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INVESTIGATING THE POSSIBLE RELATIONSHIP BETWEEN PINK GRAINS AND FUSARIUM MYCOTOXINS IN 2004 HARVEST FEED WHEAT

by

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Contents

1. Abstract

In certain regions of the UK the 2004 wheat harvest produced an unusually high number of lots containing pink grains. As some feed compounding companies were rejecting wheat that contained more than five visible pink grains per kilogram, unusually high levels of feed wheat were being rejected in autumn 2004.

Pink grains are not necessarily associated with fusarium infections or with the mycotoxins that can be produced by some *Fusarium* species. Consequently, with the higher than usual level of rejections of feed wheat due to the presence of pink grains, it was questioned whether it was appropriate to exclude 'pink grain' lots on the basis of the risk of mycotoxin presence? The subject was discussed at a meeting called by AIC on 3rd September 2004. The outcome was to request the HGCA to undertake fusarium mycotoxin analysis of feed wheat samples containing pink grains to see if a relationship between pink grain number and deoxynivalenol (DON) existed.

Sixty-eight feed wheat samples with visible pink grains were submitted by AIC member companies and tested for both trichothecene mycotoxins and *Fusarium* species. The aim was to obtain samples from around the UK roughly in the proportion to wheat acreage by region. In the event, sample numbers were biased toward eastern regions where the problem was perceived to be worst.

The analyses showed that there was no relationship between pink grain number and either DON or *Fusarium* species. The number of pink grains present in a sample does not allow any prediction of mycotoxin levels.

DON levels ranged from 20 to 11,500 parts per billion (ppb). Only 1.5% of samples exceeded 5,000 ppb. The sample with the highest value was submitted as having too many pink grains to count. When the results are considered in relation to the maximum levels for feed discussed at a Commission Working Group meeting in Brussels in mid September then only the single, highly contaminated, sample (11,500ppb) was above the maximum level discussed of 6,000-8,000ppb.

Other work, not focussed specifically on rejected feed wheat samples shows that *Fusarium* mycotoxin levels in 2004 were generally low and similar to previous years. The level of fusarium ear blight (FEB) in 2004 was also normal when compared with previous years in the Defra winter wheat disease survey.

This short project must be considered with reference to the full R & D programme funded by HGCA, Food Standards Agency, Defra and LINK that is presently active in the area of FEB and associated mycotoxins.

2. Introduction

In certain geographic regions the 2004 harvest produced an unusually high number of loads of feed wheat containing pink or red coloured grains. The problems for farmers, merchants and consumers caused by an increased presence of pink grains in the 2004 wheat crop were discussed at the AIC on September 3rd 2004 by industry buyers, traders, researchers and HGCA. The problem of pink grains and their rejection was identified as being most severe in parts of East Anglia and Lincolnshire. One area of concern from some end-users was whether the presence of pink grains indicated high levels of fusarium mycotoxins such as deoxynivalenol (DON).

The general perception is that these pink grains are associated with fusarium mould species and that there is a significant risk that they will have high fusarium mycotoxin levels. This is not necessarily the case for two reasons:

1. Pink grain in itself is not necessarily an indication of the presence of fusarium. Pink discoloration of cereal grains can be due to a number of reasons including:
    - naturally occurring plant pigments;
    - other diseases not associated with mycotoxins;
    - incidental infection by other fungal species and bacteria.
2. Even if fusarium is present on grain this does not necessarily indicate the presence of mycotoxins. Even in years with high incidence of *Fusarium* species, levels of mycotoxins in UK grain are usually low.

Fusarium mycotoxins can be produced by species such as *Fusarium graminearum* and *F. culmorum*. If present, levels do not decline during drying and storage. High levels of DON in feed material can have a detrimental effect on animal health. Most widely affected are pigs with symptoms such as reduced appetite, reduced weight gain and, in some cases, vomiting. The presence of pink grains can lead to rejection at feed mill intake.

Both merchanting and feed compounding sectors of AIC felt that it was important to understand the full impact of the pink grain problem. In previous years pink were shown not to represent a feed safety risk. Therefore, a study was undertaken to establish if there is a relationship between the number of pink grains/kg in a sample and fusarium mycotoxins, especially DON, for harvest 2004.

The study involved merchants and compounders submitting samples from all areas of the UK for analysis through an independent laboratory. Efforts were made to try to ensure that the samples submitted for testing reflected the distribution of wheat acreage across the country by region.

The results were analysed with reference to the maximum levels for feed grain discussed recently in Brussels between industry and Commission representatives. The report[1] of a Commission Working Group meeting dated 24th September 2004 indicated that maximum levels of DON for raw materials for use in compound feed may be in the region of 6,000 to 8,000µg/kg or parts per billion (ppb). These proposed levels reflect the position in some other countries including EU member states.

