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Validation of a model to avoid conditions favouring Ochratoxin A production during ambient-air drying

by

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ABSTRACT

Ochratoxin A (OTA) is a fungal mycotoxin that may form when grain moisture content exceeds 18%. EU food limits are set at 5ppb. In a previous HGCA project the risk of OTA formation during drying was assessed using the simulation tool 'Storedry', substituting the old biodeterioration criteria of visible mould or germination loss with a new model for avoiding OTA formation. This model was based on the time taken before fungal growth enters the rapid growth phase. Shorter safe storage times were predicted using the new model. The present study aimed to compare these predictions with real, full scale drying processes.

Four bins each of 30 tons of wheat were dried using ambient air in two seasons. Physical parameters (e.g. moisture and temperature in the wheat and biological changes including mycotoxin production) were measured to provide data to validate the model. Valuable data on changes in populations of fungi were also collected.

The simulation, 'Storedry', gave good predictions of the general drying behaviour and final moisture content. However, it underestimated drying time by about 20%, mainly because the exhaust air in the simulation was less saturated than in the experiment and so the prediction of exhaust humidity by Storedry needs to be improved.

The prediction of biodeterioration using the new OTA model was found to be too rapid. Experimental data indicated a safe storage time at least two times that predicted by the model even when the wheat had been inoculated with *Penicillium verrucosum*, the main cause of OTA in UK cereals.

Based on this, simulations were run with safe storage times increased by a factor of two. From these simulations it was concluded that, for drying with continuous ventilation, the new OTA model was no more demanding than the well established model based on visible mould and significant loss of viability. Therefore, when drying by continuous ventilation, the risk of OTA appears no greater than the risk of visible mould or significant loss of viability.

For drying by continuous ventilation, the time for the drying front to pass through the bed does not need to be reduced. Hence, recommendations for bed depth and airflow rate in The Grain Storage Guide (HGCA, 2003) remains appropriate for continuous ventilation.

Drying rate does not need to depend on the amount of inoculum present.

SUMMARY

Introduction

Mycotoxins are toxic chemicals produced by fungi. In grain harvested and stored in the UK the main threat of mycotoxin contamination is from ochratoxin A (OTA) which is most likely to be produced by *Penicillium verrucosum*. While *P. verrucosum* grows at 80% relative humidity (r.h.), it does not produce OTA below 85% r.h. At 25°C, grain at 18.7% moisture content gives an r.h. of 85% and so grain with moisture contents below 18.7% will be safe from OTA production.

In a previous HGCA-funded project (Bruce *et al.*, 2006), the risk of OTA formation during drying was assessed using the simulation tool 'Storedry', substituting the old biodeterioration criteria of visible mould or germination loss with a new model for time before onset of rapid fungal growth and possible OTA formation. This new model was developed by Jonsson (Olsen *et al.* 2004) and was based on small scale measurements. Shorter safe storage times were predicted using this new model and near ambient drying was identified as the operation during which grain was most at risk from OTA.

The original aim of the present study was to validate the new OTA model at full scale by comparing measured toxin production with predictions by the new model. This aimed to ensure confidence in the strategies derived in the previous project using the combined model of drying and biodeterioration and indicate where improvements to the model might be appropriate.

Changes in physical parameters including temperature and moisture content, and biological changes including mycotoxin production, were observed in four beds of drying wheat in each of two drying seasons to provide data to validate the model at full scale. Valuable data on other biological changes such as populations of fungi were also collected.

Year 1

Approximately 30 tonnes of freshly harvested wheat was placed in each of four open top bins each 3x3m in plan view. Each bin was fitted with a fan suitable for ambient air drying, connected to two air ducts located just below the parallel-sided part of the bin. Two of the bins contained grain with average initial moisture contents of 19.9% and 21.4% (all moisture contents are given on a wet basis). The other two bins contained grain at higher moisture levels, with average initial moisture contents of

23.3% and 23.9%. These moisture contents had been chosen to cover the range in which OTA formation would be expected. Bed depths were 3.5 to 4m.

As the grain was dried, the temperature was monitored in each bin using thermocouples and r.h. of the air in each aeration duct and at the surface of the grain was measured. Pressure switches were placed in the duct to monitor the timing of the fan operation and the static pressure in the air ducts was measured. Temperature, pressure and r.h. data were logged every 10 minutes onto three dataloggers.

Sampling of grain at five depths, down to 2m, was done on a daily basis except at weekends and bank holidays and the sampled grain was analysed for moisture content by oven drying. Airflow was measured daily for each bin at the inlet to the fan using a vane anemometer and at five locations on the grain bed surface using a Casella meter.

Drying was continued until the moisture content of the grain close to the surface of the bin had fallen, indicating that the drying front had passed through the grain.

Samples were collected at start and end of drying for analysis of fungi and OTA.

Measurements of moisture content of the grain sampled at various depths showed the expected form, i.e. for a period no significant changes were seen because the drying front, moving up through the bin from the air inlet towards the surface, had not reached the zone from which moisture samples were taken. Then the moisture content at the lowest sampling position fell towards an equilibrium moisture value as the drying front passed. This process was repeated at each sampling position in turn as the drying front progressed toward the surface of the bin.

Temperature measurements in all four bins showed a similar pattern, in which the temperature of the 'ambient' air was increased due to the heating imparted by the fan, and then reduced by evaporative cooling as the air passed up through the damp grain. The temperature in the plenum ducts was between 3.6 and 4.0°C hotter than the ambient temperature measured near the bins. This was a greater temperature rise than would normally be found in near ambient drying equipment and was due to the heating of the air by turbulence caused by right-angled bends in the inlet ducts. This meant that, in effect, drying was carried out with a heater constantly on.

The inlet air r.h., initially at about 75%, fell over the experiment to about 65%, while the exhaust remained above 95% throughout. The outlet r.h. varied with moisture content, the exhaust r.h. being 91% for the two driest bins and 97 and 98% for the

two wettest. The exhaust r.h. remained at this level until the drying front passed through the bed surface at which time it dropped to the level of the inlet r.h.

The average airflow through the bins was $32m^3$/min.

Table 1 gives counts of *P. verrucosum*, field and storage fungal species. Counts of field fungi, mainly *Cladosporium* and *Aureobasidium* spp, varied between 10^3 and 10^5 colonies per g at the start of the test and changed little. Counts of storage fungi, mainly *Penicillium* spp but also consistently including *Wallemia*, *Eurotium* and *Asperguillus* spp were of the order 10^2 colony forming units (c.f.u.) per g in the two bins at the lowest moisture content and rose to around 10^4 per g by the time drying was complete and in the two damper bins from 10^3/g to over 10^5/g by the time drying was complete. *P. verrucosum* was only detected in one sample at the start of the test.

Table 1. Counts of *Penicillium verrucosum*, field and storage fungal species in the four bins.

Initial moisture content (%)	Average count ($\log_{10}$ c.f.u. g^{-1})					
	P. verrucosum		Storage species		Field species	
	Initial	Final	Initial	Final	Initial	Final
19.9	0	1.7	2.9	4.0	3.6	4.5
21.4	0	2.4	3.8	5.4	4.9	4.5
23.3	1.8	3.9	3.0	4.0	4.7	4.4
23.9	0	4.3	3.6	5.9	5.1	4.7

Table 2 gives the final OTA levels and associated counts of *P. verrucosum*. The grain was sampled using two methods. Firstly, the grain was sampled using the regulatory sampling method (Commission Directive No. 2002/26/EC), which involves taking 100 samples of 100g of grain. Ten samples of 100g of grain were taken from each of ten columns. The samples from each column were analysed separately. Secondly, the grain was sampled at the surface and at a depth of 0.5m at five positions.

Table 2. Final OTA levels (ppm) and associated counts of *Penicillium verrucosum* ($\log_{10}$ c.f.u g^{-1}).

Moisture content of the grain		19.9	21.4	23.3	23.9
Regulatory Sampling from 10 columns	Ave (ppm)	0	0	0.08	3.83
	Nos +ve	0	0	4	8
	Range (ppm)	-	-	0.11-0.33	0.08-16.31
Samples taken from 5 locations	**surface** Ave (ppm)	0.15	0	1.31	3.42
	Nos +ve	1	0	2	3
	Range (ppm)	0-0.75	-	0-3.35	0-16.1
	P.verrucosum	1.5	2.7	4.1	4.3
	0.5m Ave (ppm)	0.37	0	1.14	1.78
	Nos +ve	1	0	1	3
	Range (ppm)	0-1.83	-	0-5.69	0-5.56
	P.verrucosum	1.9	0	3.8	4.3

The average level of *P. verrucosum* was 51c.f.u g^{-1} in the bin with a moisture content of 19.9%. This level of *P. verrucosum* was apparently associated with occurrence of OTA in only 1 in 5 samples. Where OTA did occur it was at less than 1ppb. Higher counts of *P. verrucosum* at the surface of the bin at 21.4% moisture content did not coincide with detection of OTA. At 0.5m, neither *P. verrucosum* nor OTA were detected.

P. verrucosum numbers reached around 10^4c.f.u g^{-1} in the damper bins by the time drying was complete, coinciding with 1 to 3 samples out of five containing OTA at an average of over 1ppb and with individual samples exceeding 5ppb.

The regulatory sampling method, taking 100 samples of 100g from 10 columns detected no OTA in the two driest bins whereas the individual samples from 5 positions taken at the surface and 0.5m did detect OTA. In the two bins at the higher level of moisture content, more columns produced positive OTA detections than did samples taken from 0 and 0.5m, but, as before, averages were higher using the smaller, non-regulatory samples. This suggests that taking 100 samples, as required for regulatory sampling, may not be necessary and that taking a smaller number of

samples from and near the surface may be all that is required to provide a satisfactory test for the presence of OTA.

The simulation, 'Storedry', calculated well the general drying behaviour and final moisture content, but underestimated the experimental drying time by about 20%. The possible reasons include that the exhaust air in the simulation was less saturated than in the experiment and the simulated drying zone was narrower, and airflow, which is very difficult to measure but a very important determinant of drying rate. The physical parameters were better predicted when the airflow was increased by 25%, so this expedient was used to allow the biodeterioration model to be correctly tested.

The prediction of biodeterioration, using a spoilage index based on the new model of safe time before OTA production may start, was found to be too rapid. Experimental data from Year 1 pointed to a safe storage time at least 2 times longer than predicted by the model, and possibly up to 6 times. One of the possible reasons for this large difference was that the Jonsson model was based on small scale experiments in which the wheat had been inoculated with *P. verrucosum*, the main cause of OTA in UK cereals. Although spores of this fungus were present in the grain sampled after drying in Year 1 experiment, very few spores were present in the grain sampled before drying started. This might be expected on freshly harvested grain as *P. verrucosum* is considered a storage fungus rather than a field fungus. However, it was considered possible that the low spore count of the grain before drying, possibly unusually low in the UK context, may have been responsible for the lower than predicted OTA levels after drying. Consequently, the experiment in Year 2 was designed around the use of an inoculation treatment of the damp wheat with spores of *P. verrucosum*.

Year 2

The same four bins as before were used with wheat at moisture contents of 17.2, 18.9 20.0 and 21.9%. The top 1m of each bin was segregated into nine $1m^3$ cells using a grid of plywood sheeting arranged vertically so as not to affect the airflow.

As each cell was loaded it was inoculated using suspensions of *P. verrucosum* spores, dripped into the grain using a watering can as it entered the cell. Three cells in each bin were inoculated at 1000 spores/g, three cells were inoculated at 10 spores/g and the final three cells were inoculated using water.

The grain was dried as in Year 1 and the temperature, airflow, relative humidity and static pressure were monitored as in Year 1. The grain was sampled twice weekly for moisture, mould and OTA analysis to follow the production of OTA.

Measurements of temperature, airflow, moisture content, relative humidity and static pressure followed a similar pattern to Year 1. The simulation model Storedry again gave good predictions of drying behaviour, though the same areas of difference as Year 1 were again apparent. Although time for the wettest layers to dry was well predicted using the experimental airflow, a better estimate of time above the moisture content suitable for fungal growth was obtained by increasing the measured airflow by 25%, as in Year 1.

Table 3 gives details of sample timing of samples analysed for fungal counts and OTA in relation to drying time. Samples were taken from each of the nine cells in each bin from the surface and from a depth of 0.5m.

Table 3. Timing of sampling.

Initial moisture content (%)	21.9	20.0	18.5	17.2
Experimental drying time (days)	29	24	26	20
Time when samples taken for OTA (days)	22,29	21,24	26	20
Time when samples taken for fungal counts (days)	11,22	11,21	11,22	13,16

Tables 4 and 5 show the counts of *P. verrucosum* from the surface and 0.5 m.

Table 4. Counts of *P. verrucosum* at the surface.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* ($\log_{10}$ c.f.u g^{-1})			
		21.9 %	20.0 %	18.5 %	17.2 %
1000	1	4.11	2.72	1.30	1.70
	2	4.31	2.48	3.00	0
10	1	4.11	3.11	2.43	1.78
	2	4.59	2.00	2.74	0
0	1	4.11	2.36	1.30	2.11
	2	3.85	2.30	1.78	0

Table 5. Counts of *P. verrucosum* at 0.5 m.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* ($\log_{10}$ c.f.u g^{-1})			
		21.9 %	20.0 %	18.5 %	17.2 %
1000	1	5.01	2.91	2.30	2.32
	2	5.35	3.41	2.08	2.70
10	1	4.64	3.00	2.48	2.04
	2	4.67	2.30	2.60	0
0	1	4.63	2.46	2.11	1.30
	2	3.76	1.15	0	0

Counts for the three driest bins were small even in the bin with initial moisture content of 20%.

Where the average initial moisture content was 21.9% the counts were larger. In this case there is some evidence that the inoculation had an effect with the highest counts found at 0.5m in cells with the highest level of inoculum.

