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Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat

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Summary

Wheat ears were inoculated with conidia of *Fusarium* spp. at different growth stages between ear emergence and harvest and moist conditions were maintained for up to 7 days subsequently by mist irrigation. Of the fungi tested (*Fusarium culmorum, F. avenaceum, F. tricinctum, F. sporotrichioides* and *Microdochium nivale*), only *F. culmorum* produced ear blight symptoms and grain samples were found subsequently to contain deoxynivalenol. Most ear infection and deoxynivalenol formation occurred following inoculation at about mid-anthesis. Small amounts of deoxynivalenol were formed and some *F. culmorum* was isolated even in the absence of ear blight symptoms. An overnight wet period was sufficient to initiate infection and deoxynivalenol formation but both were increased by extending the wet period up to at least 3 days. Recovery of *Fusarium* spp. from harvested grain was usually possible whether or not symptoms developed. *F. culmorum* usually persisted and often increased to moderately high levels after storage for 7 wk in a range of moisture conditions.

Key words: Wheat, ear blight, *Fusarium* spp., *Fusarium culmorum*, mycotoxins, deoxynivalenol

Introduction

Fungi are important causes of deterioration of growing and stored grains. *Fusarium* spp. are of particular importance in the growing crop. Ear infection of cereals can occur from anthesis to harvest and can result in contamination by a range of mycotoxins, including zearalenone and trichothecenes. The quantity and type of mycotoxin produced appear to be influenced by the weather before and after harvest and by the fungal species present. In surveys of UK wheat, mycotoxin levels have usually been found to be low but variable (Polley *et al.*, 1991). This is not always the case elsewhere in Europe: in a survey of cereals in Bavaria from the 1987 harvest, some samples showed very high levels of contamination, including 44 mg deoxynivalenol kg^{-1} in one wheat sample (Lepschy-von Gleissenthal *et al.*, 1989). Data from other European studies also suggest that incidence and amounts in wheat might sometimes be high (e.g. Perkowski, Chełkowski & Wakuliński, 1990; Perkowski, Wakuliński & Chełkowski, 1990).

Conditions that influence the production of mycotoxins are little known although zearalenone production is initiated at relatively low temperatures (Sutton, 1982). Unusually, some growth of *Fusarium* can occur in stored grain (Lacey, 1990), but this requires moisture conditions (water activity) above about 0.87 a_w at temperatures between about 0 and 35°C, with the optimum about 25°C (Magan & Lacey, 1984). Critical studies on environmental conditions and infection have concerned particularly *F. graminearum* Schwabe on maize and wheat in Canada, where it is associated with wet weather at anthesis (Sutton, 1982) and where most of the inoculum is wind-dispersed ascospores. This was confirmed in European conditions (Suty & Mauler-Machnik, 1996). *F. culmorum* (W. G. Sm.) Sacc., the predominant species in Britain, is splash-dispersed as asexual conidia. Ear infection by *F. culmorum* and other species is also greatest in Britain in wet conditions in summer (Jones *et al.*, 1997).

This paper reports a series of field experiments designed to investigate critical infection times and wet periods during infection on the development of ear blight and mycotoxins in wheat by *Fusarium* spp.

Materials and Methods

Fungal inoculum

Fusarium species isolated from crops at Rothamsted were used in all experiments. Isolates represented *F. avenaceum* (Fr.) Sacc., *F. culmorum*, *F. sporotrichioides* Sherb. (originally identified as *F. poae* (Peck) Wollenw. (Bateman, Kwaśna & Ward, 1996; Ramakrishna, Lacey & Smith, 1996)), *F. tricinctum* (Corda) Sacc. and *Microdochium* (*Fusarium*) *nivale* (Fr.) Samuels & Hallett. Cultures were lyophilised for storage and during the period of inoculum preparation were maintained on 2% malt extract agar with subculturing as necessary. Three different isolates of each species were generally mixed for use as inoculum in each experiment. In the first year's experiment, inoculum was prepared on 2%

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Table 1. *Inoculation and irrigation treatments*