However, lower limits for feed grains were indicated in an FSA document (AFS 459)[2] of 9th August 2004. In addition, it is worth noting that the EU is well advanced in its discussions on maximum levels for cereals used for food uses and a maximum level for DON in wheat of human consumption of 1250ppb is likely to be implemented in EU legislation on 1st July 2006.

3. Samples and testing

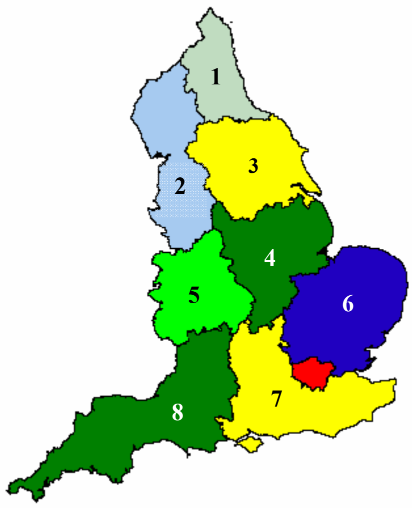
3.1. Samples from AIC members

AIC members were requested to provide samples for testing as follows:

3.1.1. Sample collection

- Feed wheat samples to be collected from merchants and feed mills (rejected for pink grains only).
- Take several samples (ideally 8 for a single lorry load) and mix to form an aggregate sample.
- The aggregate sample should be between 1kg and 2kg.
- Samples to contain up to 50 pink grains/kilogram.
- Origin of samples to reflect UK wheat production see table below:

	Regions	% production by region	No.samples
1	North Eastern	3.4	4
2	North Western	1.3	2
3	Yorks & Humber	12.7	12
4	East Midlands	19.6	20
5	West Midlands	8.1	8
6	Eastern	26.6	25
7	South Eastern	13.2	12
8	South Western	9.8	10
	Scotland	4.5	5
	Wales	0.7	1
	N. Ireland	0.2	1
	Total UK	**100.0**	**100**



Information required

- Date sampled.
- Geographic origin of bulk being sampled.
- Name and address of company.
- A contact name at the company.

3.1.2. Samples received

The following samples were received by RHM Technology and analysed for 10 trichothecenes. Ground sub-samples were then forwarded to NIAB for *Fusarium* species testing.

Regions	No.samples
North Eastern	1
North Western	-
Yorks & Humber	7
East Midlands	14
West Midlands	-
Eastern	33
South Eastern	6
South Western	5
Scotland	1
Wales	-
N. Ireland	1
Total UK	**68**

Samples ranged in pink grain content from 2 to 400 with 2 samples labelled as 'too high to be counted'. Unfortunately not all samples received had a declaration of pink grain numbers. These have been included in the data set but could not be used in the overall analysis.

3.2. Testing

3.2.1. Mycotoxins

The trichothecene mycotoxins (deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), fusarenone X (FUSX), T2 toxin (T2), HT2 toxin (HT2), diacetoxyscirpenol (DAS), neosolaniol (NEO) and T2 triol (T23)) were analysed by gas chromatography with mass spectrometry (GC/MS) using a UKAS accredited method.

3.2.2. Preparation of samples

All samples in this survey were ground and thoroughly mixed prior to analysis.

3.2.3. Analytical methodology

Extraction and clean-up: Ground samples were extracted with acetonitrile/water by shaking for 2 hours on a wrist action shaker and the extract subjected to charcoal/alumina clean-up.

3.2.4. Determination of trichothecenes by GC/MS

The sample extracts were derivatised to form the trichothecene -trimethyl silyl (TMS) derivatives and determined by GC/MS operating in selected ion mode to test for four specific ions for each trichothecene – TMS derivative.

3.2.5. Spiked samples

All analyses were conducted with a spiked sample, i.e. to each sample matrix, on each day, a known amount of toxin was added prior to extraction, clean up and detection, these samples were used to assess recovery, and recoveries of 70-110% were classed as valid. Spiked samples were also used for quantification, thus making all results recovery corrected.

3.3. Fusarium species

Representative sub-samples of the ground material were sent to NIAB for *Fusarium* species type testing. A sub sample of the ground grain was thinly spread onto the surface of potato dextrose agar, with 20 plates inoculated for each sample. Samples were incubated for seven days at 22°C (12hr UV, 12 dark). Developed colonies were examined using low power microscopy with *Fusarium* species identified using a range of colony and mycelial features including spore morphology.

