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Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to *Fusarium* head blight

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Abstract

The data available indicate that aggressiveness of *Fusarium graminearum* and *F. culmorum* depends on their deoxynivalenol (DON) and nivalenol-producing capacity: toxin-producing ability correlated closely with the level of aggressiveness measured. This agrees well with other literature findings. However, the resistance of a cultivar influenced DON production significantly. In the most resistant genotypes, toxin contamination remained near zero, whereas the same isolates and inoculum produced very high toxin levels in susceptible cultivars. As toxin levels were correlated with the ratio of *Fusarium*-damaged kernels (FDK) and this ratio is very low in highly resistant cultivars, the conclusion is that the level of resistance level is more important in governing DON accumulation in a given cultivar than is the aggressiveness of an isolate. In susceptible cultivars, DON producing ability is decisive, but in highly resistant cultivars resistance is the major factor in suppressing disease development and DON accumulation. In different years, the same FDK values were associated with different DON concentrations and this depended very much on the precipitation towards the end of May, the time of inoculation.

Introduction

Following the large Fusarium head blight (FHB) epidemics in the USA, Canada, China AND Europe (including Germany, Hungary, Romania and Croatia) significant research has been devoted to the breeding of resistant cultivars. However, alternative solutions could originate from a better understanding of the host–pathogen relationship, the nature of resistance and an understanding of the role of deoxynivalenol (DON) and other trichothecenes in pathogenesis.

The resistance of wheat to FHB is a complex phenomenon. The forms, types or components of physiological resistance (Mesterházy, 1995; 2001) are: (i) resistance to initial infection (Schroeder and Christensen, 1963); (ii) resistance to spreading (Schroeder and Christensen, 1963); (iii) resistance to kernel infection (Mesterházy, 1995; Mesterházy et al., 1999); (iv) tolerance to infection (Mesterházy, 1995; Mesterházy et al., 1999) and (v) resistance to DON accumulation (Miller et al., 1985).

Research on two other forms of resistance is in progress. Resistance to late blighting means low *Fusarium*-damaged kernel (FDK) values even when a long rainy period occurs at harvest (Mesterházy, unpublished data). Such resistance contrasts with cultivars with low FHB values, but high FDK values at harvest. The resistance to head death above the infection point of the head means that transport of assimilates is still possible following infection of the rachis. As a result, seed size is near normal. In susceptible genotypes, the transport vessels cease function, mycelial masses inhibit transport and the grains shrivel even though they are free of infection. However, the relationship between these parameters and their genetic background is not clear. The best-known component is the resistance to pathogen spread; all genetic work refers to this form of resistance. The genetic inter-relations of these

components are not known. In medium-susceptible, medium-resistant or susceptible genotypes, resistance components have been identified and provide information about the risks of the given genotypes. In the most resistant cultivars, however, there are no significant yield losses, the ratio of FDK is very low, FHB severity is small and toxin contamination is also very low. It seems that at high resistance the significance of these components is much less or negligible (Mesterházy et al., 1999).

The morphology of a cereal crop also plays a role in natural infection. Dwarf types are more susceptible than tall cultivars and wheat genotypes with awns are more at risk than tall or awnless types (Mesterházy, 1987; 1995). Cultivars with longer internodes below the heads are less infected (Parry et al., 1995). Resistance at the seedling stage, crown rot resistance and resistance of the leaves has also been demonstrated, but useful relationships with FHB resistance for breeding were not found (Mesterházy, 1983; 1987).

Materials and methods

The methods for fieldwork in resistance research, toxin and ergosterol evaluation have been described by Mesterházy et al. (1999), Lamper et al. (2000) and Mesterházy and Bartók (1996, 1997, 2001). Inoculation was made by spraying the heads and covering them with polythene bags for 24 h. Each isolate was used in three replicates. Fusarium head blight was evaluated as a percentage of the spikelets of 15–20 heads 10, 14, 18, 22 and 26 days after inoculation (full flowering). Their mean value was used in the analyses and AUDPC values were also calculated. From each group of heads, ten spikelets were separated randomly and threshed at low wind speed by a Wintersteiger head thresher 'Seedboy'. The ratio of FDK, that is, the ratio of visually infected scabby or tombstone kernels, was measured. The weight of grains was measured to provide an indication of any yield responses. The combined samples of the three replicates were evaluated for DON and ergosterol content with HPLC. For this 6 g grain was used, milled, and 5 g was evaluated.