Treatment date	Growth stage	Irrigation
1994		
23 June	63	none
24 June	63	Intermittent (non-inoculated)
29 June	67–69	Intermittent
2 July	69–71	Intermittent to 4 July, then continuous
6 July	71–75	Continuous but off 24–h, 8–9 July
14 July	77–81	Continuous
21 July	85–89	Continuous
27 July	89–91	Continuous
1995		
1 June	51–57	Continuous
9 June	59–63	Continuous
15 June	61–69	Continuous
21 June	69–71	Continuous
21 June	69–71	None following inoculation, then 3 days on, 3 days off for 12 days, to flattened crop
27 June	71–75	Continuous
3 July	75–81	Continuous
10 July	85	Continuous
10 July	85	None following inoculation, then 3 days on, 3 days off for 12 days, to flattened crop
1996		
10 June	53	None, half, 1 or 3 days (to all plots, subdivided)
16 June	65	
19 June	69	
23 June	71	
27 June	71	
30 June	73	
5 July	75	
10 July	77	

malt extract agar in Petri dishes and incubated at 25°C for 7–10 days. When required, conidia were suspended in sterile distilled water by washing the agar surface with gentle teasing using a disposable plastic spreader. Conidial suspensions were filtered through muslin before use. In the two subsequent years, cultures were grown in Bilai's liquid medium (Joffe, 1963). Spore concentrations were standardised at approximately 10^6 ml^{-1}.

Field experiments

There were three experiments in successive years on two adjacent sites in the same field at Rothamsted farm. Each was in a first winter wheat crop, cv. Hussar sown at 380 seeds m^{-2}, following winter oilseed rape (1994 experiment) or winter oats (1995 and 1996 experiments). The 1996 experiment was on the same site as the 1994 experiment. Each experiment had a randomised block design with two complete blocks of nine main plots. Plots were 14–17 m × 4 m with 2–m sown guard areas between plots and a 1–m path between blocks to accommodate irrigation lines. Randomised subplots for inoculation treatments were marked out within each plot. In 1994, each of six subplots (1 m × 1 m), in two rows of three, was inoculated with a 100–ml spore suspension of one of the five *Fusarium* spp. (a mixture of isolates) or used as the uninoculated control. In 1995, each of four subplots (1 m × 4 m) was inoculated with a 200–ml spore suspension of one of three *Fusarium* spp., or left uninoculated. In 1996, each of four subplots (1 m × 4 m) was inoculated with a 200–ml spore suspension of *F. culmorum*, a fifth with *F. sporotrichioides* and a sixth was left uninoculated.

Spore suspensions were applied using a hand-held pressure sprayer. Mist irrigation (Access Irrigation Ltd, Northampton) was provided after inoculation by three rows of misting nozzles on 1–m poles at 2–m intervals along the length of the plot, one row along each side of the plot and one along its centre. Mist application was controlled by a wet-leaf sensor placed in the crop to maintain moist conditions after inoculation to enhance infection. The misting lines were moved from plot to plot as required, with two replicate plots (the most that could be accommodated by the apparatus) misted in immediate succession at intervals determined by the wet leaf sensor in one of the plots. During 1994 and 1995, ears were inoculated at different growth stages from ear emergence to harvest and were irrigated for 6 days after inoculation (Table 1). In 1996, plots were inoculated from just before anthesis and each was divided into four parts receiving, respectively, no irrigation, overnight irrigation only (half day), 1 day of irrigation or 3 days of irrigation (Table 1). Plots inoculated with *F. sporotrichioides* were situated in the 3–day irrigation section.

Ear blight was assessed by counting diseased (with necrotic lesions visible on one or more spikelets) and healthy ears along four randomly chosen rows across the plot at the time that it was most evident and before all the ears markedly changed colour.

All the ears from each subplot were harvested by hand when the grain was ripe (growth stage, GS, 92; Zadoks, Chang & Konzak, 1974) and subsamples of the harvested grain were used for fungal isolations (1995) and mycotoxin analyses.

Fungal isolations

The percentage of grains contaminated by *Fusarium* spp. was determined in at least 50 grains taken from 10 ears from each subplot 1 wk after inoculation in 1994 and 1995. This was done usually by placing 25 grains that were surface-sterilised in 10% Chloros (sodium hypochlorite) for 5 min and 25 unsterilised grains from each plot on to 2% malt extract agar. Potato dextrose agar containing the fungicide dichloran (2 mg litre^{-1}) was used additionally on most occasions and gave similar results. Colonies were identified and counted after incubating for 7 days at 25°C.