4. Results

4.1. Mycotoxin results

Table 1. Levels of fusarium mycotoxins (ppb) found in feed wheat contain pink grains

% samples in the range (ppb)	DON	3AcDON	15AcDON	NIV	T2	HT2
Not detected*	0	86.8	69.1	17.7	98.5	95.6
0-100	10.0	13.2	30.9	79.4	1.5	4.4
101-250	5.9	-	-	2.9	-	-
251-500	17.9	-	-	-	-	-
501-1000	16.3	-	-	-	-	-
1001-5000	48.5	-	-	-	-	-
>5000	1.5	-	-	-	-	-
Max level	11,500#	31	77	142	11	18

* limit of detection =10ppb

one sample, intended for on-farm feeding contained 11,500ppb.

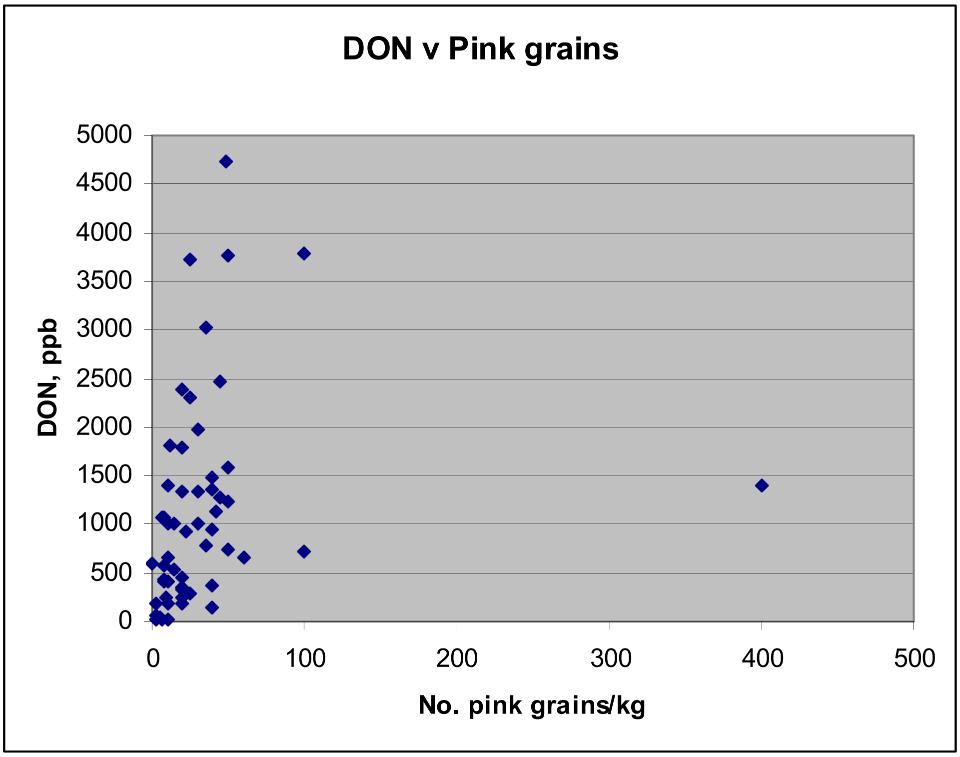
No samples had detectable levels of DAS, FUSX, NEO and T23.

Table 2. DON values by region

Region	No of samples	No of pink grains*	DON ppb	
			Range	Mean
All	67	3-TMTC	20-11,500	1349
Eastern	33	8-TMTC	614-11,500	1746
East Midlands	14	11-45	33-3088	1414
Yorks & Humb	7	10-50	57-1334	586
South East	6	3-50+	22-1589	568
South West	5	3-100	58-1800	766
North East	1	7	11	-
Scotland	1	10	20	-
N Ireland	1	40	148	-

* data not available for all samples. TMTC = too many to count.

The scatter plot below shows DON levels against the number of pink grains in 1kg for the samples submitted with declared numbers of pink grains. There was no significant relationship between the number of pink grains in a 1kg sample and the level of DON and hence pink grain number cannot be used as a predictor of DON or other fusarium mycotoxin presence. The sample with the very high value (11,500ppb DON and TMTC pink grains) has been excluded for the sake of clarity.



The full results can be found in Appendix 1.

When examining the results it must be appreciated that the samples represent an extreme set and as the number of samples and ranges of pink grains, by region, differ substantially no direct comparisons can be made between regions. However, as was originally stated at the AIC meeting which instigated this project it appears that the high DON values and numbers of pink grain are associated with Eastern regions. Other work is underway to investigate regional differences in mycotoxin levels in wheat.

The one sample that had an exceptionally high level of DON, exceeding limits under discussion for feed wheat, was intended for on-farm feeding to dairy cattle.