No OTA was detected in the incoming grain or in the two bins with the lowest moisture content when drying in those bins was complete. In the bin with an initial moisture

content of 20.0%, no samples from day 21 showed detectable OTA but one sample from day 24 was found to contain OTA at 0.4ppb, marginally above the limit of detection of 0.2ppb and well below the permitted level of 5ppb.

In the bin with highest initial moisture content, OTA was detected in 22 out of 36 samples. Although no samples exceeded the EC regulatory limit, this result shows that the safe life of the wheat has certainly been exceeded. Table 6 shows the average and maximum levels of OTA found at the surface and a depth of 0.5m in this bin.

Table 6. OTA in the bin with an initial moisture content of 21.9 %.

Position	Level of inoculum (spores/g)	Average level of Ochratoxin A (ppb)	Maximum level of Ochratoxin A (ppb)
Surface	1000	1.01	2.96
	10	0.60	3.10
	0	0.92	1.50
0.5 m	1000	0.56	2.40
	10	0.66	3.13
	0	0.17	0.50

The average level of OTA detected after 22 days was 0.6ppb and the average level after 29 days was 0.7ppb. The drying front had passed through the surface before either of the two sets of samples had been taken and so all the grain would have been below the moisture at which activity of the *P. verrucosum* fungus would occur and therefore the levels of OTA would not be expected to increase. However, there was no correlation between the OTA results for a given location between samples taken after 22 days and those taken after 29 days. By this stage the levels of OTA would not be expected to increase but neither would the levels be expected to decrease with time as OTA is stable. The lack of correlation suggests that the OTA was not uniformly distributed in the grain in each cell, otherwise point sampling would have given a consistent result.

No correlation between the level of inoculum and the level of OTA was found, nor was there a correlation between the count measurements for *P. verrucosum* and the level of OTA. That results from the three inoculation levels were not significantly different is

a positive result. If there had been a difference, it would have been necessary to know the amount of inoculum present in wheat in store in order to select the best drying strategy. Clearly this is not practicable.

Six months after drying three of the bins were sampled using the statutory method for OTA analysis. The level detected in the bin with the initial moisture content of 21.9% was 1.9ppb. No OTA was detected in the other bins.

Experimental data from Year 2 confirmed that the safe storage time predicted by the model was too conservative. For continuous ventilation drying, the data indicated a safe storage time between 2.1 and 4.3 times longer than predicted by the model.

Earlier simulations were re-run using a less conservative criterion for successful drying and results were compared with simulations using the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability. Of the 3980 simulations, 3337 dried within the time limit of which 47% were spoiled with the Fraser & Muir model and a spoilage index ≥ 1 while 42% were spoiled with the Jonsson model and a spoilage index ≥ 2. Based on this, the new model with the higher spoilage index was no more demanding than the Fraser & Muir model.

Scientific conclusions

1. The experiment used moisture contents and airflows that allowed OTA development in the wettest treatment, but prevented it in the lower moisture content treatments. It produced a good set of data, appropriate for use in validating both the physical and biodeterioration elements of the simulation model.

2. Neither the presence or the amount of OTA, or the counts of fungal colonies of *P. verrucosum* showed any significant relationship to level of inoculum.

3. The simulation, 'Storedry', gave good predictions of the general drying behaviour, final moisture content and time for the wettest layers, near the surface of the bed, to dry. For example, at 1m, the measured and predicted final moisture content of the four bins dried in year 1 agreed to within 0.5% moisture content.

4. The exhaust air in the simulation was less saturated than in the experiment. The relationship between wheat equilibrium moisture content and air relative humidity is responsible for this aspect of the simulation, so its accuracy at high relative humidity needs to be reviewed and improved.

5. The difference between the simulated and measured r.h. of the exhaust air led to the drying time being underestimated by 20%. A better estimate was obtained by

increasing the measured airflow by 25%. This allowed the biodeterioration model to be correctly tested.

6. The new model of safe time before OTA production may start was too conservative. For continuous ventilation drying, the data indicated a safe storage time between 2.1 and 4.3 times longer than predicted by the model. It should be noted that the new model has not been tested in intermittent ventilation, as used by most farm drying systems.

7. The criterion for successful drying, that spoilage index should not exceed 2, was applied to earlier simulation runs and results were compared with simulations using the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability. The new model with the higher spoilage index was found to be no more demanding than the Fraser & Muir model.

Industrial conclusions

1. When drying by continuous ventilation, the risk of OTA appears no greater than the risk of visible mould or significant loss of viability.

2. For drying by continuous ventilation, the time for the drying front to pass through the bed does not need to be reduced. Hence, recommendations for bed depth and airflow rate in the Grain Storage Guide remain appropriate for continuous ventilation.

3. Drying rate does not need to depend on the amount of inoculum present.

INTRODUCTION

Mycotoxins are toxic chemicals produced by fungi that are thought to suppress competition from other species, possibly even arthropods. In grain harvested and stored in the UK the main threat of mycotoxin contamination is from ochratoxin A (OTA) as conditions are too cool to permit the formation of aflatoxins. Under UK conditions OTA is most likely to be produced by *Penicillium verrucosum* (Frisvad and Filtenborg, 1989). While *P. verrucosum* grows at 80% r.h., it does not produce OTA below 85% r.h. (Northolt and Bullerman, 1982). At 25°C, grain at 18.7% moisture content gives a relative humidity of 85% (Henderson, 1987) and so grain with moisture contents below 18.7% will be safe from OTA production.

Cereals contribute 50 to 80% of the OTA intake among European consumers (SCOOP, 2002) and OTA occurrence in blood and urine is almost universal (MAFF 1999). The European Commission has defined maximum legislative limits for OTA in unprocessed cereals at 5 ppb (Commission Regulation 1881/2006). In the UK, 2% of grain tested is reported as having concentrations exceeding 5 ppb and 21% of samples are reported to have OTA at detectable levels (Scudamore *et al.*, 1999).

Since the highest moisture content for trading of grain in most markets is 15-16%, the toxin can only be produced when the grain is damp. These conditions can occur:
1. During ambient air drying,
2. Before drying by continuous driers, due to harvest backlogs,
3. When surface grain absorbs moisture during the winter or
4. During moisture translocation in un-cooled grain, owing to moisture in hot air rising from the bulk and then condensing on cold surface grain.

Of these possibilities, the ambient-air drying process was deemed the area of highest risk. In this system, ambient air is blown through the bulk by a fan via ducts beneath the grain. This causes a drying front to form and move from the bottom of the grain to the surface. The grain ahead of this front remains at or higher than the initial moisture content until the drying front passes through which can take 10 days even in the best designed systems (Armitage and Wildey, 2003). OTA was found on 4 out of 24 farms during ambient-air drying and in 8 out of 240 samples where the initial moisture content was above 19% (Scudamore and Wilkin, 1999).

A previous HGCA project (Bruce *et al.*, 2006) assessed the risk of OTA formation during drying using the simulation tool 'Storedry', substituting the old biodeterioration criteria of visible mould or germination loss with a new model for OTA formation and fungal growth, based on the time taken before fungal growth enters the logarithmic phase (Olsen *et al.* 2004).

Initial comparisons made using the new fungal model indicated shorter safe storage times. For instance at 20°C and 20% moisture content, 10 days were available for drying before germination loss and visible fungi were likely while there were only 6.5 days before significant risk of OTA. Simulations were run of a 3.0m deep, 110m² bed of 250 tonnes of wheat ventilated at 0.05m³/s.t using the two spoilage criteria, weather data for 20 years from five locations in England, 4 moisture contents, 2 start dates and 7 drying strategies of fan and heater control.

Of those treatments successfully dried using the old criteria, only 2 in 3 succeeded using the new spoilage model, so spoilage by OTA in near ambient drying was considered a potentially serious issue.

A useful measure of drying performance was the maximum bed depth, which would allow drying success in all 20 years simulated. When comparing drying using the new and old criteria, a reduction in bed depth of about 1m was required to ensure success even in the worst drying year. For instance when drying grain at 20% moisture content, running fans continuously and switching heat in at 80% relative humidity (r.h.), grain up to 3.2m deep was successfully dried using the old criteria but grain up to only 2.3m was permissible with the new spoilage model.

Many simulations were then run to see how far performance of existing systems could be improved by changing the fan/heater control strategies, with some success. For example, changing the r.h. at which fans were switched on and off in one of the strategies gave bed depths that were that were 112% higher, costs were 10% lower and drying times were 6% longer. A larger fan allowed an extra 0.2m depth. Even so, a reduction from current bed depth of as much as 1m would be needed to avoid risk of OTA in poor drying seasons.

The original aim of the present study project was to validate the new OTA model at full scale by comparing measured toxin production with predictions by the new model.

This aimed to ensure confidence in the strategies derived in the previous project using the combined model of drying and biodeterioration and indicate where improvements to the model might be appropriate.

Experiments were designed to measure changes in physical parameters such as moisture and temperature in a bed of drying wheat and relate them to biological changes such as mycotoxin production to provide data to validate the model at full scale. Valuable data on other biological changes such as populations of fungi were also to be collected.

YEAR 1

METHODS

Approximately 120 tonnes of freshly harvested wheat was placed in Bins 3 to 6 in the CSL grain store. These are square open top bins of 30 tonne capacity, each fitted with a MR280 5.5kW fan from Air Control Industries Ltd, suitable for ambient air drying. Each fan was connected to two parallel ducts running the length of the bin. Bins 3 and 5 contained grain with average initial moisture contents of 19.9% and 21.4% respectively. Bins 4 and 6 contained grain at a higher moisture level, with average initial moisture contents of 23.3% and 23.9% respectively.

Drying of Bin 5 was started on 8 August 2006, bins 4 and 6 were started on 11 August and Bin 3 was started on 16 August.

The temperature of the grain was monitored at two positions per bin at 2cm above the surface and at depths of 0.3, 0.6, 0.9, 1.2 and 1.5m using type T thermocouples. Relative humidity of the air in one of the aeration ducts per bin was measured using two Honeywell HIH-3610 series humidity sensors. Pressure switches were placed in the duct to monitor the timing of the fan operation and the static pressure in the duct was measured using Furness type FCO40 differential pressure transducers. Temperature, pressure and humidity data were logged every 10 minutes onto three dataloggers, which were downloaded with sufficient regularity to ensure that the loggers did not fill up. On one occasion, over the August bank holiday long weekend, data was not collected so about 1.5 days of data was lost.

Part way through the trial one of the two r.h. sensors in each duct was moved to the surface of the grain.

Sampling of grain at five depths, down to 2m, was done on a daily basis except at weekends and bank holidays. The sampled grain was analysed for moisture content using the International Organisation for Standardisation (ISO) routine reference method for the determination of moisture content for cereals and cereal products (ISO 712:1998). The oven moisture procedure was carried out incorrectly for many of the samples. Fortunately it was possible to correct the erroneous values using a calibration derived by analysing a range of samples analysed using the two methods.

Air speed was measured daily for each bin using a vane anemometer with five repetitions at the inlet to the fan and using a Casella meter at five locations on the grain bed surface.

Drying was continued until the moisture content of the grain close to the surface of the bin had fallen, indicating that the drying front had passed through the grain. This occurred between 7 and 12 September 2006.

Samples were taken for analysis of OTA using two methods. Firstly, the grain was sampled using the regulatory sampling method (Commission Directive No. 2002/26/EC), which involves taking 100 samples of 100g of grain. Ten samples of 100g of grain were taken from each of ten columns. The samples from each column were analysed separately. Secondly, the grain was sampled at the surface and at a depth of 0.5m at five positions. OTA analysis was done using the method of Sharman *et al.* (1992) modified to use a sample size of 25g with 100ml of extraction solvent (Acetonitrile/Water mixture (6:4, v/v)).

Samples were also taken for fungal counts. These were taken at the surface and at 0.5m at the same five positions used for OTA. For each mould analysis 40g of grain was weighed directly into a stomacher bag, 360ml of 0.1 % peptone water added and the grain soaked for 30 minutes. Post soaking the grain was stomached for 1 minute and the suspension serially diluted to 10^{-4}. For each dilution two DG18 agar plates were labelled and 0.1ml of the appropriate suspension spread plated onto the agar surface. DG18 plates were incubated at 25°C for 10 days after which colonies were

identified and counted (counts were expressed as colony forming units (cfu) per gram grain).

RESULTS

1. Moisture content data

Figures 1 to 4 show, for Bins 3 to 6, the time course of moisture content of grain sampled at depths from 0 to 2.0m below the grain surface. The lines connect measured data points, assuming a linear transition between data points.