Results

Instability of DON production

The DON-producing ability of isolates was not consistent. Very large differences occurred under different conditions. One of the main factors was the interrelationship with cultivar resistance. Table 1 shows the mean results of a three-year test for DON with 25 genotypes and four isolates each of *Fusarium graminearum* and *F. culmorum* (Mesterházy et al., 1999). The mean toxin production in the cultivars in response to the same isolates was different, ranging from 0.32 $\mu g\, g^{-1}$ (ppm) in the most resistant genotypes to 42 $\mu g\, g^{-1}$ in the most susceptible genotypes. Striking differences were also found for the mean performance of isolates with different toxin-producing capacities. It seems that the characterization of DON production for an isolate is much more accurate than for a randomly chosen wheat cultivar. Even when correlations between DON data for isolates and genotypes are significant, there remain differences in toxin concentrations between wheat genotypes when values for mean toxin concentrations in isolates are very similar (for example, see data for *F. graminearum* isolates 40 and 44). We do not know the cause of the three-fold difference in DON concentrations in wheat cultivars for isolates from Bence or Csaba, but the conclusion is clear: to understand fully the influence of cultivar on toxin production we need to study more isolates. The same conclusion is valid for isolate aggressiveness where more cultivars are necessary to gain reliable conclusions. This work is usually not done in this way. Usually one isolate or a mixture of isolates are used on several genotypes, or several isolates are tested on one or more cultivars. As a result, the reliability of the data is low. For this reason, data from such tests are less suitable for drawing general conclusions. It should be pointed out that the *F. graminearum* and *F. culmorum* isolates produced very similar resistance rankings, with correlations normally above $r = 0.90$; $P = 0.001$.

Influence of cultivars

The cultivars influence symptom development and other traits (Table 2). The fact that DON concentrations closely correlate with the ratio of FDK and the extent of FHB proves that toxin contamination is a consequence of the amount of disease observed. Low FDK ratios mean low toxin contamination. When we calculate the ratios for DON/FDK or DON/FHB, we see that in resistant genotypes significantly higher values are needed to produce 1 $\mu g\, g^{-1}$ DON than in susceptible cultivars. In the highly resistant group, 2–8% FHB and 2–4% FDK produces 1 $\mu g\, g^{-1}$ DON. In the more susceptible group, 0.82–1.50% FHB or 0.8–1.5% FDK is needed to produce the same amount. It appears, therefore,

Table 1. FHB resistance of 25 wheat genotypes to *Fusarium* isolates, mean data for DON concentrations in μg g⁻1 ppm, 1994–1996

Genotype	Isolates								Mean
	89.4 Fc	12375 Fc	12377 Fg	207 Fg	12551 Fc	44 Fg	40 Fg	223 Fc	
Sgv-NB ∗ MM-Sum3	0.3	0.0	0.4	0.6	0.3	0.8	0.0	0.2	0.3
Arina	0.4	0.8	1.3	0.0	1.0	1.1	4.2	1.6	1.3
Sgv-NB ∗ MM-Sum3	0.3	1.2	1.5	1.0	1.3	1.4	1.8	5.2	1.7
Ringó Star	0.8	1.9	1.9	2.4	3.0	3.0	1.3	2.2	2.1
RSt- MM/NB	2.1	0.0	0.9	5.8	0.6	3.1	1.5	4.1	2.3
HD84.42	2.3	5.3	3.4	4.2	3.9	4.0	11.0	10.6	5.6
81.60-NB/Kö	4.5	5.4	3.0	3.8	5.2	8.2	5.6	12.8	6.1
P4371.88	2.3	6.2	3.4	4.2	6.1	5.9	10.9	10.2	6.2
SK8090	4.2	2.2	8.5	0.5	1.3	14.1	13.0	8.7	6.6
Siouxland	1.1	1.6	6.4	4.0	4.9	9.4	11.9	19.0	7.3
P2118.89	11.4	8.1	7.3	4.4	2.5	10.9	17.3	14.5	9.5
Sum3ˆ2-81.60	2.5	4.1	2.3	10.0	10.4	12.0	18.2	18.2	9.7
Rechsler	11.5	4.2	3.3	14.8	11.3	18.1	22.8	9.1	11.9
Sgv-GT//Pdj2/Uhr	1.7	8.7	3.9	9.7	12.8	9.9	29.8	25.2	12.7
Kincsö	7.1	8.6	8.1	5.0	13.5	15.7	15.6	35.9	13.7
Kende	9.9	5.8	7.9	13.7	19.9	16.1	22.7	19.2	14.4
Bence	8.2	9.6	7.8	21.8	25.0	43.6	14.8	16.0	18.3
Szöke	5.4	8.9	8.6	27.4	25.7	30.3	29.2	16.5	19.0
78.1.4	11.5	3.8	25.9	7.5	10.7	12.2	39.0	41.7	19.0
Góbé	14.3	11.2	25.4	6.2	18.5	22.9	16.9	39.1	19.3
Jbj-50	9.0	16.2	18.2	19.0	31.8	41.9	19.8	33.6	23.7
Csaba	12.3	17.6	16.3	21.2	38.4	23.2	49.7	67.1	30.7
Zugoly	8.8	19.6	15.6	20.9	35.1	60.3	38.4	59.2	32.2
Örség	9.3	20.5	16.1	30.9	47.1	56.2	53.2	54.6	36.0
Zombor	17.7	18.3	24.1	60.6	52.7	58.1	65.5	41.1	42.3
Mean	6.4	7.6	8.9	12.0	15.3	19.3	20.6	22.6	14.1
LSD 5%									8.6