4.2. Fusarium species

As the technique used was based on the ground samples used for mycotoxin analysis and not whole grains it was necessary to devise a simple scale of infection. This is given below.

Overall infection levels per sample.

Light	0.5%	to	20%
Medium	20%	to	45%
Heavy	45%	to	70%
Very heavy		above	70%

Table 3. Percentage (%) of fusarium infection.

	F. graminearum	*F. culmorum*	*F. poae*	*F. spp**
None	73.8	27.7	96.9	18.5
Light	9.2	33.8	3.1	36.9
Medium	9.2	18.5	0.0	35.4
Heavy	3.1	9.2	0.0	4.6
V heavy	4.6	10.8	0.0	4.6

* This includes other colonies which were clearly *Fusarium* spp. on the basis of colony morphology and spores, but where individual species were not identified. It includes species such as *F oxysporum* and *F solani*.

Table 4. Regional results of fusarium infection (%) with (heavy & v heavy)

	Number of samples	*F. graminearum*	*F. culmorum*	*F. poae*	*F. spp*
Eastern	33	36(12)*	76(24)	3	88(9)
E Midlands	14	21	79(14)	-	93(7)
Yorks & Humber	7	-	71(29)	14	86
South East	6	33	50	-	50
South West	5	20(20)	80	-	60

* Percentages with heavy and v heavy infections are given in brackets. No value means no instances of heavy or v heavy were recorded.

These results are discussed in section 6.

5. Additional information

5.1. Incidence of Fusarium ear blight in winter wheat (2004) and CropMonitor

The Defra-funded survey of winter wheat diseases carried out annually by ADAS and CSL shows that the incidence of fusarium ear blight (FEB) at GS75 in the UK has been sporadic over a number of years. The level (% ears affected) found in 2004 was lower that that in 2003 and appears to be within the normal variation seen since 1997.

Table 5: National incidence of FEB

Year	% ears affected
10 year mean (1994-2003)	3.5
2000	4.9
2001	2.2
2002	3.1
2003	7.5
2004	3.0

Source: Commercial crop monitoring: winter wheat survey. CSL 2004.
http://www.cropmonitor.co.uk/commercialsurvey/cswheat/fuspress04.cfm

However, there is a shift in the FEB pathogens isolated from diseased wheat ears. The dominant pathogen alters between years and in 2004 *Fusarium langsethiae* was the most prevalent. There were significant levels of *F. graminearum, F. avenaceum* and *F. poae* but *F. culmorum* and *M. nivale* were at low levels.

Table 6. Species responsible for FEB symptoms 2000-2004

Year	*F. avenaceum*	*F. culmorum*	*F. graminearum*	*F. poae*	*F. nivale*	*F. langsethiae*
2000	14	16	15	22	55	-
2001	6	13	7	61	0	26
2002	8	18	29	30	36	5
2003	24	11	35	39	32	1
2004	25	6	29	29	6	51

Source: Commercial crop monitoring: winter wheat survey. CSL 2004.
http://www.cropmonitor.co.uk/commercialsurvey/cswheat/fuspress04.cfm

F. langsethiae is a newly described *Fusarium* species which causes a discrete lesion on the glume similar to that caused by *F. poae*. As a result it is unlikely to have a dramatic effect on yield, however it does produce the type A trichothecenes mycotoxins T-2 and HT-2.

Mycotoxins are secondary metabolites produced by some of the *Fusarium* pathogens. Mycotoxins produced by FEB pathogens in the UK include:

***Fusarium* species**	**Main mycotoxins produced**
F. culmorum	Deoxynivalenol, nivalenol, zearalenone
F. graminearum	Deoxynivalenol, nivalenol, zearalenone
F. avenaceum	Enniatins
F. poae	HT-2 and T-2 toxins, diacetoxyscirpenol, nivalenol
M. nivale	None confirmed

Source: Effect of fungicide on mycotoxin production. CSL 2004
http://www.csl.gov.uk/science/organ/environ/entom/fusarium/contr7.cfm

The full articles can be found at:
http://www.csl.gov.uk/science/organ/environ/entom/fusarium/inc2.cfm
http://www.cropmonitor.co.uk/commercialsurvey/cswheat/fuspress04.cfm

5.2. Results of HGCA project 2452

The five year HGCA/FSA project ‘Investigation of fusarium mycotoxins in UK wheat production’ is investigating mycotoxin levels in wheat grown under a range of agronomic inputs from organic to full fungicide protection. The initial 150 results from 2004 (year 4) show that, for DON, the mean level is slightly higher than it was previous years. Within this small sample set only 1% exceed the 1250ppb level for unprocessed cereals for use in the food chain proposed by the EU.