In 1995, the survival of *F. culmorum*, the most effective inoculant fungus, on grain harvested from selected plots was tested at 15°C and 25°C at a range of water activities (cf. Magan & Lacey, 1984). Petri dish

Table 2. Fusarium *on grain 1 wk after inoculation and concentration of deoxynivalenol (DON) in ripe grian after inoculation with* F. culmorum, *1994*

Growth stage at inoculation	Logit % grains with fungus (back transformation)[a]				DON (μg g^{-1})
	F. avenaceum	*F.culmorum*	*F. sporotrichioides*	*F. tricinctum*	
63	−1.62 (3.3)	−1.68 (2.8)	−2.03 (1.2)	−2.31 (0.5)	2.45
67–69	−0.60 (22.6)	−0.06 (46.5)	−1.27 (6.8)	−0.88 (14.1)	1.16
69–71	−0.35 (32.7)	0.42 (69.2)	−1.40 (5.2)	−0.70 (19.4)	0.97
71–75	−0.38 (31.6)	−0.04 (47.5)	−0.62 (21.8)	−0.68 (20.0)	1.07
77–81	−0.42 (29.9)	0.50 (72.6)	−1.31 (6.2)	−1.22 (7.5)	0.35
85–89	−0.55 (24.4)	−0.16 (41.5)	−1.75 (2.4)	−1.39 (5.3)	–
89–91	−0.12 (43.4)	1.01 (87.8)	−1.13 (8.9)	−0.79 (16.5)	–
SED (6 df)	0.243	0.432	0.333	0.296	0.157 (4 df)

[a]The data in each column show frequencies only of the species inoculated (shown in column heading); a separate analysis of variance was done for each inoculated species and excluded subplots that were inoculated with other species or were uninoculated.

– zero values, excluded from the analysis of variance.

lids, each containing a layer of grain, were placed over seed trays containing saturated salt solutions to maintain the required water activities. A solution of K_2SO_4 was used for 0.98 a_w at both temperatures, KNO_3 (15°C) or $Pb(NO_3)_2$ (25°C) for 0.95 a_w and $MgSO_4.7H_20$ (15°C) or $BaCl_2$ (25°C) for 0.90 a_w. Ten grains from each treatment were removed after 1, 2, 3 and 7 wk and incubated on malt extract agar.

Mycotoxin analysis

Mycotoxins were analysed in grain subsamples, harvested from subplots inoculated with *F. culmorum* and from non-inoculated control subplots, using gas chromatography linked to mass spectrometry with selected ion monitoring (Mirocha *et al.*, 1998). Samples were analysed as trimethylsilyl ether derivatives on a Shimadzu QP–5000 GC/MS using a 30 m fused silica gas chromatography column. Injector and detector inlet temperatures were, respectively, 300 and 280°C. The oven temperature was programmed to increase from 80°C to 280°C at 25°C min^{-1} and then held at 280°C for 5 min with a constant flow of helium at 1 ml min^{-1}. A standard curve was run with each batch of grain samples, with the standards interspersed among the samples being analysed. Selected ion monitoring utilised fragment ions (m/z values) of 235.15, 422.25, 392.20, 482.30, 377.20, 480.30, 570.30, 585.30, 333.10, 289.15 and 151.15 as determinants. The m/z value 235 was used as the general quantitative fragment for most of the deoxynivalenol derivatives, 235, 422 and 392 for deoxynivalenol, 392 and 482 for 3–acetyldeoxynivalenol and 15–acetyldeoxynivalenol, 289 and 482 for nivalenol, 480 and 570 for fusarenon-X and 333 and 151 for zearalenone.

Table 3. *Incidence of ear blight and concentration of deoxynivalenol (DON) in harvested grain after inoculation with* Fusarium culmorum, *1995*[a]

Growth stage at inoculation	Logit % ear blight (back-transformation)	DON μg g^{-1}
Not inoculated	–	0.04
51–57	−1.27 (6.8)	0.16
59–63	−1.37 (5.6)	0.81
61–69	−0.15 (42.1)	12.12
69–71	−1.76 (2.4)	0.40
71–75	–	1.69
75–81	–	0.55
85	–	0.10
SED	0.088 (3 df)	0.848 (7 df)

– zero values, excluded from the analysis of variance.

[a] Data are from those plots in which the crop was not flattened artificially (see text and Table 2).