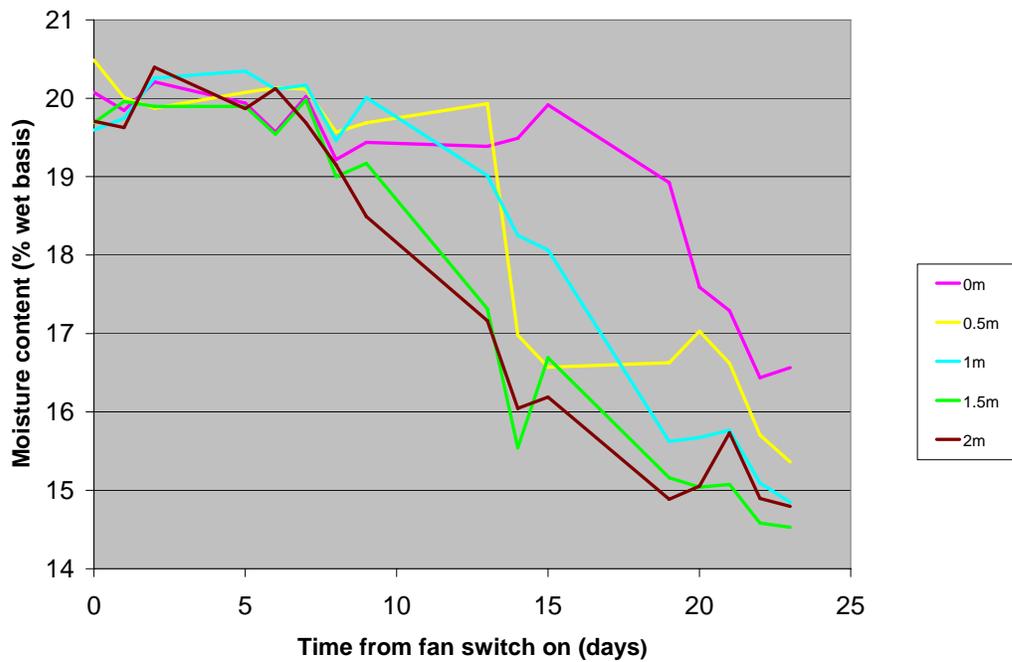**Figure 1. Moisture content of grain sampled at depths from 0 to 2.0m below the grain surface in Bin 3 against time.**

Figure 2. Moisture content of grain sampled at depths from 0 to 2.0m below the grain surface in Bin 4 against time.
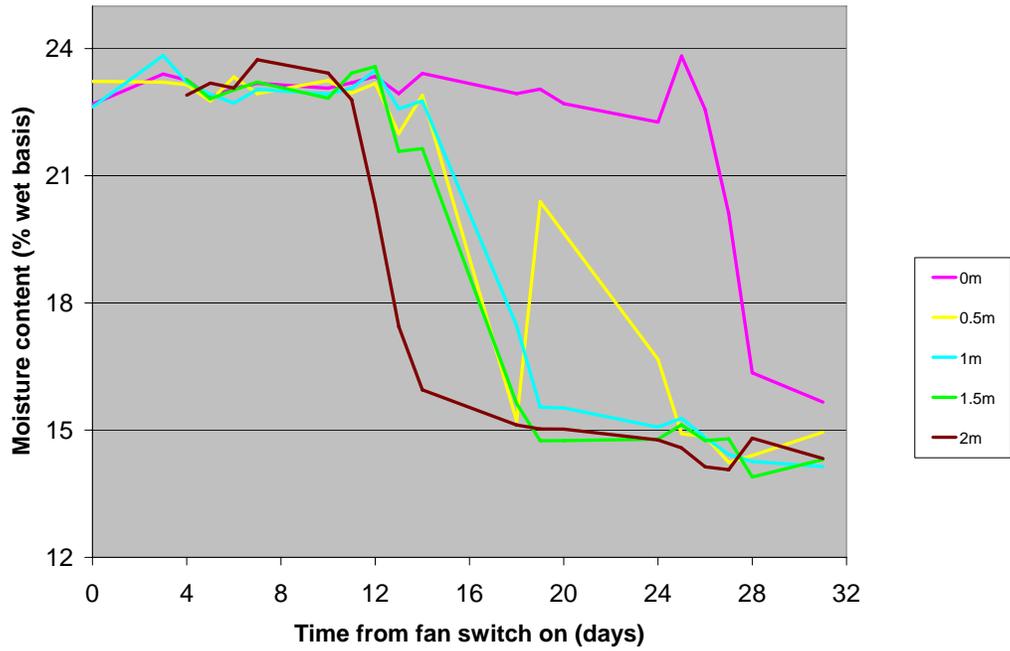

Figure 3. Moisture content of grain sampled at depths from 0 to 2.0m below the grain surface in Bin against time.
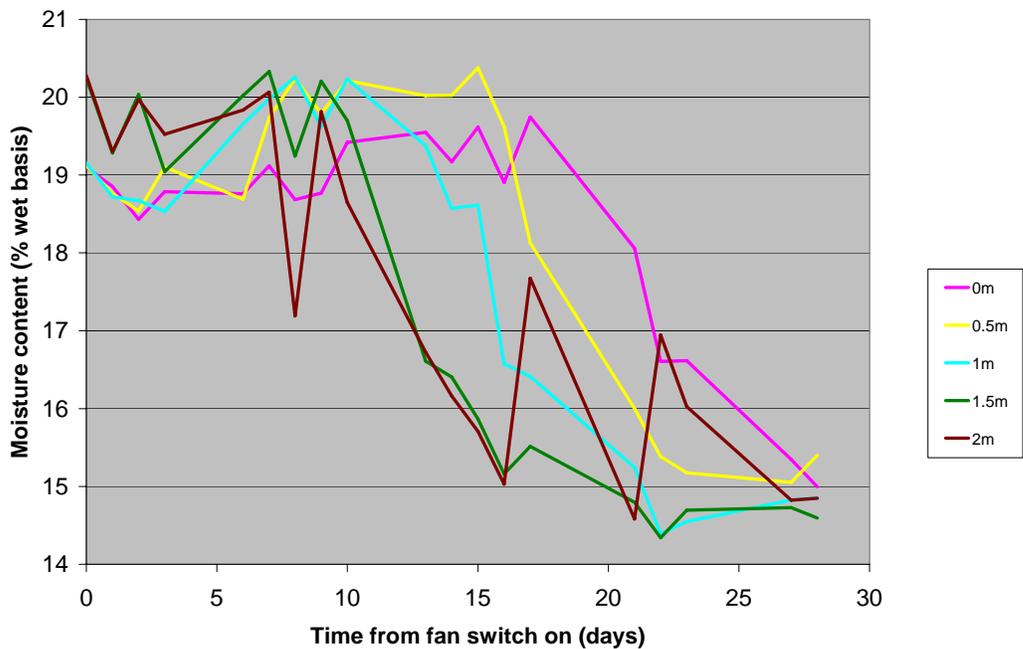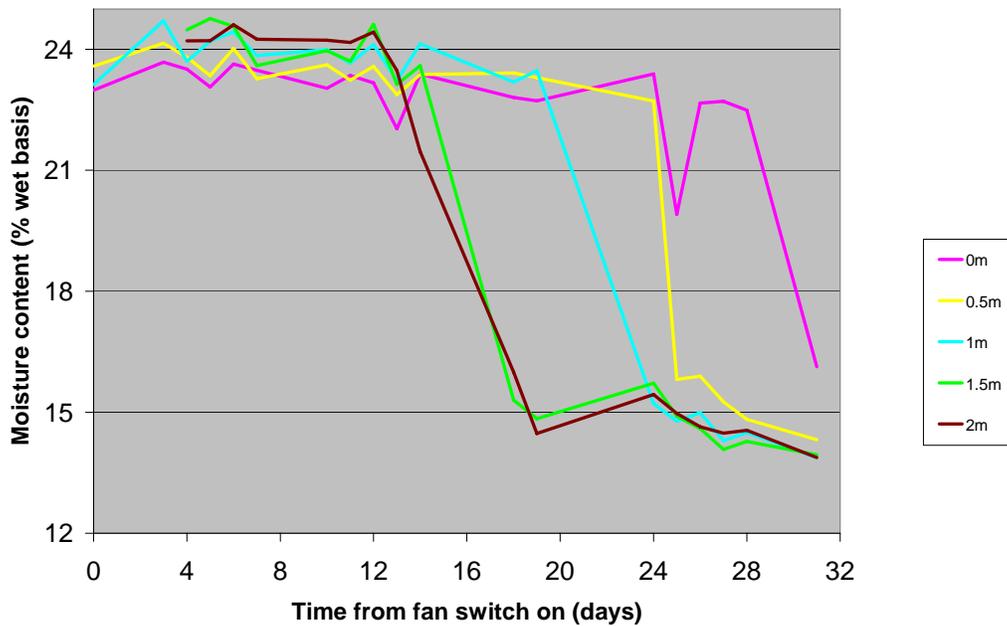
Figure 4. Moisture content of grain sampled at depths from 0 to 2.0m below the grain surface in Bin 6 against time.

The graphs all show the expected form, i.e. for a period no significant changes were seen because the drying front, moving up through the bin from the air inlet towards the surface, has not reached the zone from which moisture samples were taken. Then there was a rapid fall in moisture content towards an equilibrium moisture value as the drying front passed. (Measured values rarely show a very steep fall because the data points are one day apart, at closest.) If the experiment was continued long enough, even the uppermost layers approached moisture in equilibrium with the air. This behaviour is seen more clearly in the wetter bins, 4 and 6, than in the drier bins, 3 and 5. In this experiment, sufficiently low moisture content was achieved for the grain to be safe for storage, so the drying was successful in that basic and important respect.

All the graphs show some considerable fluctuation in the moisture content records. This can be explained partly by uncertainty as to precisely where the samples were taken from in relation to the drying front - moisture gradients are steep close to the leading edge of the drying front. Also, the inevitable disturbance of the grain in the upper 2m owing to sampling devices being inserted through this zone on a daily basis,

and thus the sample taken from a particular depth may not have been at that depth throughout.

2. Temperature, relative humidity and static pressure data

Temperature data was logged every 10 minutes. The pattern of data is dominated by the diurnal swings in temperature, which propagate through the whole bed. This fluctuation makes the detail of the changes at individual levels in the bed difficult to discern. It was found that much of this disturbance could be removed by averaging the data values from each location over a 24 hour period, so that the behaviour is clearer. Most of the temperature data referred to has been averaged for this reason.

Figure 5 shows the data measured by sets of seven thermocouples located in a vertical line from 0.02m above the surface to 2.5m below in Bin 6. The values shown are averaged for all of the locations at each depth at which each temperature was measured and over 24 hours. A data point calculated in this way is plotted at the mid point time of that 24 hours. The gap in the data is due to data being lost due to the logger not being downloaded over a bank holiday weekend.

Figure 5. Temperature in Bin 3 against time.
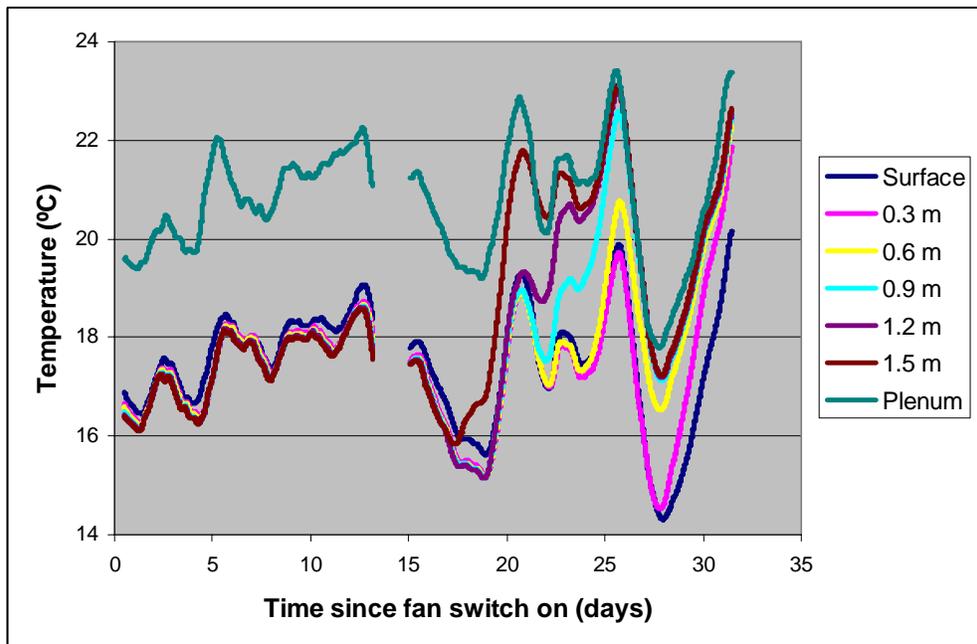
Both vertical thermocouple positions in all four bins show a similar pattern, in which the temperature of the 'ambient' air is increased due to the heating imparted by the

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fan, and then reduced by evaporative cooling as the air passes up through the damp grain.

Ambient temperature is not shown in Figure 5 but the temperature in the plenum ducts was between 3.6 and 4.0^{o}C hotter than the ambient temperature measured near the bins. This was a greater temperature rise than would normally be found in near ambient drying equipment and was due to the heating of the air by turbulence caused by right-angled bends in the inlet ducts. This meant that, in effect, drying was carried out with a heater constantly on. After 17 days, the temperature at 1.5m below the grain surface started to rise as the drying front arrived at that location, and by 21 days this temperature was close to the plenum air temperature, showing that the drying front had passed. As the drying front reaches each location the temperature at that location rises until by 23 days the data are clearly separated, in order of depth. They reconvene until, by 31 days, they are all together and close to the duct temperature, except for the surface sensor which started at 0.02m above the surface but, due to grain bed shrinkage, was by then much more exposed.

This pattern was also seen in all the other bins, and confirms that a drying front was formed in each grain bed, was propagated through the bed and had at least partially emerged from the bed before the experiment was stopped by the fan being turned off.

Figure 6 shows r.h. versus time for Bin 6. Initially both r.h. sensors were in the plenum duct after the fan, but one was moved to the surface of the grain bed on day 6 to monitor the air exhausting from the bed. Averaging over 24 hours shows that the inlet air r.h. was initially at about 75% and fell over the experiment to about 65%, while the exhaust remained above 95% throughout.