5.3. Mycotoxin survey data from 1999/2000

To ascertain the position of UK grain with respect to mycotoxins CSL were commissioned by the HGCA to test UK grains in storage for ochratoxin A (OA), and deoxynivenol (DON). Samples of grain were collected across the UK from farms, central stores, mills, maltings and ports from the beginning of February to the beginning of April 2000. A total of 320 samples were collected, consisting of 201 wheat, 106 barley and 13 oats, which is approximately proportional to the hectares of each grown in the UK.

The sampling method used was based on that suggested in Commission Directive 98/53/EC for sampling grain for aflatoxins, in which 100 small samples are taken to produce one aggregate sample. The grain was sampled using purpose-built five-compartment grain spears which were adjusted so that each compartment could hold 30g of wheat. By sampling at 20 locations in a grid pattern across the surface of the bulk, at depths of 0.2 to 1.7m, 100 sub-samples of 30g each were obtained, giving an aggregate sample of 3kg. Since the spears were set for 30g of wheat per compartment, extra sampling was necessary with barley and oats to obtain the required 3kg.

DON was detected in 88% of samples but the majority (83%) contained less than 100ppb. Only one sample exceeded 500ppb, with a level of 600ppb, and none exceeded 750ppb (see table 7.). However, another 6 samples fell between 250 and 499ppb. Of these 7 samples, three were intended for feed, one for malting and three for "other uses".

Table 7. Levels of deoxynivalenol (DON) found in each type of grain

Grain	Number of samples tested	Number of samples in each range ppb) of DON							Maximum value ppb
		Not detected*	20-49	50-99	100-249	250-499	500-749	750 or more	
Wheat	201	3	45	101	47	4	1	0	600
Barley	106	31	48	24	1	2	0	0	370
Oats	13	13	6	3	1	0	0	0	108
Total	320	37	99	128	49	6	1	0	
	% =	**11.6**	**30.9**	**40.0**	**15.3**	**1.9**	**0.3**	**0**	

*Not detected = below the measurable detection limit of 20ppb.

The levels of DON, which are dictated by growing conditions rather than storage conditions, were all acceptable in terms of legislative limits under discussion in 1999 (750ppb) and well below the current discussion level for food grade cereals of 1250ppb. Although much of the country experienced above average rainfall in 1999 and the occurrence of FEB on winter wheat was higher than average (MAFF data), the species of *Fusarium* involved were not overly dominated by those which are regarded are mycotoxin-producing species. In the previous year (1998) ear blight had been twice as high as in 1999 but very few predominantly mycotoxin-producing species were present. Therefore, one should consider that 1999 was neither a particularly serious year for trichothecene production, nor an especially 'clean' one.

5.4. Seed testing data

NIAB provides a commercial seed testing service and kindly made the data below available. Results are given for *M. nivale*, *F. culmorum*, *F. poae* and other *Fusarium* spp grouped together.

Table 8. Percentage incidence of *M. nivale* and *Fusarium* spp in UK seed wheat

	2001	2002	2003	2004
M. nivale	0.57	9.08	6.82	3.10
F. culmorum	0.42	0.06	0.13	0.51
F. poae	1.80	0.11	0.68	0.15
Fusarium spp.	4.53	4.72	6.78	3.65

Results from 2004 show, with the exception of *F. culmorum,* incidence of *Fusarium* spp is lower than 2003 and similar to previous years. This is broadly in agreement with ear blight assessments made as part of the Defra-funded CropMonitor wheat disease survey (see section 5.1).

6. Discussion

6.1. *Interpretation of results*

6.1.1. Mycotoxin analyses

All samples, whatever the level of pink grains had measurable DON levels. The range was 20-11,500ppb. Thirty eight percent of samples exceed the 1250ppb maximum level for unprocessed cereals destined for human consumption proposed for enforcement across the EU on 1st July 2006. However, if we look at the results in the light of the discussions held in Brussels on 24th September 2004 where the Commission were reported as "moving towards setting maximum levels for compound feed, varying according to the species. As for raw materials, either there would be no limit, or they would correspond to safe, sound and merchantable limits, that is 7 to 8 ppm." then only one sample would exceed this range. However, it must be stressed that many of the samples submitted must be considered as worse case samples as they had visible pink grains as high as 400/kg and in 2 cases they were described as 'too many to count'.

The major objective of the project was to establish if a relationship between pink grain count (in 1kg) and the level of DON existed. These results show that this is clearly not the case. For example, with samples containing 8 pink grains/kg DON varies from 416 to 1079 ppb whereas when 40 pink grains /kg were present the DON levels ranged from 148 to 1490 ppb.