Results

1994

Ear emergence was between 10 and 16 June but, because of problems with the irrigation equipment, inoculation was delayed until 23 June (GS 63) when the first subplots to be inoculated (with *F. avenaceum*, *F. culmorum*, *F. sporotrichioides*, *F. tricinctum* or *M. nivale*) were those in the two non-irrigated control plots. Initially, only intermittent irrigation was possible because of equipment faults. On most days irrigation was applied four to six times for 3–5 min. The first plots to be both inoculated and irrigated were inoculated on 29 June. Continuous misting was possible after 4 July, but harvest prevented inoculation of the final two plots in the experiment. Table 1 shows a full list of treatments.

No disease identifiable as ear blight caused by *Fusarium* was seen in any plot. However, some ears had a bleached appearance at about GS 81–85. This was most frequent in plots inoculated with *F. culmorum* at GS 63 but not irrigated, and affected half the plants. The effectiveness of inoculation was assessed by isolating *Fusarium* spp. from grain 1 wk after treatment; inoculation at GS 67–69 or later resulted in greatest frequency of isolations of

F. culmorum (Table 2). Seed from inoculated subplots also carried a range of *Fusarium* spp. other than the inoculant, mainly *F. culmorum*. Such contaminants were isolated from up to 50% of seeds but were fewest after inoculation with *F. culmorum*. The frequencies of isolation, over all inoculation times, also differed among the inoculated species after harvest. These frequencies were: *F. avenaceum*, 47.3%; *F. culmorum*, 79.0%; *F. sporotrichioides*, 7.4%; *F. tricinctum*, 29.7%; *M. nivale*, 0.3% (a single isolation). Considering all isolation methods, approximately 20–25% of harvested seeds carried *Fusarium* spp. *F. culmorum* was also isolated from almost 25% of surface-sterilised seeds, suggesting seed infection, but less than 8% of surface-sterilised seeds inoculated with other species yielded the inoculant species.

Samples taken from each plot inoculated with *F. culmorum* were analysed to determine their deoxynivalenol content. Most deoxynivalenol (2.45 $\mu g\ g^{-1}$) was found in grain inoculated at GS 63, despite lack of irrigation (Table 2). From GS 67–91 to GS 71–75, deoxynivalenol contamination remained close to 1 $\mu g\ g^{-1}$ and none was recovered after GS 85–89. None was found in uninoculated wheat with irrigation.

1995

Ear emergence occurred at the beginning of June and the first inoculation treatment was on 1 June (GS 51–57). Thereafter, two plots were inoculated each week until GS 85 was reached, with a further two plots inoculated at GS 69–71 and 85 (Table 1). Because of their poor recovery after inoculation in 1994, *F. sporotrichioides* and *M. nivale* were not used in 1995. Ear blight was assessed on 7 July at about GS 83. At this stage, there was considerable disease (42% of ears affected) in plots inoculated at GS 61–69 with *F. culmorum* and much less in plots inoculated earlier or later, with none seen for inoculations after GS 69–71 (Table 3) or in plots inoculated at any growth stage with *F. avenaceum* or *F. tricinctum*. Despite the amount of ear disease from the GS 61–69 inoculation, recovery of *Fusarium* spp. from inoculated ears was low. However, recovery for inoculations at GS 69–71, except for *F. tricinctum*, was greater than for earlier inoculations (Table 4). *Fusarium* spp. were recovered at trace levels where no inoculum was applied and was absent from most such plots.