Fc = *F. culmorum*; Fg = *F. graminearum*.

that DON decomposing mechanisms are present in resistant genotypes (see also Miller and Arnison, 1986).

Climatic effects

Another cause of variation in DON production are the differences in weather and other factors, that occur in different years. The same isolate may, in different years, cause highly significant differences in disease development which are associated with differences in DON concentrations. The highly significant year–isolate interactions show this clearly. This means that more accurate data would be obtained for an isolate if inocula were tested over several years. The influence of year on DON contamination has another aspect. When the general means for the disease parameters and DON production are compared on an annual basis, we see that there are ten-fold differences between the amount of FDK or FHB needed to produce 1 μg g^{-1} DON. What is the cause of this year effect?

We compared the mean values of FHB, FDK and DON, and yield loss for 1990–2000 with the meteorological data. A close correlation was found between the amount of DON produced and the amount of precipitation between May 20 and 31 ($r = 0.6422$; $P = 0.05$). The amount of precipitation for the whole of May showed no significant relationship with DON contamination level. The between year DON–FHB and DON–FDK correlations were between 0.4 and 0.5. Yield loss was strongly influenced by FHB ($r = 0.7651$; $P = 0.01$) and kernel infection ($r = 0.7996$; $P = 0.01$). Interestingly, June precipitation did not show a relationship with DON or with the other traits. However, the large precipitation from 1st to 20th July significantly increased kernel infection ($r = 0.6520$; $P = 0.05$) and yield loss ($r = 0.7688$; $P = 0.01$). The mean temperatures for the three months separately or together did not significantly influence DON levels or the other traits. The significance of the precipitation in the last third of May was supported by the data set from fungicide

Table 2. Mean data for the FHB test with eight Fusarium isolates, 1994–1996

Genotype	Trait				Ratio for	
	DON $\mu g\,g^{-1}$	FHB %	FDK %	Yield loss %	FHB/DON	FDK/DON
Sgv-NB ∗ MM-Sum3	0.32	2.61	0.92	3.68	8.16	2.87
Sgv-NB ∗ MM-Sum3	1.7	3.45	2.03	7.54	2.03	1.19
Arina	1.28	5.28	3.88	7.61	4.12	3.03
RSt- MM/NB	2.26	7.95	5.02	12.9	3.52	2.22
Rsztár	2.06	10.61	7.08	11.75	5.15	3.44
Siouxland	7.28	13.1	25	25.93	1.8	3.43
SK8090	6.55	14.82	15.54	22.65	2.26	2.37
Sum3$\hat{2}$-81.60	9.72	15.07	11.59	24.11	1.55	1.19
P2118.89	9.54	16.92	30.42	19.8	1.77	3.19
P4371.88	6.15	19.09	17.19	27.32	3.1	2.8
HD84.42	5.59	20.58	17.26	17.43	3.68	3.09
81.60-NB/Kö	6.07	20.83	11.97	24.31	3.43	1.97
Bence	18.34	20.91	16.06	23.97	1.14	0.88
Rechsler	11.9	23.33	28.09	29.86	1.96	2.36
Kincsö	13.67	24.1	18.43	28.42	1.76	1.35
Szöke	18.99	26.15	19.49	33.19	1.38	0.97
Kende	14.41	27.9	22.32	42.53	1.94	1.55
Sgv-GT//Pdj2/Uhr	12.71	28.31	15.73	40.81	2.23	1.24
Jbj-5o	23.69	30.78	42.34	44.69	1.3	1.79
Góbé	19.31	31.51	32.09	37.11	1.63	1.66
78.1.4	19.03	31.72	40.1	39.31	1.67	2.11
Örség	42.25	34.52	43.17	48.21	0.82	1.02
Zombor	18.43	35.74	35.43	39.67	1.94	1.92
Zugoly	32.23	38.37	50.43	46.17	1.19	1.56
Csaba	30.72	38.92	26.45	46.89	1.27	0.86
Mean	13.37	21.7	21.48	28.23	2.43	2.00
LSDS 5 %	8.58	0.98	2.7	3.59		