Other tricothecenes.
NIV is present in measurable levels in 82.3% of all samples in the range 10-142ppb. High NIV values do not necessarily mean high DON values and vice versa.

3-AcetylDON is present in 13.2% of the samples in the range 10-31ppb. 15-AcetylDON is present in 30.94% of the samples in the range 10-77ppb. There does not appear to be any useful correlation of either of these mycotoxins with the number of pink grains in a 1kg sample.

T2 and HT2. Only one sample contained T2 with three samples having a measurable level of HT2. The levels were, T2 11ppb and for HT2 12.4-18ppb. At present the proposed EU limit for unprocessed cereals has not been set.

6.1.2. Fusarium species

The results are given in tables 3 & 4. The dominant *Fusarium* species was found to be *F. culmorum* and this observation is in line with seed testing results on this year's wheat. *F. culmorum* also had the highest percentage of samples exhibiting heavy or very heavy infections. A close examination of the data shows that there is no relationship between the number of pink grain or DON and any of the *Fusarium* species. It must be borne in mind that the fusarium type testing was performed on a ground sample and not whole grains as would normally be done. This meant that the testing had to be adapted to the ground sample format and may have influenced the confidence which can be placed on these data.

When we look for regional differences (Table 4) we find that no clear distinction can be made. It is necessary to treat these results with some caution as there are very few samples in most of the regions and hence a single sample has a disproportionate affect on the percentages. It is perhaps not surprising that no clear regional difference is apparent as the samples submitted for testing all had visible pink grain and as 'worse case' samples are not a true representative cross-section of the wheat grown in each region.

6.1.3. Additional information

Section 5 includes additional information from other testing performed on 2004 harvest wheat samples and those from previous years. The results of mycotoxin testing show that the 2004 harvest was not significantly different from prior years. In addition, the levels of fusarium infection are again broadly similar to previous years although data from the survey of incidence of FEB in winter wheat (2004) shows a shift in the pattern of *Fusarium* species with *F langsethiae* at high levels. This *Fusarium* species is said to be responsible for the mycotoxins T-2 and HT-2 but the results from the 'pink grain' samples in the present study do not bear this out.

6.2. Other work on Fusarium ear blight in the UK

This rapid response investigation was not work done in isolation as there is a large body of current and ongoing research into FEB and the associated mycotoxins. As discussed in section 5, extensive previous and ongoing HGCA/FSA-funded projects show that levels of DON in UK wheat are generally very low. In addition to the studies outlined above, there are also a number of ongoing projects covering:

- due diligence testing for mycotoxins;
- prevention of FEB and associated mycotoxins through breeding and improved fungicide application;
- effects of processing on the fate of mycotoxins in cereal products;
- rapid and cost-effective measurement of mycotoxins.

These on-going projects are summarised below.

HGCA project 2804: Review of food safety issues relating to the supply and market acceptability of UK malting barley and UK malt. HGCA project 2819: Monitoring contaminants in wheat grains. HGCA project 3033: Monitoring the wholesomeness of grain and grain-derived co-products destined for animal feed. These three projects monitor a range of grain contaminants including mycotoxins and hence provide the respective industry sectors with a due diligence approach to their raw materials and a data base of results that can be used in discussion with regulatory bodies.

HGCA project 2726: Reduced fusarium ear blight and mycotoxins in UK wheat through improved resistance (REFAM). This Sustainable Agriculture LINK (SAL) project aims to identify the genes that confer resistance to FEB. By identifying the genes responsible from known resistant varieties from different parts of the world and mapping their positions the project will provide breeders with tools to develop FEB resistant UK wheat varieties. This is seen as the most likely route to controlling fusarium infections and hence mycotoxin levels.

HGCA project 2743: Improving the deposition and coverage of fungicides on ears to control fusarium ear blight and reduce mycotoxin contamination of grain. While there are no truly resistant varieties in the UK there is a need to ensure that ear sprays are as effective as possible against FEB and hence the production of mycotoxins. This project is investigating nozzle type and spray action to ensure good ear coverage and relates this to mycotoxin levels. The output will be recommendations for spraying that will control and hence minimise fusarium infections.

HGCA project 2981: The fate of fusarium mycotoxins in the cereal food chain. This Defra LINK/FSA investigation which started in September 2004 will look at the effects of processing on the levels of mycotoxins from raw materials to food products and by-products. It will produce guidance on process conditions that will reduce mycotoxin levels in food products.

HGCA project 3008: Rapid analytical systems for raw produce quality and safety attributes: Phase 2 mycotoxins. With the high profile of mycotoxins in cereals there is a need to provide a rapid, robust and inexpensive test that can be applied to cereals. This two year Defra LINK project aims develop an instrument based on an immuno-biosensor that coupled with a novel extraction technique will measure mycotoxin to a tenth of legislative limits.