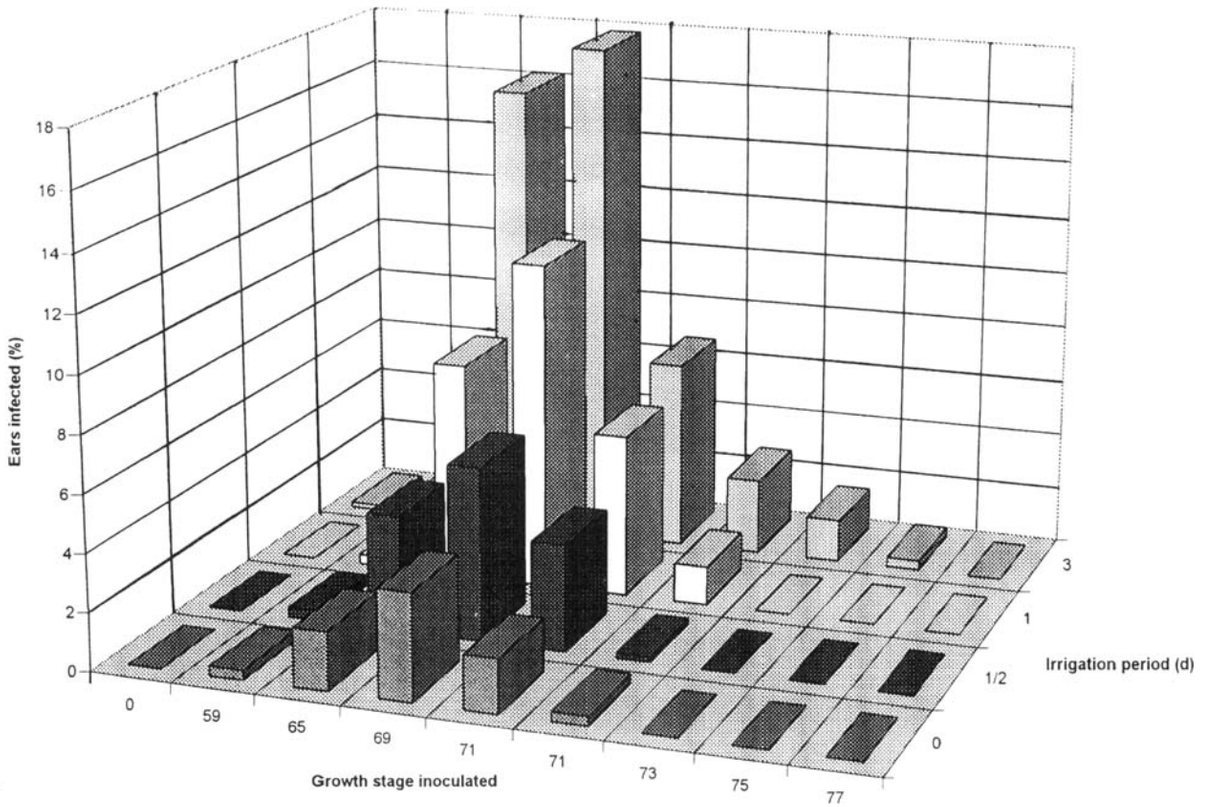

Fig. 1. Ear blight in wheat after different periods of irrigation following inoculation at different times with *Fusarium culmorum*, 1996. SEDs: 1.06 (15 df) for comparisons between different periods of irrigation; 1.44 (9 df) for comparisons between different inoculation times.

Most deoxynivalenol contamination of grain (12 μg g^{-1}) occurred after inoculation with *F. culmorum* at GS 61–69; about 1 μg g^{-1} occurred for earlier and later dates (Table 3). It was evident that low levels of deoxynivalenol were present in the absence of visible ear blight. No deoxynivalenol was found in grain inoculated at GS 69–71 or GS 85 without irrigation immediately afterwards and then flattened to simulate lodging before receiving alternating 3–day wet and dry periods over the 12 days immediately before harvest.

The behaviour of *F. culmorum* in stored grain was tested by incubating samples of infected grain from irrigated plots, inoculated at GS 61–69, at different water activities and temperatures. *F. culmorum* was recovered from 41–45% of grains from inoculated plots after incubation for 1 wk at 15°C with further increase up to 7 wk at 0.98 a_w (Table 5). *F. culmorum* behaved similarly on grain from plots inoculated with *F. avenaceum*, although its recovery was more variable on grain from plots inoculated with *F. tricinctum* (results not shown). The results at the two temperatures showed small differences; in particular, infection in treatments other than *F. culmorum* inoculation was usually less at the higher temperature. Greatest frequency occurred in grain from plots inoculated at GS 61–69, except after incubation for 7 wk at 15°C and 0.98 a_w when most grains from plots inoculated at any growth stage were heavily colonised (results not shown).

1996

The experimental procedure was changed to allow assessment of the effects of different wet periods up to 3 days on infection by *F. culmorum* and deoxynivalenol production. Inoculations were also concentrated over a shorter period by inoculating at 3–6–day intervals instead of weekly (Table 1). A further plot receiving irrigation for 3 days was inoculated with *F. sporotrichioides*. Ear emergence started at the beginning of June and was complete by 10 June when the first inoculation treatment was applied. Anthesis progressed much more quickly than in 1995 and the next inoculation was on 16 June.