Figure 6. Relative humidity against time in Bin 6.
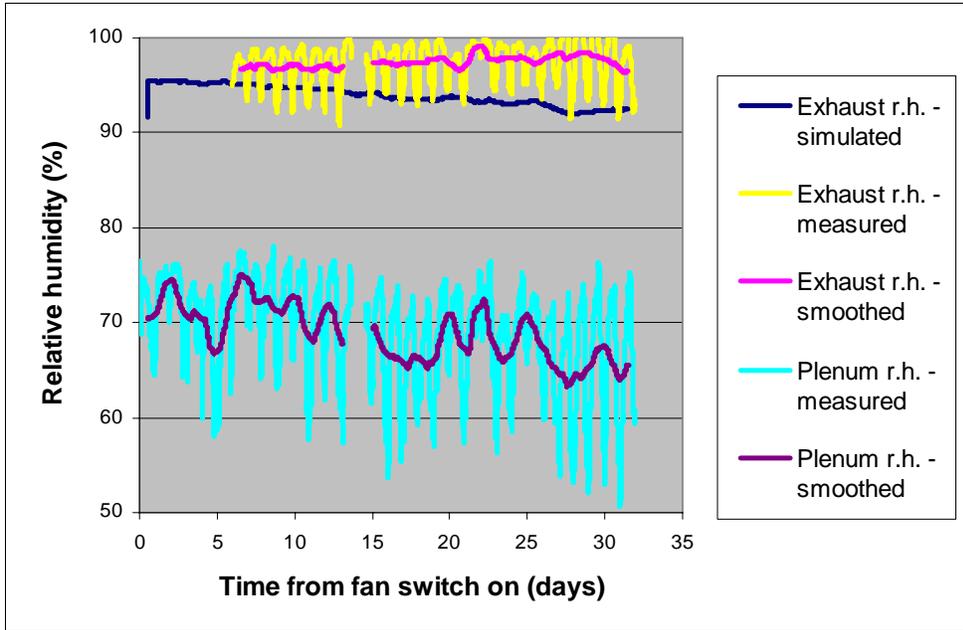
The data for the other bins followed a similar pattern until the drying front passed through the surface and the exhaust r.h. dropped (Figures 7 to 9).

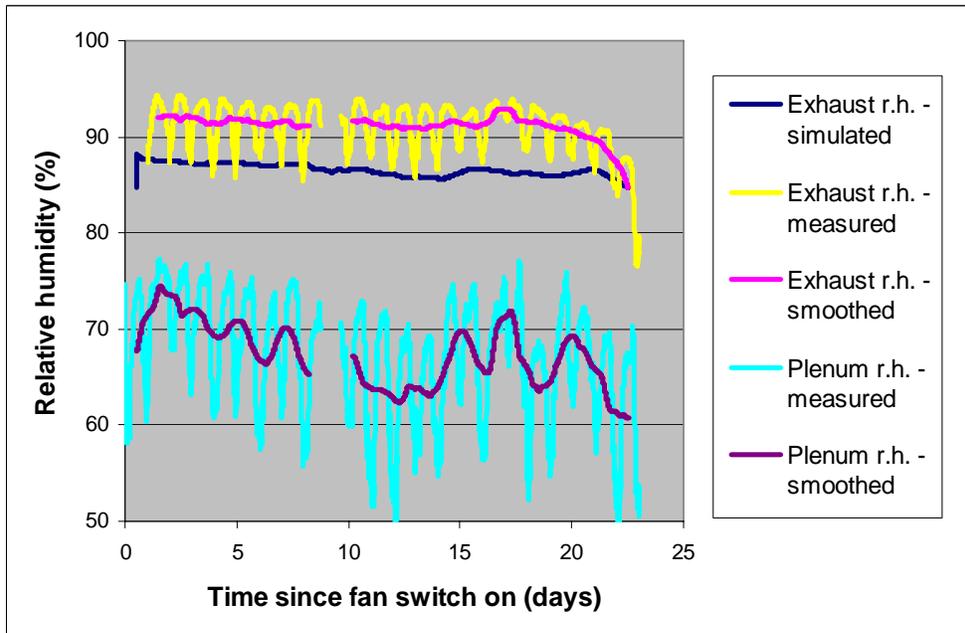**Figure 7. Relative humidity against time in Bin 3.**

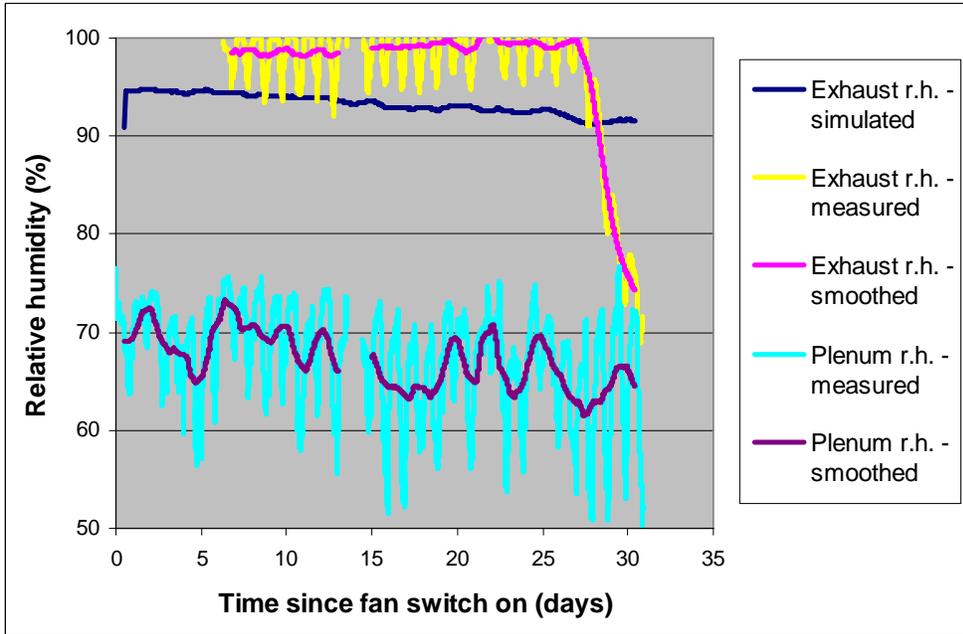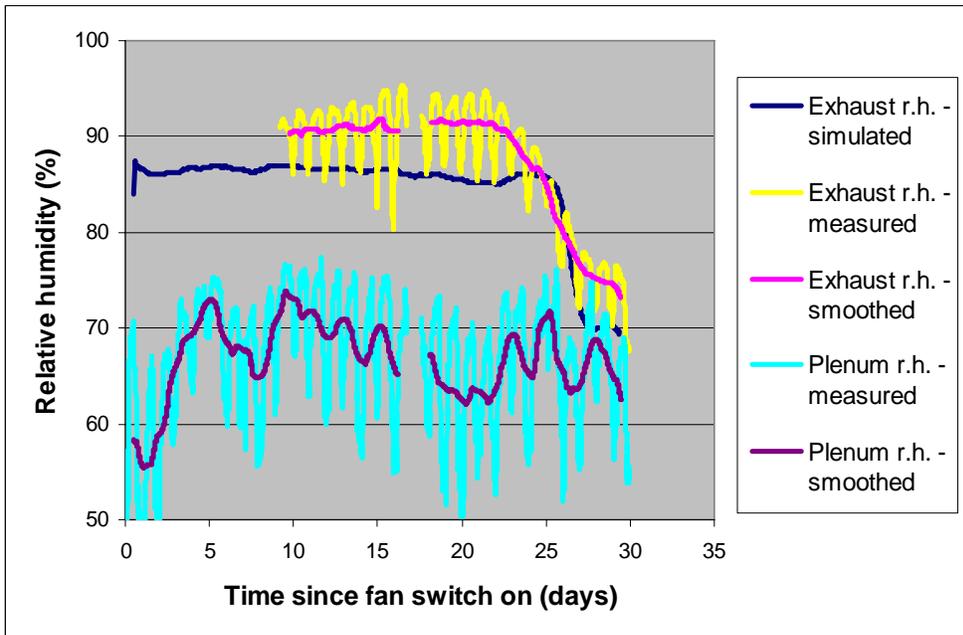**Figure 8. Relative humidity against time in Bin 4.**

Figure 9. Relative humidity against time in Bin 5.

Static pressure in the plenum duct ranged between 180 and 190mm water gauge during the experiment for Bins 4 and 6, and between 190 and 200mm water gauge for Bins 3 and 5.

3. Airflow measurements

Air speed was measured daily for each bin using a vane anemometer with five repetitions at the inlet to the fan and using a Casella meter at five locations on the grain bed surface. Table 1 gives the average flow readings at the fan inlet and at the surface of the grain for each bin.

Table 1. Average flow readings at the fan inlet and at the grain surface in the four bins.

Bin	Flow into fan measured using vane anemometer (m^3/min)	Flow at the grain surface measured using Casella meter (m^3/min)
3	29.4	33.7
4	32.3	33.7
5	29.8	32.2
6	31.6	31.8

4. OTA and fungal data

Table 2 gives counts of *P. verrucosum*, field fungal species and total storage fungal species including *P. verrucosum*. Counts of field fungi, mainly *Cladosporium* and *Aureobasidium* spp, varied between 10^3 and 10^5 colonies per g at the start of the test and changed little. Counts of storage fungi, mainly *Penicillium* spp but also consistently including *Wallemia*, *Eurotium* and *Asperguillus* spp were of the order 10^2 per g in bins 3 and 5 and rose to around 10^4 per g by the time drying was complete and in damper bins 4 and 6, from 10^3/g to over 10^5/g by the time drying was complete. *P. verrucosum* was only detected in one sample at the start of the test.

Table 2. Counts of *Penicillium verrucosum*, field and storage fungal species in the four bins.

Bin	Average count (log_{10} c.f.u. g^{-1})					
	P. verrucosum		Storage species		Field species	
	Initial	Final	Initial	Final	Initial	Final
3	0	1.7	2.9	4.0	3.6	4.5
4	1.8	3.9	3.0	4.0	4.7	4.4
5	0	2.4	3.8	5.4	4.9	4.5
6	0	4.3	3.6	5.9	5.1	4.6

Table 3 gives the final OTA levels determined using the regulatory sampling method and sampling from five locations at the surface and a depth of 0.5m. Counts of *P. verrucosum* associated with the samples taken at the five locations are also shown.

Table 3. Final OTA levels (ppm) and associated counts of *Penicillium verrucosum* (log$_{10}$ cfu g^{-1}).

Bin No			3	5	4	6
Moisture Content (%)			19.9	21.4	23.3	23.9
Regulatory sampling from 10 columns		Average (ppm)	0	0	0.08	3.83
		Nos +ve	0	0	4	8
		Range (ppm)	-	-	0.11-0.33	0.08-16.31
Samples taken from 5 locations	Surface	Ave (ppm)	0.15	0	1.31	3.42
		Nos +ve	1	0	2	3
		Range (ppm)	0-0.75	-	0-3.35	0-16.1
		P. verrucosum	1.5	2.7	4.1	4.3
	0.5m	Ave (ppm)	0.37	0	1.14	1.78
		Nos +ve	1	0	1	3
		Range (ppm)	0-1.83	-	0-5.69	0-5.56
		P. verrucosum	1.9	0	3.8	4.3

In Bin 3, the average level of *P. verrucosum* was 51c.f.u g^{-1} which was apparently associated with occurrence of OTA in only 1 in 5 samples. Where OTA did occur it was less than 1 ppb. Higher counts of *P. verrucosum* at the surface of Bin 5 did not coincide with detection of OTA. At 0.5m, neither *P. verrucosum* nor OTA were detected in Bin 5.

P. verrucosum numbers reached around 10^4 g^{-1} in the damper bins 4 and 6 by the time drying was complete, coinciding with 1 to 3 out of 5 samples containing OTA at an average of over 1ppb and with individual samples exceeding 5ppb.

The regulatory sampling method (Commission Directive No. 2002/26/EC), taking 100 samples of 100g from 10 columns detected no OTA in Bins 3 and 4 but individual samples of the 5 taken at the surface or 0.5 m did detect OTA. In Bins 5 and 6, more

columns produced positive OTA detections than samples taken from 0 and 0.5m, but, as before, averages were higher using the smaller, non-regulatory samples.

5. Consistency of data

Within each of the four bins, there were only small differences between temperatures recorded from the two sensors at a given depth. This is important because it establishes that the airflow, on which the temperature changes depend, was similar at both locations, and by reasonable extension, at all locations in the bin.

Overall airflow passing through the grain bed is difficult to measure accurately and yet it is important because drying speed depends directly on airflow. In the experiment, airflow was measured using a vane anemometer with five repetitions at the inlet to the fan and using a Casella meter at five locations on the grain bed surface. The airflow did not alter systematically during the runs. Values averaged over the run from each of the two methods were consistent, the largest difference between the averages being 14%.

One can also estimate the airflow from measured static pressure rise across the fan. The static pressure rise between atmosphere and the air duct beneath the grain bed was measured at around 180 to 200mm water gauge. If this is assumed to be the pressure rise from the fan, the fan curve (Air Control Industries, 2006) gives a flow of around 45m^3/min, which is 40% higher than the measured flow. However, the air path from the fan outlet to the air duct required the air to turn through two right angles within a short length, and in such flow turbulence would be generated and energy dissipated, as evidenced by the large rise in air temperature due to the fan. The static pressure measured in the duct would be lower after turbulence than it would be with a smooth air path. Using this lower static pressure as the true value generated by the fan would, with a centrifugal fan curve, give too high an estimate of airflow leading to the observed discrepancy between the measured flow and the flow calculated from the fan curve.

COMPARISON WITH SIMULATION

1. Analysis of the data

Analysis was done to generate values, for the input to the Storedry simulation, of initial grain parameters and air parameters over the course of the experiment. The approach used and the derived values are set out in Appendix 1. Input data files and 'weather data' for the simulation were prepared based on both measured data and 'standard values' such as shrinkage coefficient and bulk density for which no data was available from this experiment. The runs generated simulated moisture content and temperatures values at locations used in the experiment for comparison. Also produced were values of the spoilage index. This index, calculated as drying proceeds, indicates the fraction of the safe storage life used up. Hence, when the index reaches a value of 1, the safe storage life for the grain, according to the spoilage model being used, has been reached. The index continues to accumulate until the simulation ends, which may be either because the target moisture content has been reached or because the time for which weather data is available has been used. In this work, the spoilage model was that of Jonsson based on Ochratoxin A (Olsen *et al*. 2004). The spoilage index was calculated by Storedry for all layers of the grain bed individually. The output was the index averaged for the grain bed as a whole and also for whichever layer had the highest index, usually the uppermost layer. This part of the bed remains moist for longest so spoilage is usually most advanced there.