The above summary indicates the considerable effort that is being spent on both reducing fusarium mycotoxin levels and understanding the current situation. It can be concluded that the level of knowledge and expertise within HGCA and its contractors is high and able to provide useful information to all its levy payers.

7. Conclusions

- This very selective survey focussed on feed wheat samples rejected for presence of ‘pink grains’. These wheat samples with high numbers of visible pink grains contained DON at higher concentrations than has been found in random, stratified survey of the UK wheat crop undertaken in 1999. Even though higher than usual levels of DON were found these cannot be related to the number of pink grains in the sample.
- Although elevated DON levels have been found in these feed wheat samples, when they are considered in the light of on-going discussions regarding possible EU legislative limits for feed grains then only one sample, which contained too many pink grains to be counted, would be considered a particular cause for concern.
- Other work, for example HGCA/FSA project 2452, which is not focussed specifically on rejected feed wheat samples, shows that *Fusarium* mycotoxin levels in 2004 are generally low and similar to previous years.
- The level of FEB in 2004 was also normal when compared with previous years in the Defra FEB incidence survey.
- This short project must be considered with reference to the full R&D programme funded by HGCA, FSA, Defra and LINK that is presently active in the area of FEB and associated mycotoxins.

8. References

1. Anon. COCERAL. Report of the meeting of the Cereals Advisory Group. 24th September 2004.

2. G Jones. FSA EC developments concerning animal feed. 9th August 2004.

3. Project Report 230. Survey of mycotoxins in stored grain from the 1999 harvest in the UK; July 2000. Prickett, AJ, MacDonald, S, Wildey, KB, Home-Grown Cereals Authority, London

9. Appendix 1: Full analytical results

Region	No of pink grains	DON	DAS	3AcDON	15AcDON	FUSX	NIV	NEO	T23	T2	HT2	*F. graminearum*	*F culmorum*	*F poae*	*F* spp
E	50	743	<10	<10	<10	<10	29	<10	<10	<10	<10	0	Light	0	Medium
E	60	649	<10	<10	<10	<10	28	<10	<10	<10	<10	0	0	0	Light
E	TMTC	11500	<10	31	77	<10	93	<10	<10	<10	<10	0	Very Heavy	0	0
SW	3	183	<10	<10	<10	<10	17	<10	<10	<10	<10	0	0	0	0
Scot	10	20	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	0	0	0
E	TMTC	1839	<10	<10	<10	<10	27	<10	<10	<10	<10	0	0	0	0
SW	100	720	<10	<10	<10	<10	36	<10	<10	<10	<10	0	Medium	0	0
E	35	3016	<10	22	18	<10	50	<10	<10	<10	<10	0	Light	0	Medium
SW	7	1069	<10	<10	<10	<10	11	<10	<10	<10	<10	0	Light	0	Medium
SE	22	923	<10	<10	<10	<10	36	<10	<10	<10	<10	0	Medium	0	Medium
SW	3	58	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	Light	0	Medium
SE	5	39	<10	<10	<10	<10	<10	<10	<10	11	12	0	0	0	0
SE	3	22	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	Light	0	0
E	10	1001	<10	<10	<10	<10	139	<10	<10	<10	<10	Medium	Medium	Light	Light
E	42	1122	<10	<10	<10	<10	89	<10	<10	<10	18	0	Light	0	Light
E Mids	45	1276	<10	<10	10	<10	30	<10	<10	<10	<10	0	Medium	0	Light
Y & H	30	1334	<10	<10	10	<10	31	<10	<10	<10	<10	0	0	0	Light
E Mids	11	179	<10	<10	<10	<10	38	<10	<10	<10	<10	0	Light	0	Medium
E Mids		261	<10	<10	<10	<10	26	<10	<10	<10	<10	0	Light	0	Light
E Mids		1866	<10	<10	14	<10	36	<10	<10	<10	<10	Light	Light	0	Very Heavy
E		1058	<10	<10	<10	<10	28	<10	<10	<10	<10	0	0	0	Heavy
Y & H		57	<10	<10	<10	<10	20	<10	<10	<10	<10	0	0	0	Light
E Mids	40	1490	<10	<10	<10	<10	36	<10	<10	<10	<10	0	Heavy	0	Medium
E Mids	45	2472	<10	11	16	<10	34	<10	<10	<10	<10	0	Medium	0	Heavy
E Mids	20	344	<10	<10	<10	<10	<10	<10	<10	<10	<10	Light	0	0	Light