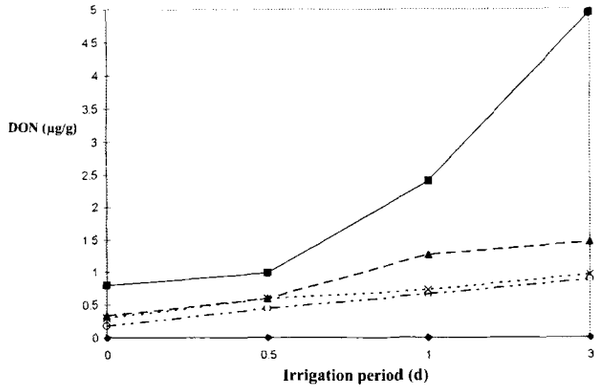

Fig. 2. Deoxynivalenol (DON) concentrations in wheat grain after different periods of irrigation following inoculation at different times with *Fusarium culmorum*, 1996. SEDs: 0.123 (12 df) for comparisons between different periods of irrigation; 0.129 (12 df) for comparisons between inoculation times. Inoculations were at GS 53 (◆), 65 (■), 69 (▲), 71, 23 June (X), 71, 29 June (O).

Table 4. Fusarium *spp.* [a] *on grain 1 wk after inoculation, 1995*

transformation)	Logit % grains with fungus (back-		
Growth stage	*F. avenaceum*	*F. culmorum*	*F. tricinctum*
69–71[b]	−2.03 (1.2)	−1.33 (6.1)	−2.03 (1.2)
51–57	−1.62 (3.3)	−1.62 (3.3)	−1.30 (6.4)
59–63	−0.74 (18.1)	−0.68 (20.0)	−1.30 (6.4)
61–69	−1.22 (7.5)	−1.18 (8.1)	−1.62 (3.3)
69–71	−0.65 (20.9)	−0.26 (36.8)	−1.81 (2.1)
71–75	−0.44 (28.9)	−0.44 (28.8)	−0.44 (28.8)
75–81	−0.65 (21.1)	−0.24 (37.9)	−0.82 (15.8)
85	−1.81 (2.1)	−0.33 (33.4)	−1.81 (2.1)
SED (7 df)	0.245	0.217	0.338

[a] The data in each column show frequencies only of the species inoculated (shown in column heading); a separate analysis of variance was done for each inoculated species and excluded subplots that were inoculated with other species or were inoculated.
[b] no irrigation

Ear blight was assessed after GS 77 from 12–20 July. Plots inoculated with *F. culmorum* at GS 65 and 69 and irrigated for 3 days were the most heavily affected (16.2 and 17.8% of ears, respectively) (Fig. 1). The amount of ear blight fell sharply on either side of these inoculation dates. It also decreased with decreasing wet period although 3.7% of ears were affected after inoculation at GS 69, even without irrigation.

Deoxynivalenol also decreased with decreasing wet period although 0.8 μg g^{-1} was produced without any irrigation (Fig. 2). Deoxynivalenol was measured only for 3–day irrigation treatments for inoculations made after GS 71. After irrigation for 3 days, most deoxynivalenol was produced by inoculation at GS 65, the concentrations decreasing sharply for later inoculations. Concentrations (μg g^{-1}) for inoculations at GS 65, 69, 71 (23 June), 71 (27 June), 73, 75 and 77 with 3 days' irrigation were, respectively, 4.95, 1.47, 0.96, 0.88, 0.71, 0.11 and 0.33 (SED = 0.414, 6 df).

Discussion

These experiments have shown that the time of infection by *F. culmorum* that produces the greatest amount of ear infection and of deoxynivalenol contamination is restricted to a short period during anthesis. Little or no disease resulted from inoculation between ear emergence and the start of anthesis and amounts decreased steeply for inoculations after about mid-anthesis (GS 65). Contamination with deoxynivalenol was greatest after inoculation at about

Table 5. Fusarium culmorum *on grain stored for 1, 2 or 3 wk in different conditions*