The simulation, Storedry, first calculates the physical parameters of the grain bed, specifically grain temperature and moisture content at all locations in the bed over the drying period. Then, based on the calculated physical parameters, the biodeterioration of the grain is calculated using a model to which elapsed time, grain temperature and moisture are inputs.

First, then, a comparison must be made between the physical parameter values measured and those predicted by Storedry.

2. Physical parameters

Figure 10 shows for Bin 3 the predicted moisture content at depths of 2.0m and 1.0m, chosen to match two of the sampling depths. Also shown are the lines connecting measured data points at the same two depths. The form of the graph from simulation and measurement is similar, and towards the end of the run both sets of lines are

converging towards equilibrium though the experiment was not continued long enough to allow convergence. The main differences are that the simulated moisture at each depth remains near its initial value for longer, and then descends more steeply than measured data. Possible reasons why the fall is less sharply defined in measured data have already been given. But the simulated moisture content lines appear to be 4-5 days behind the measured lines at this, the top of the bed, i.e. simulated drying at the top of the bed is slower than shown by measured data, although the simulated and measured moisture curves arrive at the final moisture content at much the same time.

Figure 10. Measured and predicted moisture contents at depths of 1 and 2 m in bin 3.
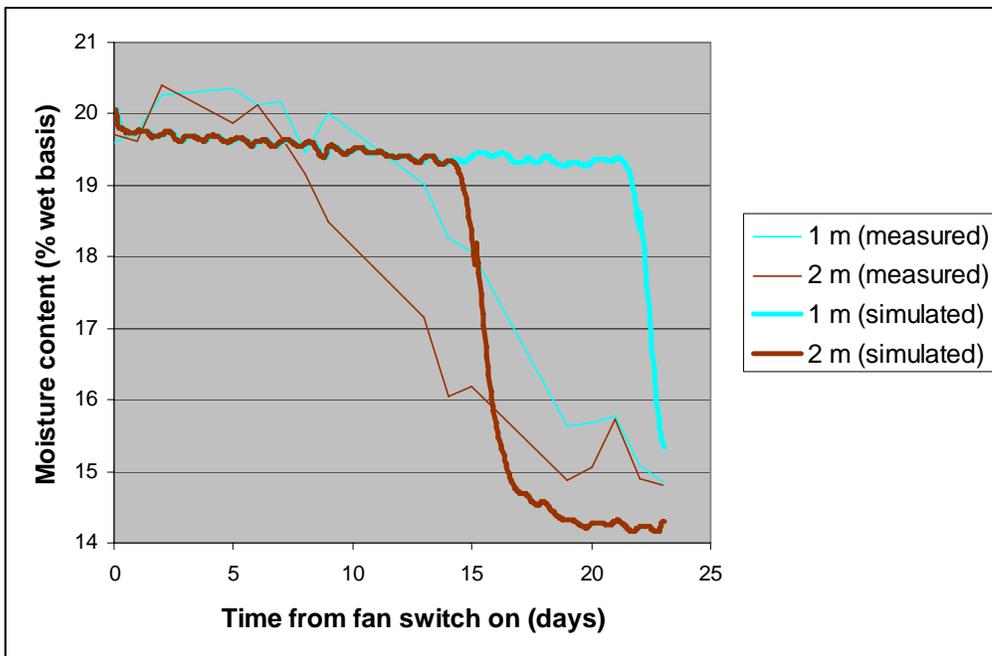
Figures 11 to 13 show the same comparisons for Bins 4, 5 and 6. The replicates show similar responses to each other, and the same conclusions can be drawn as for Bin 3, i.e. that the predicted drying of the top layers of the bed was slower by some 4 to 5 days or some 20% than measured.

Figure 11. Measured and predicted moisture contents at depths of 1 and 2 m in Bin 4.
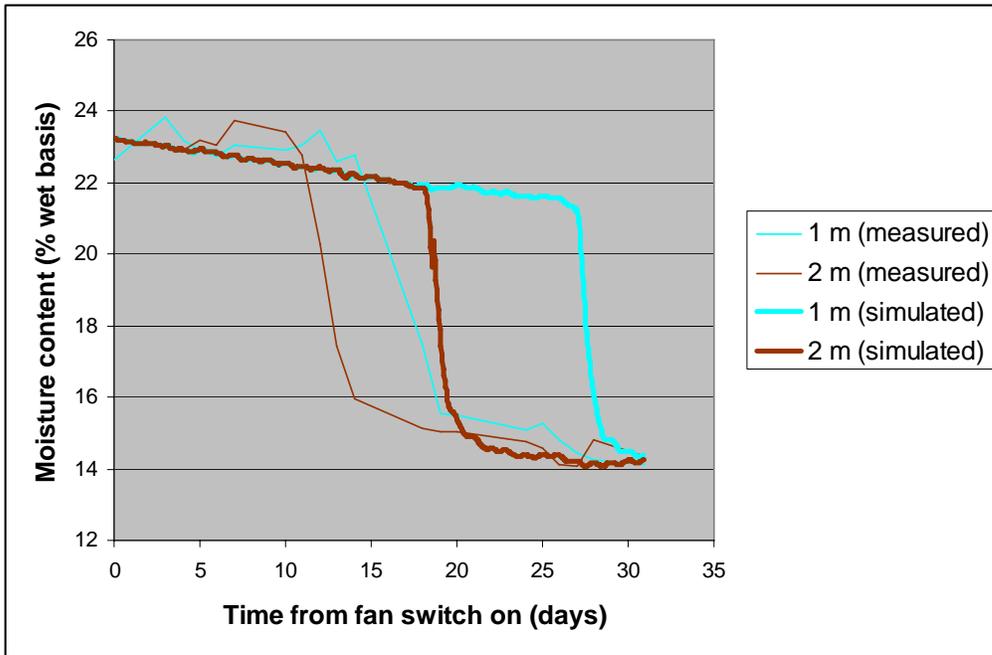

Figure 12. Measured and predicted moisture contents at depths of 1 and 2 m in Bin 5.
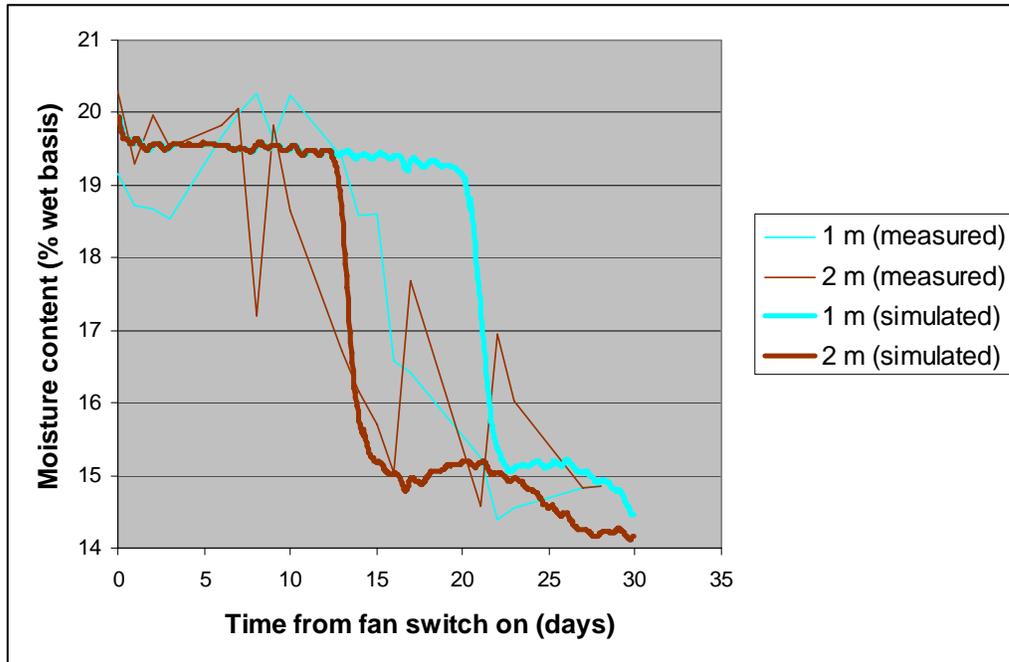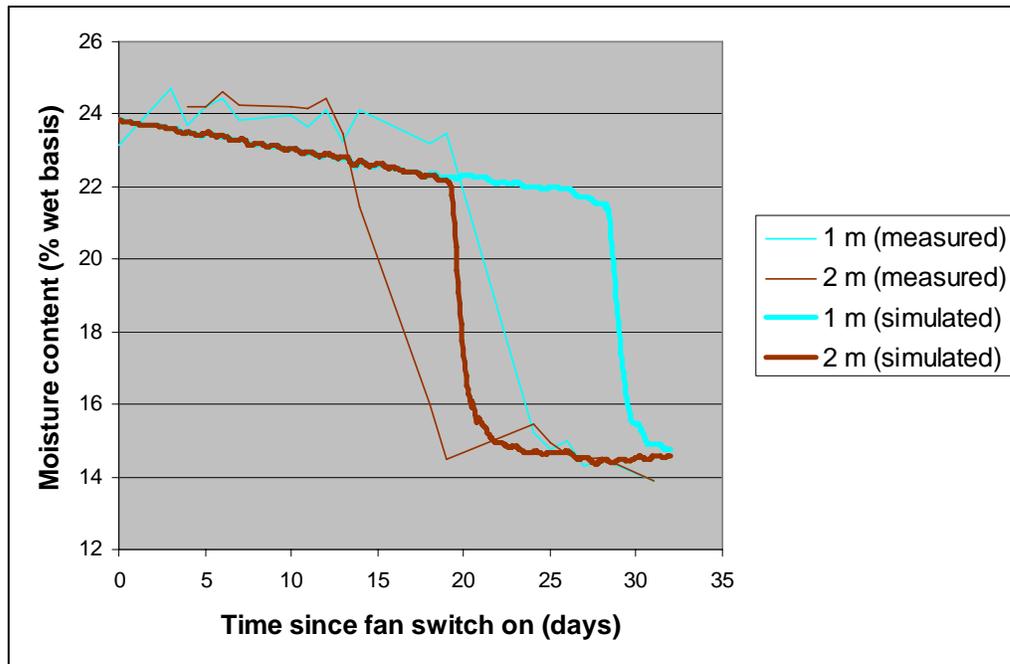
Figure 13. Measured and predicted moisture contents at depths of 1 and 2 m in Bin 6.


The fact that the simulated moisture content falls to the measured level shows that the moisture reduction is correct. The rate of drying once the drying front reaches a particular location is faster than measured, but this is at least partially explained by grain disturbance and uncertainty about sampling depth, as discussed above. The difference between the speed of the drying front through the grain bed may be due to a combination of (a) each unit of air removing more moisture than simulated, or (b) more air flowing though the bed than specified to the simulation from the measurements of airflow. The evidence for each of these hypotheses is now examined.

The more water is evaporated by an airstream, the higher the r.h. and the lower the temperature of the exhaust air will become, until equilibrium is reached. Air within the bed can only saturate as far as the surrounding grain moisture allows, so this moisture determines the equilibrium condition. If the relationship in Storedry that describes the equilibrium between air and grain is not ideal, errors in the predicted evaporation, and hence in the predicted temperature and r.h. would be expected. Looking at the exhaust air r.h., it is seen that the values produced by the Storedry model are indeed lower than measured for all the bins, as shown in Figures 6 to 9 for Bins 3 to 6. (It is noted that, in Bin 5, the exhaust r.h. falls when the drying front

emerges from the top of the bed, as is correctly predicted by the simulation, but a few days later than measured. In the other bins the drying was not continued long enough to see this change.) The fact that the exhaust r.h. was lower in the simulation does not establish that the air carried less water. The temperature must also be examined, since the two are linked. Figure 18 shows the temperature of exhaust air, averaged over 24 hours, for simulation and measurements, in bin 6. The simulated and measured temperatures of the exhaust air from bins 3 to 5 followed a similar pattern.