Correlation analysis

	DON $\mu g\,kg^{-1}$	FHB %	FDK %
FHB %	0.8672		
FDK %	0.8199	0.8358	
Yield loss %	0.8693	0.9527	0.8294

All are significant at $P = 0.001$.

tests performed between 1992 and 1999. We can conclude that DON contamination is influenced more by the amount of precipitation after inoculation than by later rainfall. The late July rainfall increased FDK and also DON, but the correlation for DON was not significant. It seems that FDK and FHB influence DON less than precipitation, though these correlations were not significant. This means that epidemics with similar FDK values can result in ten-fold different levels of DON contamination. Such findings will have important consequences for health.

DON and aggressiveness

The next question concerns the relationship between the DON-producing ability of the pathogen and its disease-causing capacity. A close relationship was usually found between the aggressiveness of an isolate and its DON production (Mesterházy et al., 1999). The exception was *F. culmorum* isolate 89.4F. Here, high aggressiveness was associated with low DON production. Further analysis revealed that this isolate was a nivalenol-producing type with lower DON production. When nivalenol and DON were considered together, the toxin production (total trichothecene) corresponded to the aggressiveness we measured (Figure 1). In 1997, the results were very similar (Table 3). However, in that year, the nivalenol-producing isolate (89.4F) was also a good DON producer. It seems that the production of nivalenol or DON can be dependent on ecological parameters.

The data for the whole period 1990–2000 support these conclusions: correlations between DON

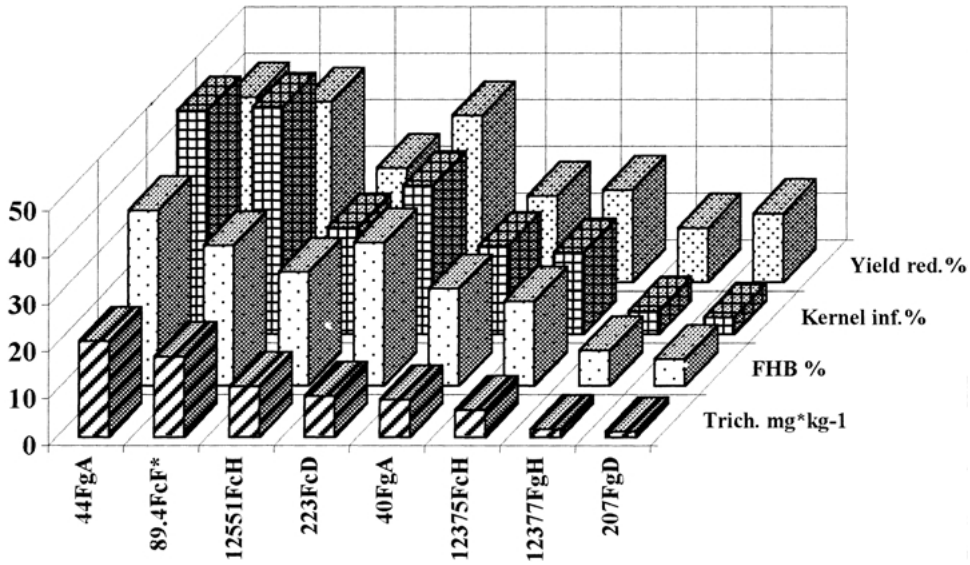

Figure 1. Aggressiveness and trichothecene (DON + NIV) content in FHB tests, 1994.

Table 3. Mean aggressiveness and DON production of *Fusarium* isolates on 22 cultivars, 1997

Isolates	FHB %	FDK %	Yield loss %	DON $\mu g\,g^{-1}$
12551Fc	4.97	5.3	8.51	1.55
39.01Fc	6.89	3.77	3.8	0.84
12377Fg	10.38	22.85	21.26	5.57
223Fg	19.03	35.78	35.24	9.62
40Fg	19.86	38.49	28.31	8.13
89.4FFc	21.52	25.21	31.58	0.16
207Fg	24.48	38.46	39.56	10.73
12375Fc	25.22	37.84	42.88	10.12
Mean	16.54	25.96	26.39	5.84

Correlation analysis

	FHB %	FDK %	Yield loss %
FDK %	0.9075***		
Yield loss %	0.9568***	0.9370***	
DON $\mu g\,kg^{-1}$	0.6635***	0.8478***	0.7591***

*** $P = 0.001$. Fc = *F. culmorum*; Fg = *F. graminearum*.

production and disease development were very close. However, correlations between DON concentration and FDK values were generally closer than between visual symptoms (FHB) and DON. This is to be expected: the same FHB severity may result in very different kernel infection severity and DON concentrations are determined from the grains. Here, resistance to kernel infection plays a crucial role.