Temperature (°C)	Water activity (a_w)	Logit % grains with *F. culmorum* (back-transformation)			
		1 wk	2 wk	3 wk	7 wk
15	0.98	–0.09 (45)	0.30 (64)	0.24 (61)	0.64 (78)
15	0.95	–0.08 (45)	–0.34 (33)	–0.41 (30)	–0.30 (35)
15	0.90	–0.17 (41)	–0.45 (28)	–0.43 (29)	–0.60 (23)
25	0.98	–0.06 (46)	–0.02 (49)	0.04 (52)	0.30 (64)
25	0.95	–0.29 (36)	–0.29 (36)	–0.34 (33)	–0.88 (14)
25	0.90	–0.38 (31)	–0.16 (42)	–0.17 (41)	–0.31 (34)
SED (16 df)		0.205	0.154	0.188	0.212

mid-anthesis, but small amounts (approximately 1 μg g^{-1}) were produced following inoculation at other times, at least to the late milk-early dough stages (GS 77–83), without visible disease. Disease and deoxynivalenol contamination increased with increasing wet period from ½ to at least 3 days and it is possible that the differences in the maximum deoxynivalenol contamination found each year might be explained by the amount of wetting that they received: intermittent in 1994 (2.4 μg g^{-1}) and continuous for 6 days in 1995 (12 μg g^{-1}) and for 3 days in 1996 (4.9 μg g^{-1}). Inoculation without immediate misting followed by misting under simulated lodging conditions failed to produce any deoxynivalenol contamination. Nivalenol was never detected. *F. avenaceum* and *F. culmorum* persisted on stored grain after inoculation, even without visible disease, in a range of conditions, that might also influence mycotoxin content by the time the grain is used. Amounts of deoxynivalenol were found not to change after 6 wk in storage in normal commercial conditions (Young *et al.*, 1984). Moist grain, as tested here, also allows invasion by *F. graminearum* when stored in cool conditions (Sherwood & Peberdy, 1974). Isolates previously thought to be *F. poae* have been found to be atypical isolates of *F. sporotrichioides* (Bateman, Kwaśna & Ward, 1996; Ramakrishna, Lacey & Smith, 1996) and appear to represent the first record of this species in Britain. This raises the possibility of contamination by T-2 toxin although this has, so far, not been demonstrated.

Deoxynivalenol is one of the less toxic trichothecenes produced by *Fusarium* spp. The isomers, 3–acetyldeoxynivalenol and 15–acetyldeoxynivalenol are about twice as toxic to humans and animals and nivalenol is 10 times more toxic (Snijders, 1990). However, only 0.3 μg deoxynivalenol g^{-1} of feed is sufficient to decrease consumption by pigs and cause poor weight gain and economic losses. Agriculture Canada advises that grain fed to animals should contain less than 1 μg g^{-1} and grain fed to pregnant or lactating animals should be uncontaminated (Snijders, 1990). Contamination in the winter wheat experiments regularly exceeded these levels. Swedish regulations limit the contamination of swine feed to 0.5 μg g^{-1} and of cattle feed to 2 μg g^{-1} (Snijders, 1990). A tolerable daily intake for humans is 3 μg kg^{-1} body weight for adults and 1.5 μg kg^{-1} for children (Snijders, 1990). Deoxynivalenol, at 0.02–0.4 μg g^{-1}, was found in 32 of 199 UK homegrown wheat samples taken in the three years, 1980–1982 (Osborne & Willis, 1984).

Rainfall has been shown to be an important factor in determining the occurrence of ear infection by *Fusarium* although there were also correlations with relative humidity and prevalence of infection in the previous season (Snijders, 1990). There have been no previous detailed studies on the conditions necessary for *F. culmorum* to infect winter wheat and cause deoxynivalenol contamination. However, Sutton (1982) studied the epidemiology of *F. graminearum* ear blight in wheat and maize. Unlike *F. culmorum*, *F. graminearum* produces wind-dispersed ascospores as well as splash-dispersed conidia. Both have a role in the disease in Canada. Susceptibility of wheat to *F. graminearum* infection is well known and anthers have sometimes been associated with infection. Infection is considered to occur between anthesis and the soft dough stage (GS 85). However, our results suggest that the period of susceptibility to *F. culmorum* is much more limited. Such a restricted period of susceptibility presents difficulties for timing a chemical control. Infection by *F. graminearum* requires relatively high temperatures (20–30°C) and 48–60 h surface wetness. Few infections occur with less than 24 h surface wetness. *F. culmorum* generally predominates in climates cooler than those most favourable for *F. graminearum*. A few infections of *F. culmorum* were able to occur without misting and the frequency increased with increasing wet period to at least 72 h. However, toxin production also occurred without visible symptoms of infection.

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