Figure 14. Measured and simulated temperature of exhaust air, averaged over 24 hours, for Bin 6.
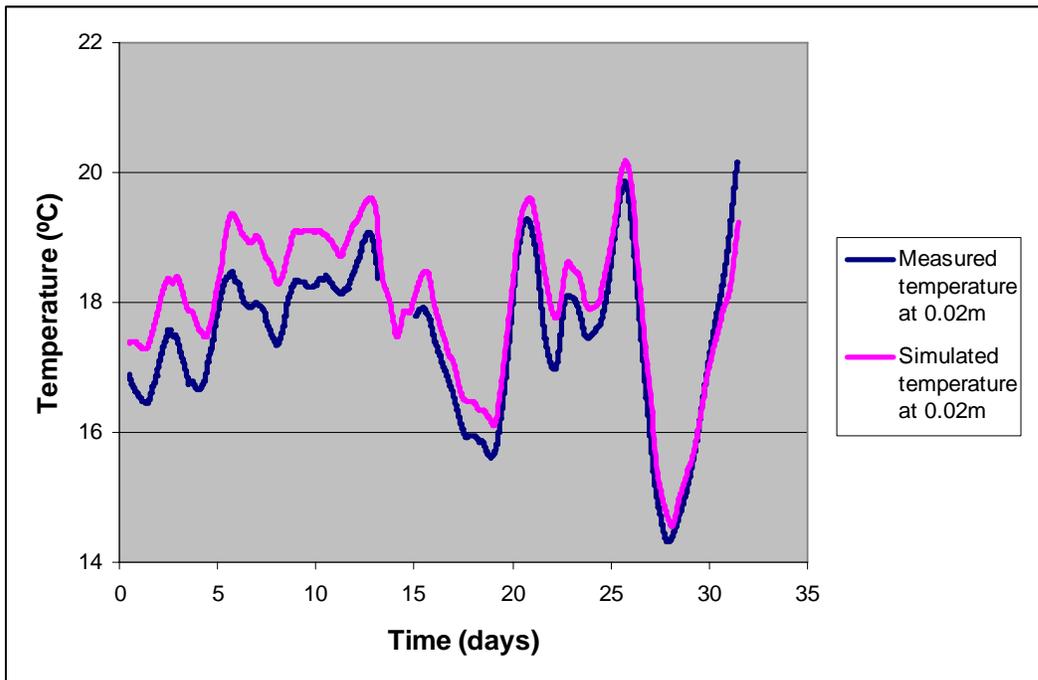

It is clear that for the first half of the drying at least, the measured temperature values are lower than simulated by around 1 ^{o}C. At a temperature of around 18°C, such a temperature difference if caused by additional evaporation would lead to a rise in relative humidity of 5-10% points, consistent with the difference observed and commented on above. So a higher equilibrium relative humidity than used in simulation would allow the exhaust air to become more saturated, and thus each unit of air would remove more water from the grain. The additional removal per unit of air relates to the increase in absolute humidity, but is proportional to the increase in relative humidity over a small range. Starting from an average of 68% r.h. in the plenum, and arriving at a simulated exhaust r.h. of 93% for Bins 4 and 6, and 86% for Bins 3 and 5, an increase of 5% r.h. would lead to an increase in evaporation rate

of 20 to 27%. This difference in drying rate can account completely for the difference in drying time observed. It assumes (not unreasonably) that all the measured values are correct.

Figure 15 shows the simulated exit temperature and the measured temperature at a depth of 0.3m in bin 6. It shows that the difference in temperature was not due to an effect such as cooling of the air once it emerged from the surface. No values of r.h. are available for locations beneath the surface.

Figure 15. Simulated exit temperature and the measured temperature at a depth of 0.3m in bin 6
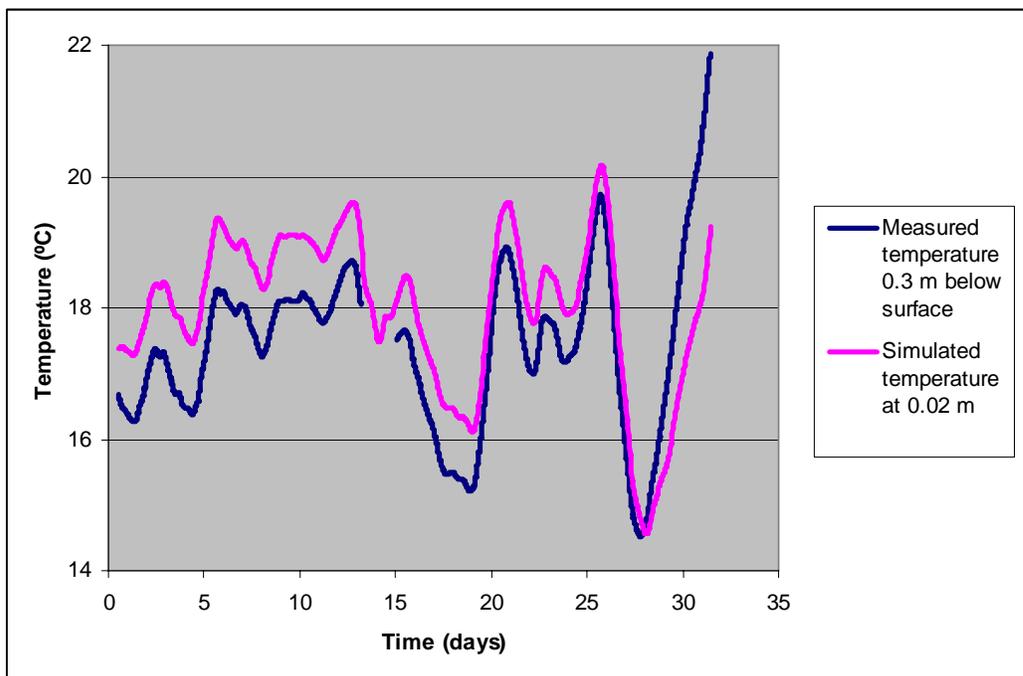
The speed of the drying front up through the bed depends on the mass of water per unit volume that must be removed from the grain, i.e. the grain bulk density as well as its moisture content is relevant. No measurements were made of the bulk density or of the mass of grain in each bin from which the bulk density could be calculated, so this parameter is only a standard value. If the bulk density of the experimental grain was different, the drying rate would be altered.

3. Biodeterioration

The biodeterioration model calculates progress towards spoilage using temperature and relative humidity at each layer in the grain bed for each time step. To test the biodeterioration model versus the measured spoilage data, physical conditions close to

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those measured must emerge from the simulation otherwise the source of the error cannot be ascribed to the biodeterioration model. As has been established, drying of the uppermost layers was some 20% faster in the experiment than in the simulation when it was run using the best estimates of all parameters. To bring the temperature and moisture predictions for the bed closer to those measured; airflow used for simulation was increased by 25%. By this means the drying time for the uppermost layers was reduced to that measured, and hence the time for the development of spoilage was corrected in this zone of the grain bed where spoilage is highest and hence critical. Figures 16 to 19 show for Bins 3-6 the predicted and measured moisture contents for depths of 1.0 and 2.0m below the grain bed surface, with the additional 25% airflow. Although the fall in moisture is steeper than measured, likely reasons being discussed earlier, the simulated drying time is equivalent to that measured. Biodeterioration predicted using this increased airflow can now be compared with measured values.

Figure 16. Predicted and measured moisture contents for depths of 1.0 and 2.0 m Bin 3 with 125% measured airflow
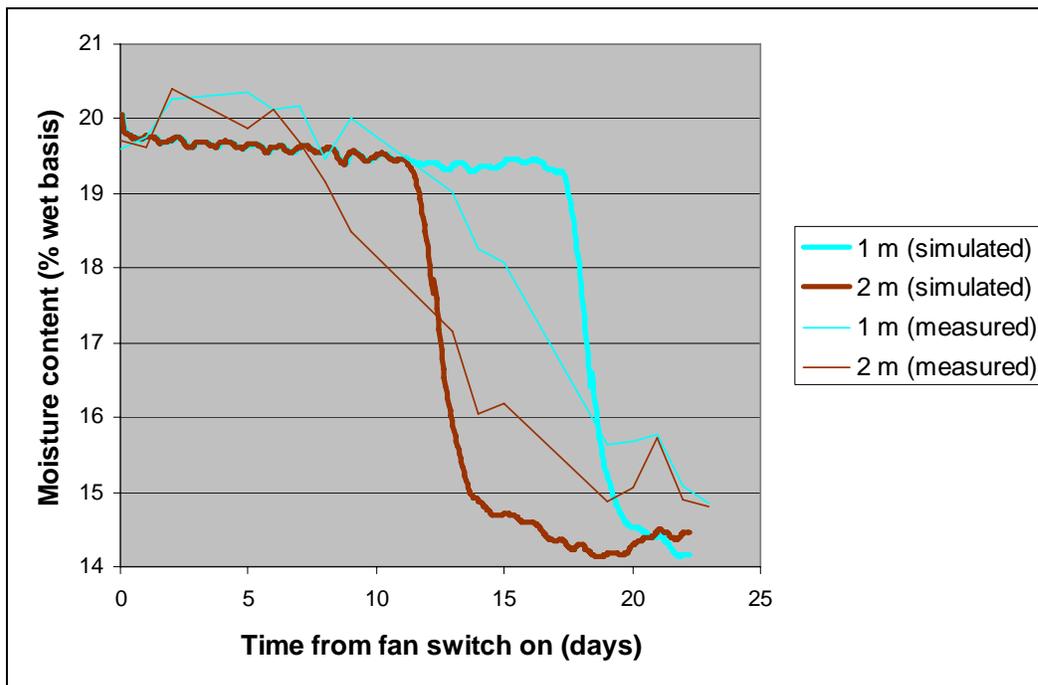
Figure 17. Predicted and measured moisture contents for depths of 1.0 and 2.0 m Bin 4 with 125% measured airflow.
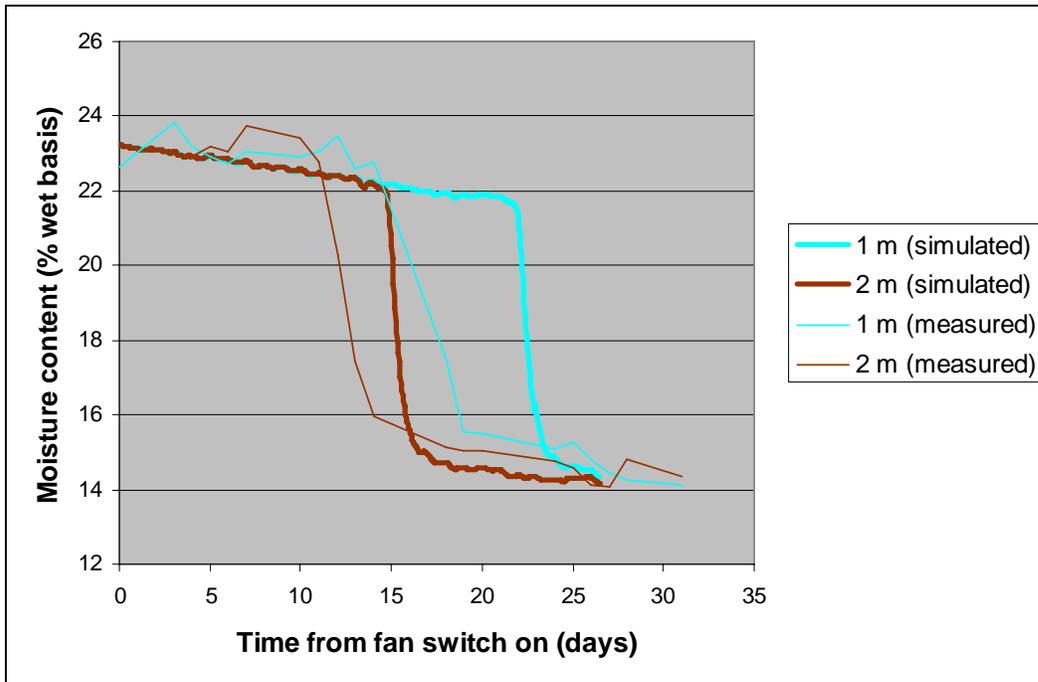

Figure 18. Predicted and measured moisture contents for depths of 1.0 and 2.0 m Bin 5 with 125% measured airflow.
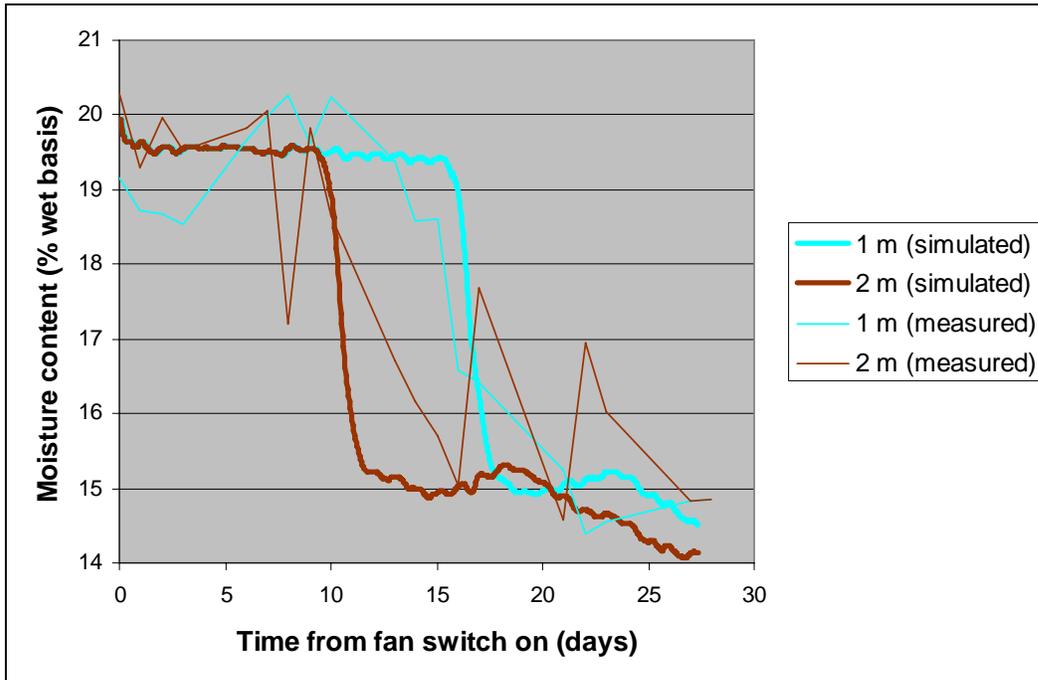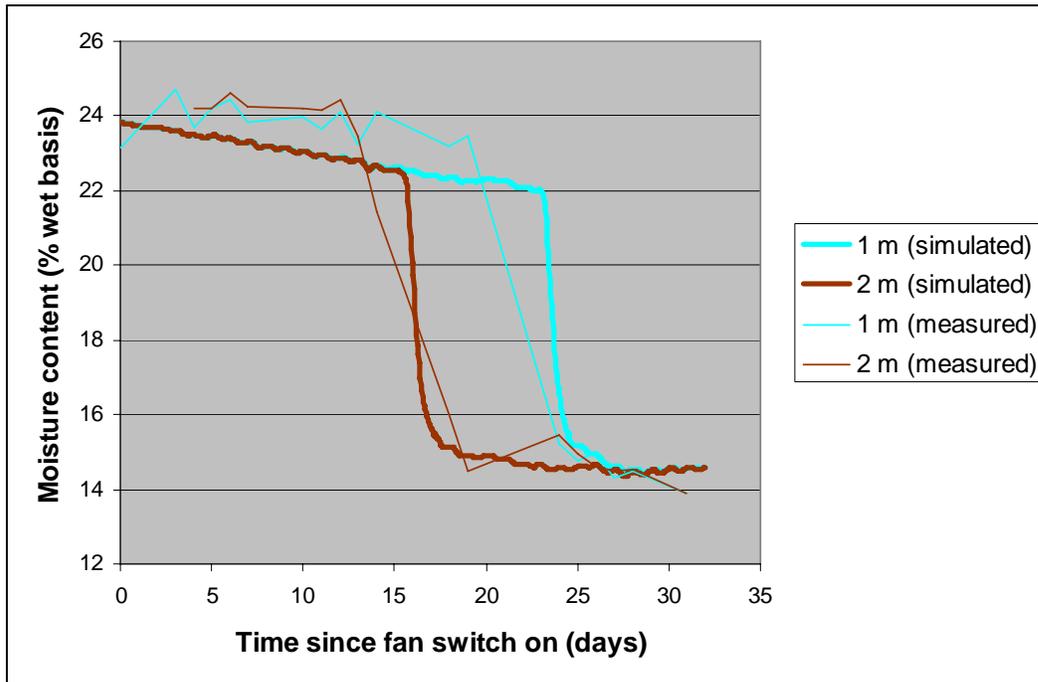
Figure 19. Predicted and measured moisture contents for depths of 1.0 and 2.0 m Bin 6 with 125% measured airflow.

