 and ZEA amount that this isolate accumulated was 7 µg/kg (average 21.69 µg/kg) .

After artificial inoculation of Golia wheat variety, it was observed that M-103a, L-201a and B-211 isolates cannot accumulate detectable amount of ZEA in grains. These isolates could cause certain proportions of disease symptoms on the head after inoculation and cause a slight decline in yield (Table 2). This result showed that ZEA production in host tissues is not specifically required for pathogenicity.

It was observed that *F. culmorum* isolates that had high virulence in both wheat varieties did not produce higher amount of ZEA. As a result of the correlation analysis ZEA does not appear to be correlated with any parameter that was taken into account for the analysis. According to these results, there is no relationship between the disease severity and ZEA; therefore we conclude that ZEA does not play a role in the pathogenesis

F. culmorum is a fungal species which is also capable of producing DON. It was determined that there is a positive correlation between the virulence of the isolates of *F. culmorum* and DON content (Tunalı *et al*. 2006, Perkowski *et al*. 1995, Gang *et al*. 1998). However in a study conducted by Mesterházy and Bartok they emphasized that the resistance of one wheat variety to Fusarium head blight can be correlated with the potential mycotoxin contamination but there are many exceptions. Hence, high mycotoxin production in an infection site can have a low disease symptom or vice versa (Mesterházy and Bartók 1992).

It was determined that after inoculation into the head, DON can be detected after 48 hours. Late detection of DON in the development of disease emphasizes that it does not play a role at the time of first infection (Mirocha *et al*. 1997). Gaffoor *et al*. has reported that ZEA is not necessary for *F. graminearum* to infect wheat (Gaffoor *et al*. 2005).

In contrast to the reports above, some reports show that despite there is a small amount of disease appearance in a wheat variety, there could be a high level of DON and ZEA in infected tissues (Šip et al. 2007). In contrast low amount of DON and ZEA was quantified in moderately diseased heads (Bai 2004). In another study conducted with 35 wheat varieties, after the inoculation with *F. culmorum* there was a positive correlation found between DON content and disease severity, but no correlation for ZEA (Chrpová *et al*. 2007).

Apart from the mentioned studies above, it has not been reported that there is any other studies about the relationship between the virulence of *F. culmorum* isolates and ZEA production. *F. culmorum* is one of the most dominant *Fusarium* species found in the region that cause both FHB and crown rot. The region is also important especially for animal growing and cheese production. Mycotoxin accumulation in cereal tissues could have impact specially in animal farms. Therefore as it is shown in this research, culturing resistant or more tolerant varieties could increase yield as well as reduce the mycotoxin risks for animals.

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