Studying the data, we see that there are differences between isolates. Some isolates of *F. culmorum* produce relatively more DON than others and we see differences also in their ability to influence FHB, FDK and yield. Some can cause more FDK, some less FHB and others less yield loss. It seems, therefore, that the *Fusarium* population consists of different individuals or lines having different pathogenic characters including toxin-producing ability. The deviations are not large and the very close correlations show the basic trends that is, higher isolate aggressiveness results in higher FHB severity, higher FDK, higher yield loss and higher DON production.

It seems that ergosterol production (Lamper et al., 2000) is also closely linked with DON and FDK, indicating that aggressiveness is proportional to the fungal biomass in the infected grains (Figure 2). More highly aggressive isolates produced more severe infection, more fungal mass, more ergosterol and more DON.

From 1998, experiments were carried out with other *Fusarium* species. Figures 3 and 4 show the FDK and DON results from the 1998 multi-isolate tests only. Table 4 shows summary data for the 1998 cultivar tests, which indicate a very close relationship between traits. Results show that resistance to *F. graminearum* or *F. culmorum* is linked to resistance to *F. sambucinum, F. sporotrichioides* and *F. verticillioides*. The data for 1999 and 2000 are very similar and confirm that this also applies to *F. poae*, *F. avenaceum* and *F. nivale*.

Pathogen strains which do not produce DON have low aggressiveness ($r = 0.9448$ between FDK and DON). The small amount of DON associated with infection by these species is due to background

infection under epidemic field conditions. This also occurred in other field tests (Bai et al., 2000).

In the fungicide tests, where four *Fusarium* isolates were used, a significant reduction in symptoms was recorded (Table 5). This reduction, measured by FHB, FDK, yield loss and DON contamination, was proportional to the anti-Fusarium activity of the fungicides used. However, Amistar (azoxystrobin) and Kolfugo (carbendazim) produced an increase on susceptible cultivar in DON even though symptom severity was decreased.

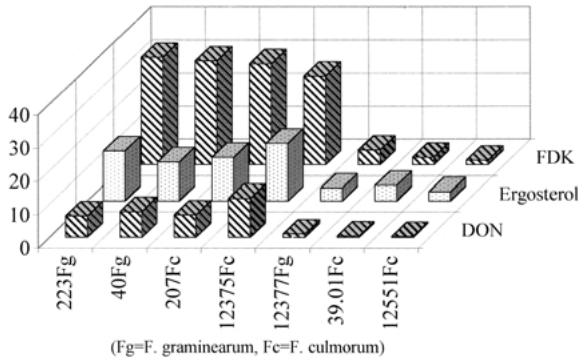

Figure 2. Ergosterol ($\mu g\, g^{-1}$), DON ($\mu g\, g^{-1}$) and FDK (%) values for *Fusarium* isolates, 1998. Fg = *F. graminearum*; Fc = *F. culmorum*.

Discussion

The data describe a complex influence of DON on the disease process. DON and other trichothecenes appear to play an important role in the aggressiveness of *F. graminearum* and *F. culmorum*. DON is a strong protein inhibitor (Snijders, 1994), and this may cause inhibition of enzymatic activity in susceptible hosts, leading to a rapid increase of the disease. This conclusion is supported by Muthomo et al. (2000), who reported a close correlation between aggressiveness and DON production in *F. culmorum* isolates. The results also show that aggressiveness closely correlated