The simulation used the model of Jonsson (Olsen *et al*. 2004) to calculate the spoilage index as drying proceeded. Once this index, which increases over time at a rate dependent on grain moisture and temperature, exceeds unity, the safe life is considered to have expired and the risk of OTA is then significant. The spoilage index approach does not predict the level of OTA, simply its likely absence or presence. For the predicted to agree with the measured result, the spoilage index from the Jonsson model would be less than unity for Bins 3 and 5 (though perhaps close to unity given the presence of OTA at one corner), and greater than unity for Bins 4 and 6. Table 4 shows the maximum spoilage index anywhere in the bed (it is normal for this to be found close to the surface because that grain remains moist longest), for two end points for drying, the average moisture being equal to that measured, and for the surface moisture to be equal. It is clear that the Jonsson model predicted spoilage index values well in excess of unity in all runs. For Bins 5, it was above 2.2, *i.e.* more than twice the safe life had expired by the end of the experimental run, and yet no OTA was detected. For Bin 4, with only 3 samples with detected OTA, the spoilage index was 6.3 so only a small amount of OTA had been produced even though the spoilage index had exceeded six. Bin 6 was predicted to be worse, as was the case in

practice, with an index of 7.3. This comparison shows that the spoilage model of Jonsson was conservative, by a factor of at least 2.2 and arguably up to 6.

Table 5. Comparison of spoilage models for 4 Bins, simulating drying with 125% of the experimentally measured airflow

Target for wettest moisture in bed reached	Bin 3	Bin 4	Bin 5	Bin 6
Max spoilage index by Fraser model	1.7	3.5	1.6	4.1
Max spoilage index by Jonsson model	2.5	6.3	2.3	7.3
Target for average moisture in bed reached	Bin 3	Bin 4	Bin 5	Bin 6
Max spoilage index by Fraser model	1.6	3.5	1.5	4.2
Max spoilage index by Jonsson model	2.4	6.3	2.2	7.3

For comparison, the spoilage index was also calculated using an old but widely used model of spoilage (Fraser and Muir, 1981) in which the safe storage life was based on visible mould and loss of viability. This model predicts that all bins would experience such spoilage, the worst being Bin 6 in which over four times the safe life had expired.

It is noted that, had the equilibrium relative humidity for the given moisture been higher in the simulation model in order to equal that measured and thus to bring about the faster drying seen in practice, the spoilage index calculated in the simulation by the Jonsson model would have been higher still.

INTERIM CONCLUSIONS FROM YEAR 1

1. The simulation, 'Storedry', calculated well the general drying behaviour and final moisture content, but underestimated drying time by about 20%. Investigation of the possible reasons suggests that the exhaust air in the simulation was less saturated than in the experiment. The relationship between wheat equilibrium moisture content and air relative humidity is responsible for this aspect of the

simulation, so its accuracy at the high r.h. end may need to be reviewed and improved.

2. The physical parameters were better predicted when the airflow was increased by 25%, an expedient used to allow the biodeterioration model to be correctly tested.

3. The prediction of biodeterioration, using a spoilage index based on the Jonsson model of safe time before OTA production may start, was found to be too conservative. Experimental data points to a safe storage time at least 2 times longer than predicted by the model, and possibly up to 6 times.

YEAR 2

METHODS

Results from Year 1 showed that the model used for predicting the safe time before risk of OTA was conservative by at least a factor of between 2 and 6. The main question raised by the experiment and simulation was why this large difference arose. Unless the cause could be determined, the simulation, grounded in the experimental results, could not be used with confidence to specify safe drying regimes for UK grain that were not overly conservative.

One of the possible reasons for the difference between model and practice was that the model was based on small scale experiments in which the wheat was inoculated with *P. verrucosum*, the main cause of OTA in UK cereals. Although spores of this fungus were present in the grain sampled after drying in the 2006 experiment, very few spores were present in the grain sampled before drying started. This might be expected on freshly harvested grain as spores of *P. verrucosum* originate in the store from previous harvests and do not come in from the field. However, it was considered possible that the low spore count of the grain before drying, possibly unusually low in the UK context, may have been responsible for the lower than predicted OTA levels after drying. Consequently, the experiment in Year 2 was designed around the use of an inoculation treatment of the damp wheat with spores of *P. verrucosum*.

Inoculum was produced using a known OTA producing isolate of *P. verrucosum.* The isolate was inoculated onto damp grain and grown at 20°C for 10 days before being store at −20°C until required. The spores were harvested by washing the wheat.

Approximately 120 tonnes of freshly harvested wheat was placed in Bins 3 to 6 as before. In each bin, the top 1 m of grain was divided into 9 sections using a grid constructed of sheets of plywood arranged vertically (Figure 20).

Figure 20. Plywood grid used to divide the surface 1 m of grain into 9 sections.
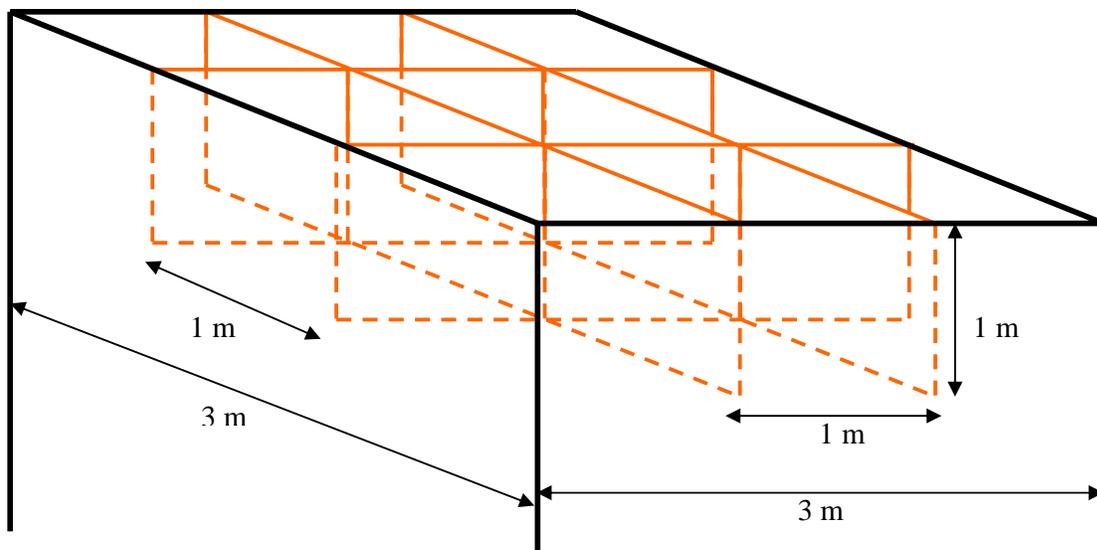
As each section was loaded with approximately 1 tonne of grain it was inoculated using suspensions of the *P. verrucosum* spores in 1 litre of water applied using a watering can as the grain left the delivery spout at the top of the bin.

Three sections in each bin were inoculated at 1000 spores/g and three sections were inoculated at 10 spores/g. The final three sections were not inoculated. Figure 21 shows the layout of the sections in the four bins and the level of inoculation in each section.

Figure 21. Layout of the sections in the four bins and the level of inoculation in each section.

Bin 5   **Bin 6**

Cell 9 1000 spores/g	Cell 6 10 spores/g	Cell 3 0 spores/g	Walkway	Cell 3 1000 spores/g	Cell 6 0 spores/g	Cell 9 10 spores/g
Cell 8 0 spores/g	Cell 5 1000 spores/g	Cell 2 10 spores/g		Cell 2 0 spores/g	Cell 5 10 spores/g	Cell 8 1000 spores/g
Cell 7 10 spores/g	Cell 4 0 spores/g	Cell 1 1000 spores/g		Cell 1 10 spores/g	Cell 4 1000 spores/g	Cell 7 0 spores/g
Cell 9 1000 spores/g	Cell 6 0 spores/g	Cell 3 10 spores/g		Cell 3 0 spores/g	Cell 6 10 spores/g	Cell 9 1000 spores/g
Cell 8 10 spores/g	Cell 5 1000 spores/g	Cell 2 0 spores/g		Cell 2 1000 spores/g	Cell 5 0 spores/g	Cell 8 0 spores/g
Cell 7 0 spores/g	Cell 4 10 spores/g	Cell 1 1000 spores/g		Cell 1 10 spores/g	Cell 4 1000 spores/g	Cell 7 10 spores/g

Bin 3   **Bin 4**

Immediately after loading the bins moisture content samples were taken from the surface and depths of 0.5, 1.0, 1.5, 2.0 and 2.5m. The average moisture contents for the four bins are given in Table 6.

Temperature was monitored as before at depths of 0.3, 0.6, 0.9, 1.2 and 1.5m at four positions in each bin. A humidity sensor was placed on the surface of the grain in each bin along with a thermocouple to measure surface temperature. Table 6 gives the initial moisture content in the bins, the date on which drying started and the positions of the thermocouples and humidity sensors.

Table 6. The approximate initial moisture content for each bin and the cells with temperature sensors, humidity sensors and the date on which drying commenced.

Bin	Approximate average initial moisture content (%)	Cells with grain temperature monitoring positions	Cells with humidity sensors and surface thermocouples	Date drying began
3	22	2, 3, 4, 9	10	02/08/07
4	20	3, 4, 5, 9	6	24/08/07
5	19	1, 6, 7, 8	5	02/08/07
6	17	4, 5, 8, 9	1	15/08/07

Humidity of the air in the aeration duct, the timing of the fan operation and the pressure in the duct were measured as before. The time period between logged data sets was increased to 30 minutes on two of the dataloggers and 60 minutes on the third to allow weekly downloading without the loss of data.

Airflows were measured on the grain surface from the centre of each cell at appropriate intervals using the Casella meter. Grain was sampled at up to five depths, down to 2m, twice a week to ensure drying was tracked and to provide samples for analysis of OTA and fungal content. Analysis of the samples for moisture content was done using the ISO oven method.

The experiment was continued until, from moisture content samples, the moisture content of the grain close to the surface of the bin had fallen, indicating that a drying front had been passed through the grain. This took between 20 days for the driest wheat to 29 days for the wettest.

P. verrucosum was isolated from the fungal count samples and tested for OTA production.

RESULTS

1. Moisture content data

Table 7 gives the initial moisture contents at 6 depths for each bin.

Table 7. Initial moisture content for each bin.

| Depth (m)	Moisture content (% wet basis)			
	Bin 3	Bin 4	Bin 5	Bin 6
0	20.3	19.3	18.5	17.3
0.5	20.4	20.1	19.3	17.0
1	20.9	20.1	18.7	17.1
1.5	23.2	19.8	19.1	17.3
2	23.5	20.0	19.1	17.3
2.5	23.2	20.1	18.4	17.1
Average	21.9	20.0	18.9	17.2

The moisture content in the initial samples of bins 4, 5 and Bin 6 are fairly uniform. However, Bin 3 shows a considerable range, with samples taken from the top 1m at approximately 20.5% and those taken from 1.5 m and below at 23.3%.

Fewer samples were analysed in Year 2 but it was ensured that there were enough samples to confirm that the drying behaviour was the same as in year 1. In this experiment, a sufficiently low moisture content was achieved for the grain to be safe for storage, so the drying was successful in that basic and important respect.

2. Temperature, relative humidity and static pressure data

All the vertical sets of seven temperature sensors in each bin show a similar pattern to Year 1, described in detail above. Figure 22 shows for Bin 3, the temperature data measured by one set of seven thermocouples located in a vertical line from just below the grain surface to 1.5m below. The data presented here has been averaged over 24 hours to remove the diurnal fluctuation as before.