E Mids	15	1016	<10	<10	<10	<10	20	<10	<10	<10	<10	0	0	0	0
E Mids	20	2380	<10	<10	14	<10	25	<10	<10	<10	<10	0	0	0	Light
E	15	538	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	Light	0	Light
E	20	241	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	0	0	Light
E	30	1010	<10	<10	<10	<10	16	<10	<10	<10	<10	0	Light	0	Medium
E	35	791	<10	<10	<10	<10	15	<10	<10	<10	<10	Light	Medium	0	Light
E	48	4723	<10	14	20	<10	44	<10	<10	<10	<10	0	Very Heavy	0	Light
Y & H		601	<10	<10	<10	<10	46	<10	<10	<10	<10	0	Heavy	Light	Medium
E Mids		2644	<10	<10	15	<10	58	<10	<10	<10	<10	0	Heavy	0	Medium
E Mids		3088	<10	<10	29	<10	36	<10	<10	<10	<10	0	Very Heavy	0	Medium
E		1257	<10	<10	10	<10	37	<10	<10	<10	<10	0	Heavy	0	Medium
E		4079	<10	25	37	<10	82	<10	<10	<10	<10	Very Heavy	0	0	Light
E	20	341	<10	<10	<10	<10	28	<10	<10	<10	<10	0	Medium	0	0
E Mids		33	<10	<10	<10	<10	27	<10	<10	<10	12	0	Light	0	Light
E	20	331	<10	<10	<10	<10	77	<10	<10	<10	<10	Light	0	0	Light
E	25	3722	<10	26	46	<10	142	<10	<10	<10	<10	Light	Medium	0	Medium
E	400	1405	<10	<10	13	<10	30	<10	<10	<10	<10	0	Very Heavy	0	Medium
Y & H	10	407	<10	<10	<10	<10	61	<10	<10	<10	<10	0	Medium	0	Medium
Y & H	20	177	<10	<10	<10	<10	13	<10	<10	<10	<10	0	Medium	0	Medium
Y & H	25	294	<10	<10	<10	<10	10	<10	<10	<10	<10	0	Light	0	0
E Mids	20	451	<10	<10	<10	<10	<10	<10	<10	<10	<10	Medium	Light	0	Light
E	20	1334	<10	<10	<10	<10	36	<10	<10	<10	<10	0	Very Heavy	0	Medium
E	10	1397	<10	<10	<10	<10	33	<10	<10	<10	<10	0	0	0	0
E	50	3775	<10	20	25	<10	75	<10	<10	<10	<10	0	Very Heavy	0	0
SW	20	1800	<10	<10	<10	<10	76	<10	<10	<10	<10	Very Heavy	Light	0	Medium
E	8	586	<10	<10	<10	<10	52	<10	<10	<10	<10	Light	Light	0	Medium
E	8	438	<10	<10	<10	<10	23	<10	<10	<10	<10	Medium	Light	0	Light
E	8	1076	<10	<10	<10	<10	52	<10	<10	<10	<10	0	Light	0	Heavy
E	8	416	<10	<10	<10	<10	34	<10	<10	<10	<10	Light	Light	0	Medium
E	100	3777	<10	10	15	<10	28	<10	<10	<10	<10	Very Heavy	Medium	0	Light
E	40	945	<10	<10	13	<10	23	<10	<10	<10	<10	0	Heavy	0	Medium
E	30	1978	<10	<10	17	<10	53	<10	<10	<10	<10	Medium	Light	0	Very Heavy

E	40	371	<10	<10	<10	<10	24	<10	<10	<10	<10	0	0	0	Light
E	40	1351	<10	<10	<10	<10	61	<10	<10	<10	<10	Heavy	Light	0	Light
E	12	1819	<10	10	11	<10	27	<10	<10	<10	<10	0	Very Heavy	0	Light
SE	9	243	<10	<10	11	<10	<10	<10	<10	<10	<10	Medium	0	0	Medium
SE	50	1589	<10	<10	11	<10	13	<10	<10	<10	<10	Medium	Medium	0	Very Heavy
NI	40	148	<10	<10	<10	<10	28	<10	<10	<10	<10	0	0	0	Light
Y & H	50	1235	<10	<10	<10	<10	75	<10	<10	<10	<10	0	Heavy	0	Medium
E	11	655	<10	<10	<10	<10	60	<10	<10	<10	<10	Heavy	Light	0	Light
E Mids	25	2300	<10	<10	<10	<10	<10	25	<10	<10	<10				
N E	7	11	<10	<10	<10	<10	<10	<10	<10	<10	<10				
SE	0	593	<10	<10	<10	<10	<10	20	<10	<10	<10				