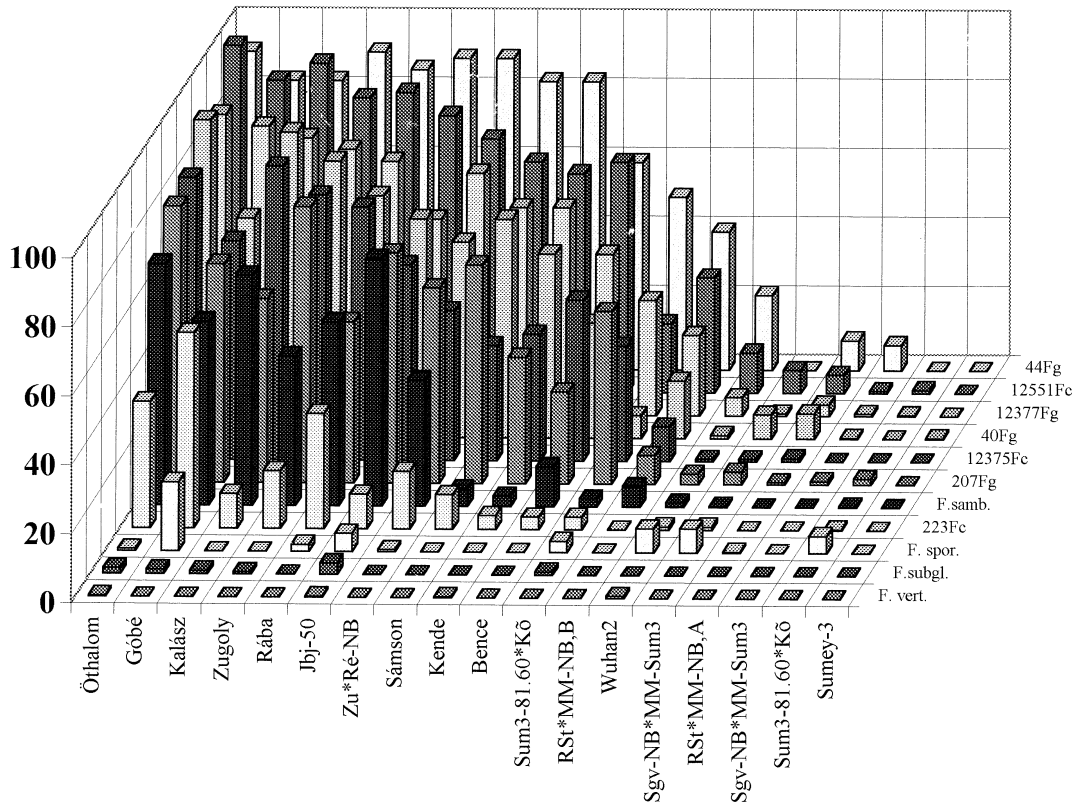

Figure 3. *Fusarium* head blight resistance in wheat to different *Fusarium* spp., FDK values %, 1998.

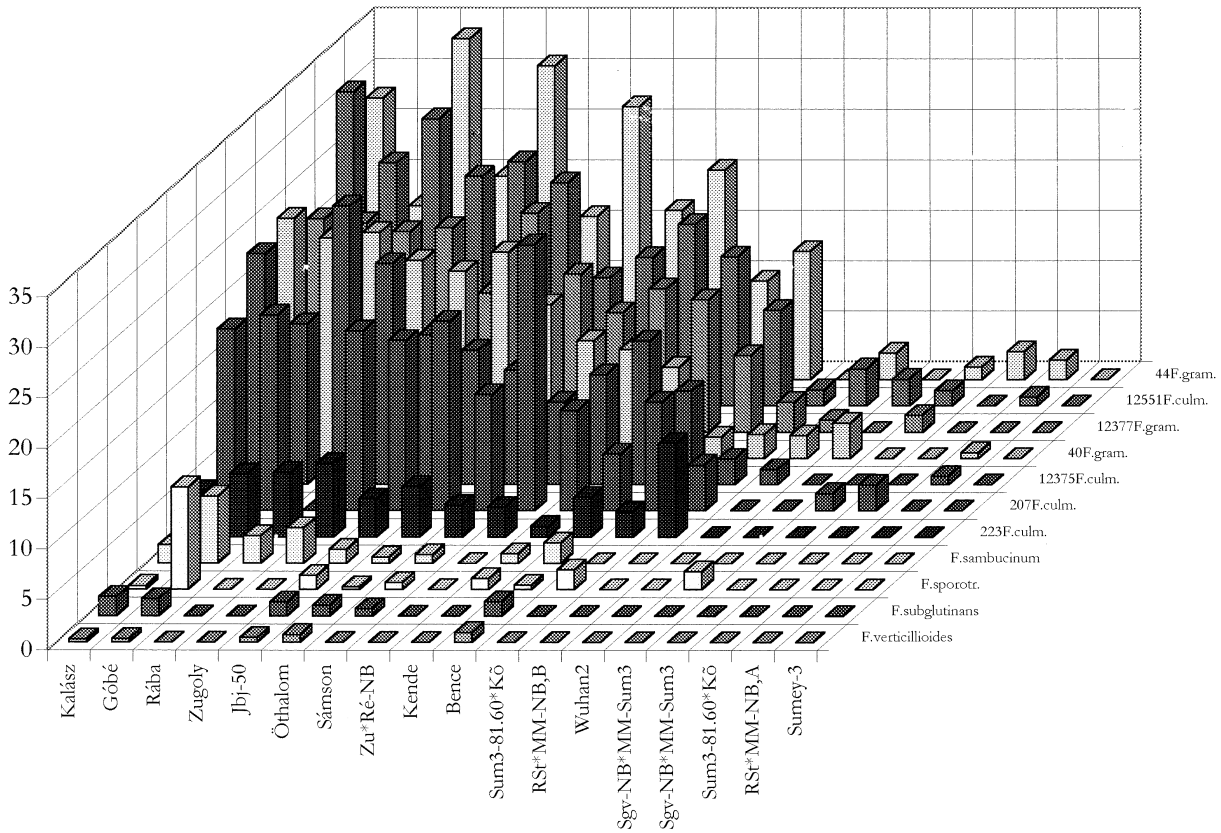

Figure 4. Fusarium head blight resistance in wheat, DON contamination (mg kg^{-1}) 1998.

with DON-producing ability of both species. Similar results were obtained for *F. culmorum* isolates (Gang et al., 1998). The data support the positive role of DON in pathogenesis. We should realize that the different trichothecene toxins have different toxicity in plants and animals. Eudes et al. (1997) reported that DON and 3-AcDON is much more toxic in plants than T-2 toxin or nivalenol.

Proctor et al. (1995) have disrupted the *Tri5* gene which encodes trichodeine synthase and plays a crucial role in the synthesis of the trichothecene skeleton. This resulted in a significant decrease in aggressiveness, again indicating that DON is essential for disease development (Bai et al., 2000; Desjardins et al., 1996; 2000). Their results showed, however, that all aggressiveness was not lost, and the amount of loss was different in different plant species. In wheat, it was more expressed, in other crops less so. These findings support the view that other components of aggressiveness, such as cell wall-degrading enzymes, exist. Alexander et al. (1997) concluded that trichothecenes are not necessary for pathogenicity, but that they increase the extent of the disease. They concluded, however, that breeding for toxin resistance could result in higher resistance to disease. To achieve this, they suggested the incorporation of the *Tr12* transporter gene in plants in the hope that the resulting toxin resistance would cause higher disease resistance and less toxin contamination. As other components also play a role in pathogenesis, the result of the gene transfer might be disappointing. The reduction of the disease could be less than anticipated.

Mirocha et al. (1997) reported that after inoculation, DON was not detected in infected tissue earlier than 48 h. Evans et al. (2000) found a similar time-course after inoculation in barley. Kang and Buchenauer (1999) came to similar conclusions with a detection time of 36 h. Chen et al. (1996) reported 24 h for the first detection of DON after inoculation. The relative late occurrence of DON in disease development suggests that DON does not play a role in the initial phase of infection. Bai et al. (2000) came to the same conclusion for barley. It is important to note that mycelium and

Table 4. Resistance against *Fusarium* species in wheat cultivars to FHB, 1998

Genotype	Traits			
	DON μg g^{-1}	FHB %	Yield loss %	FDK %
Sum3-81.60 ∗ Kõ	0.48	0.14	5.54	0.79
Sumey-3	0.00	0.14	3.97	0.03
Sgv-NB ∗ MM-Sum3	0.56	0.44	8.4	0.91
RSt ∗ MM-NB,A	0.38	0.55	4.47	2.42
Wuhan2	1.02	2.21	2.46	4.85
RSt ∗ MM-NB,B	2.11	2.94	23.77	12.52
Bence	6.85	3.45	21.77	26.85
Sgv-NB ∗ MM-Sum3	0.72	3.94	5.12	2.45
Sum3-81.60 ∗ Kõ	5.13	12.73	10.15	18.52
Kende	7.98	17.11	36.46	30.97
Sámson	8.99	17.36	46.9	35.00
Zu ∗ Ré-NB	8.41	21.73	45.83	40.36
Jbj-50	11.61	23.97	33.34	45.82
Öthalom	10.94	24.47	41.35	58.27
Rába	12.83	25.68	31.6	47.76
Zugoly	12.13	26.39	35.37	50.06
Kalász	13.97	30.20	42.97	51.15
Góbé	13.12	40.55	46.09	52.55
Mean	6.51	14.11	24.76	26.74
LSD %	4.40	1.06	5.18	3.54
	FHB %	FDK inf. %	Yield red. %	
FDK. %	0.9336***			
Yield red. %	0.8573***	0.9072***		
DON μg kg^{-1}	0.9435***	0.9799***	0.8838***	

*** $P = 0.001$.

Table 5. DON production following fungicides application in cultivar Zugoly, 1998. Data in mg kg^{-1}

Fungicide 1/ha	Isolates				
	12377Fg	40Fg	12375Fc	12551Fc	Mean
Folicur Top+ Kolf.S. 1 + 1.5	10.04	4.47	5.74	13.29	8.39
Folicur Solo 1	7.61	1.16	10.34	16.64	8.94
Folicur Top 1	8.97	3.83	9.24	14.37	9.10
Falc. 1.0	12.88	5.84	10.57	17.69	11.75
Juwel 1	12.47	3.83	14.13	17.59	12.01
Falcon 0.8	12.32	6.74	17.76	19.62	14.11
Fusarium check	14.94	8.94	21.83	22.67	17.10
Amistar 1	20.10	12.31	25.13	26.29	20.96
Kolfugo S 1.5	14.72	5.53	26.67	37.56	21.12
Mean	11.40	5.46	13.97	19.51	12.59
LSD 5%					3.58

conidia do not contain DON. Mesterházy (1978) found no DON, but there were traces of diacetoxyscirpenol and neosolaniol and larger amount of diethylnivalenol in 14-day-old shaken cultures of *F. graminearum*, even though the isolates studied produced large quantities of DON after head inoculation. New data (Evans et al., 2000) confirms this, supporting the view that DON is synthesized only during pathogenesis.

Accordingly, DON has been considered to function as a pathotoxin. This led to the conclusion that selecting for toxin resistance directly or from calluses, somaclones or microspores on toxin-containing agar or culture filtrate-containing media would lead to the production of disease-resistant plants. Such work was started in China (Chen et al., 2000), Austria (Buerstmayr et al., 1996; Lemmens, 2000, pers. comm.), the Netherlands (Snijders 1997, pers. comm.) and Hungary. Positive results were achieved in China, where highly resistant plants were selected. However, later work revealed that among the surviving plantlets there were some disease-resistant plants, but there were also plants with considerable susceptibility. In Hungary (Ahmed et al., 1992; 1996) somaclones were screened on culture filtrate and T-2 toxin media. The surviving 10% of calluses were regenerated and followed to the R_3 generation allowing selection for *Fusarium* resistance. A parallel population was also added, where no toxin selection was applied and calluses were regenerated randomly. When the two

populations were compared, it was found that in both populations there were plants with greater resistance than the parent cultivars, but there were also some plants with increased susceptibility. Monasterski (2000) found a close correlation between resistance and the regeneration ratio of calluses after selection on toxin-containing media, but the resistance of the regenerated progenies has not been tested. DON sensitivity of seedlings and resistance to disease correlated well (Liu et al., 1999) and seems, therefore, to confirm the role of resistance in suppressing DON activity, rather than to suggest that DON plays a major role in pathogenesis. Our conclusions are that neither DON nor T2 toxin are pathotoxins, and that toxin resistance and disease resistance are two different phenomena.

The effect of the cultivars on the DON-producing ability of given isolates should also be stressed. In susceptible and moderately susceptible cultivars, the disease-causing ability depends largely on DON-producing ability. However, in highly resistant genotypes this ability is strongly inhibited. Toxin production is also substrate-dependent. Under field conditions, a much narrower spectrum of toxins occurred in wheat than in rice grains in the laboratory (Szécsi and Bartók, 1996). The same phenomenon was recognized by Stack et al. (2000) and Miedaner et al. (2000). For this reason, toxin-producing ability should be measured under field conditions and to neutralize the cultivar effect, a set of genotypes with differing resistance should be used.

The level of DON concentration is also strongly influenced by the amount of precipitation following infection. Between years, ten-fold differences occurred when we considered the amount of FDK needed to produce 1 $\mu g\, g^{-1}$ DON. This means that aggressiveness and cultivar resistance are not alone in determining the final level of toxin concentration. In later phases of the disease, other mechanisms are important to control DON production. Fungicides play an important role in decreasing significantly the infection of cereals by Fusaria and their DON-producing capacity, although partially effective fungicides cause increased toxin contamination in parallel with a lower disease level.

The regulation of DON production during the development of FHB is a complex phenomenon. The aggressiveness of *F. graminearum* and *F. culmorum* correlates with production of DON and/or trichothecenes, suggesting that production of these toxins is an important component of aggressiveness. Other *Fusarium* species, which lack ability to produce DON, are poorly pathogenic. Resistant cultivars and fungicides can fully inhibit the disease-causing capacity of highly aggressive, DON-producing isolates. It seems that strategically, an increase in the availability of disease-resistant cultivars is the most important task required to combat the problems associated with toxin-producing *Fusarium* species.

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