Figure 22. Temperature against time in Bin 3.
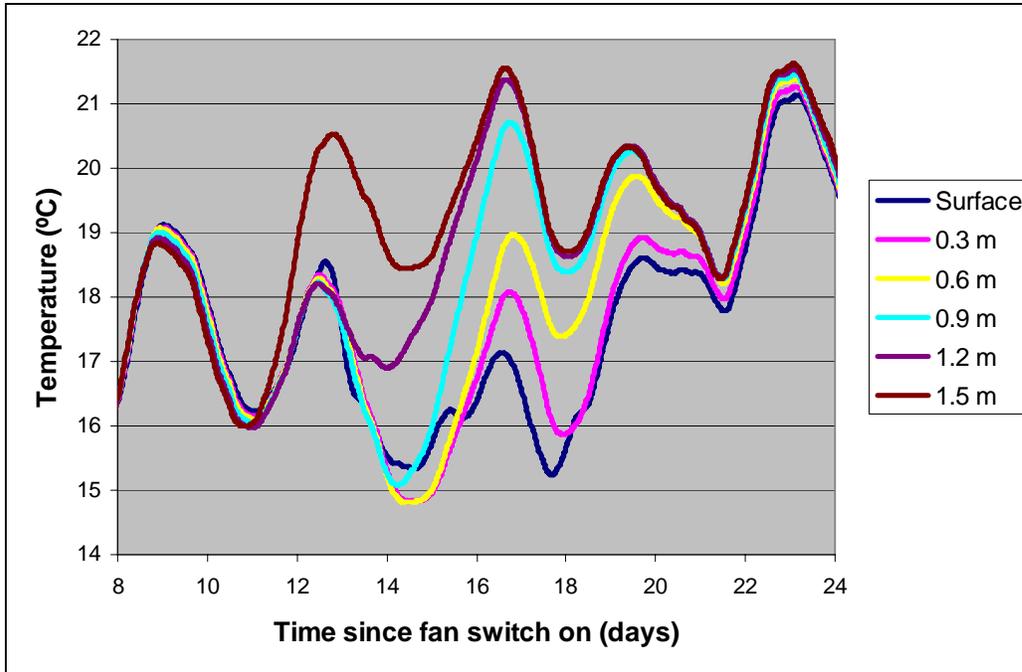
As in year 1, the temperature in the plenum ducts was between 3.5 and 3.7°C hotter than the ambient temperature measured near the bins due to the heating of the air by turbulence caused by right-angled bends in the inlet ducts.

Figure 23 shows r.h. versus time for Bin 5. Averaging over 24 hours shows that the inlet air relative humidity fluctuated between 57 and 73% during the experiment while the exhaust remained around 90% throughout the period until the surface layer began to dry at about day 15. The exhaust r.h. then fell steadily until it was similar to that in the plenum, at which point no further drying was taking place.

Figure 23. Relative humidity against time in Bin 5.
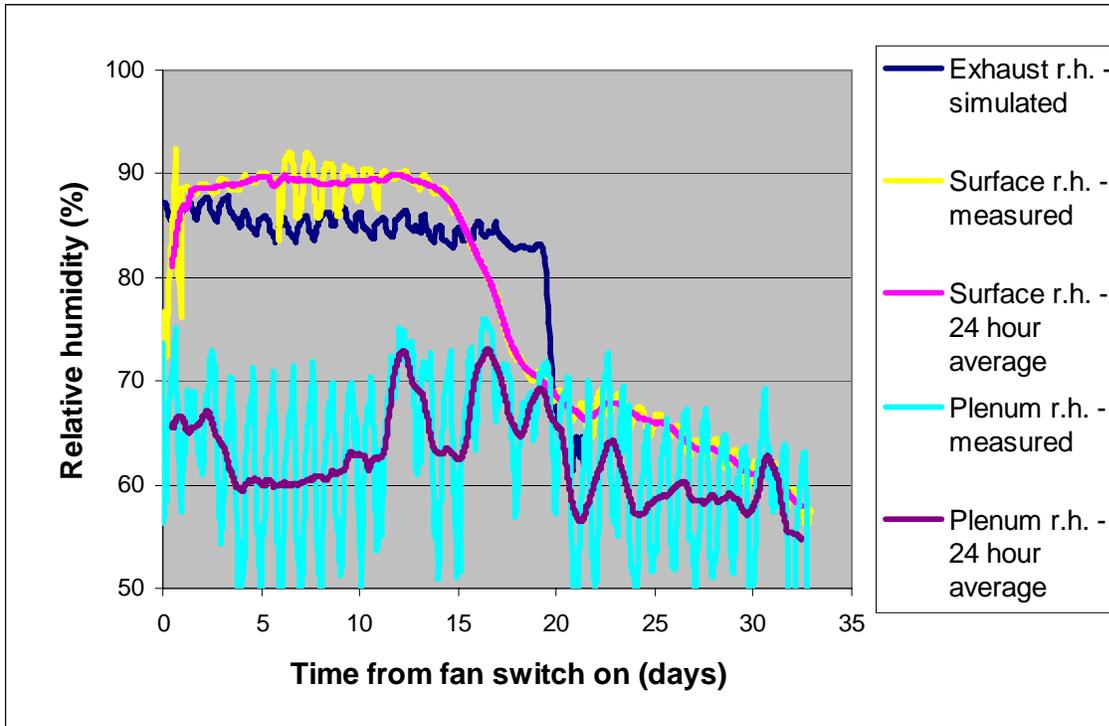
The data for the other bins followed a similar pattern and the exhaust relative humidities before the surface began to dry reflected the moisture of the grain in the bins:

Bin 3, average initial moisture content 22%, exhaust relative humidity 93%

Bin 4, initial moisture content 20.0%, exhaust relative humidity 90%

Bin 5, initial moisture content 19.1%, exhaust relative humidity 89%

Bin 6, initial moisture content 17.1%, exhaust relative humidity 83%

Static pressure in the plenum duct was constant at around 200mm water gauge during the experiment for Bin 3. The sensors in Bins 5 and 6 malfunctioned and did not give sensible readings, and no sensor was fitted to Bin 4.

3. Airflow data

Table 8 gives the average flow through the bins as measured using the Casella meter at 9 locations on the grain bed surface.

Table 8. Airflow from the grain surface.

Bin	Average airflow (m^3/h)
3	1900
4	1700
5	1800
6	2000

4. Ochratoxin and fungal counts

Table 9 gives details of sample timing of samples taken for fungal counts and OTA analysis in relation to drying time. Samples were taken from each of the nine cells in each bin from the surface and from a depth of 0.5m.

Table 9. Timing of sampling.

	Bin 3	Bin 4	Bin 5	Bin 6
Experimental drying time (days)	29	24	26	20
Time when samples taken for OTA (days)	22,29	21,24	26	20
Time when samples taken for fungal counts (days)	11,22	11,21	11,22	13,16

Tables 10 and 11 show the counts of *P. verrucosum* from the surface and 0.5 m.

Table 10. Counts of *P. verrucosum* at the surface.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* (log$_{10}$ c.f.u g^{-1})			
		Bin 3	**Bin 4**	**Bin 5**	**Bin 6**
1000	**1**	4.11	2.72	1.30	1.70
	2	4.31	2.48	3.00	0
10	**1**	4.11	3.11	2.43	1.78
	2	4.59	2.00	2.74	0
0	**1**	4.11	2.36	1.30	2.11
	2	3.85	2.30	1.78	0

Table 11. Counts of *P. verrucosum* at 0.5 m.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* ($\log_{10}$ c.f.u g^{-1})			
		Bin 3	Bin 4	Bin 5	Bin 6
1000	1	5.01	2.91	2.30	2.32
	2	5.35	3.41	2.08	2.70
10	1	4.64	3.00	2.48	2.04
	2	4.67	2.30	2.60	0
0	1	4.63	2.46	2.11	1.30
	2	3.76	1.15	0	0

Counts for the three driest bins were small even in bin 4 where the initial moisture content was 20%.

In bin 3 where the average initial moisture content was 22% the counts were larger. In this case there is some evidence that the inoculation had an effect with the highest counts found at 0.5m in cells with the highest level of inoculum.

No OTA was detected in the incoming grain or in Bins 5 or 6 after drying in those bins was complete. In Bin 4, with an initial moisture content of 20.0%, no samples from day 21 showed detectable OTA but one sample from day 24 was found to contain OTA at 0.4ppb, marginally above the limit of detection of 0.2ppb and well below the permitted level of 5ppb.

In bin 3, the bin with highest initial moisture content, OTA was detected in 22 out of 36 samples. Although no samples exceeded the EC regulatory limit, this result shows that the safe life of the wheat has certainly been exceeded.

The average level of OTA detected after 22 days was 0.6ppb and the average level after 29 days was 0.7ppb. The drying front had passed through the surface before either of the two sets of samples had been taken and so all the grain would have been below the moisture at which activity of the *P. verrucosum* fungus would occur and therefore the levels of OTA would not be expected to increase. However, there was no correlation between the OTA results for a given location between samples taken after 22 days and those taken after 29 days. By this stage the levels of OTA would not be expected to increase but neither would the levels be expected to decrease with time

as OTA is stable. So the lack of correlation suggests that the OTA was not uniformly distributed in the grain in each cell and so the point sampling did not give a consistent result.

Table 12. OTA for bin 3

Position	Level of inoculum (spores/g)	Average level of Ochratoxin A (ppb)	Maximum level of Ochratoxin A (ppb)
Surface	1000	1.01	2.96
	10	0.60	3.10
	0	0.92	1.50
0.5 m	1000	0.56	2.40
	10	0.66	3.13
	0	0.17	0.50

No correlation between the level of inoculum and the level of OTA was found nor was there a correlation between the count measurements for *P. verrucosum* and the level of OTA. That results from the three inoculation levels were not significantly different is a positive result. If there had been a difference, it would have been necessary to know the amount of inoculum present in wheat in store in order to select the best drying strategy. Clearly this is not practicable.

A third set of samples from Bin 3 were analysed for OTA. These had been taken 15 days after drying began and put in cold storage. The average level of OTA was 4.8 ppb and the maximum level was 20 ppb indicating that the samples, which had high moisture contents, had spoiled in storage probably because of a freezer malfunction.

P. verrucosum was isolated from the fungal count samples and tested for OTA production. Both isolates tested produced OTA.

Six months after drying bins 3, 4 and 5 were sampled using the statutory method for OTA analysis (Commission Directive No. 2002/26/EC). Table 13 shows the results using the regulatory sampling method and the results from cells 1, 3, 5, 7 and 9, the positions that were used for the sampling from 5 locations in Year 1.

Table 13. Final OTA levels (ppm) and associated counts of *Penicillium verrucosum* (log$_{10}$ cfu g^{-1}) for cells 1, 3, 5, 7 and 9 for bins 3, 4 and 5.

Bin No			3	5	4
Moisture content (%)			21.9	20.0	18.9
Regulatory sampling			1.9	0	0
Samples taken from 5 locations	surface	Average (ppm)	1.1	0.08	0
		Nos +ve	4	1	0
		Range (ppm)	0-3.0	0-0.4	0
		P.verrucosum	4.1	2.5	2.9
	0.5m	Average (ppm)	0.1	0	0
		Nos +ve	2	0	0
		Range (ppm)	0-0.5	0	0
		P.verrucosum	5.2	2.3	2.4

The level detected by regulatory sampling in bin 3 was 1.9 ppb. This was a higher result than the average value obtained by taking samples from 5 locations but within the range of the samples analysed. No OTA was detected in bins 4 and 5 by regulatory sampling.

These results, together with similar results obtained in Year 1 (Table 3), suggest that taking 100 samples, as required for regulatory sampling, may not be necessary and that taking a smaller number of samples from and near the surface may be all that is necessary to provide a satisfactory test for the presence of OTA.

5. Consistency of data

Airflow distribution within each bin was considered. Temperature values from the two sensors at each of 5 depths in each bin were compared. Within each of the four bins, there were only small differences between temperatures at a given depth. This establishes that the airflow, on which the temperature changes depend, was similar all locations in the bin.

The airflow measured using the Casella meter was checked against the measurement of static pressure using the fan characteristic curve of static pressure versus delivered airflow, as in Year 1. This was done for Bin 3, the only one in which a reliable value of static pressure rise across the fan was available. The measured static pressure was 195.8 mm water gauge, which would indicate an airflow of 2600m^3/h if assumed to be

the pressure rise across the fan, but as discussed above this was not suitable approach. The best estimate of airflow, measured using the Casella meter, was 1900m^3/h.

The r.h. measured in the plenum duct was 62% for Bin 3, 59% for Bin 4, 62.5% for Bin 5 and 61.6% for Bin 6 after the air had been heated by between 3.5 and 3.7^{o}C by the fan. The r.h. of the air before this heating may be calculated or read from psychrometric charts. The calculation suggests that the air r.h. before heating was, on average, in the mid 70s % throughout the experiment which is reasonable.

COMPARISON WITH SIMULATION

1. Physical parameters

Analysis was done to generate values for the input to the Storedry simulation in the same way as in Year 1. The approach used and the derived values are set out in Appendix 1.

Running the simulation Storedry using the measured initial values and air conditions in the plenum chamber, the drying time is predicted for reaching the moisture content at the bed surface measured at the end of the experimental runs. Experimental and simulated drying times are shown below.

Table 14. Drying time results of experiment and simulation at 100% of the experimental airflow.

Bin	Time to reach wettest layer moisture content target (hours)	
	Experiment	Simulation
3	694	684
4	579	621
5	622	608
6	476	473

It is clear that the simulated drying time is close to the experimental time. However from the time course of moisture it is clear that the surface of the simulated grain bed takes longer to start to dry but dries faster. This means that the wettest layers in the simulated bed are at risk of mould for a longer time even though the time to reach the

dry condition is correctly predicted. Justified by this, the airflow used in the simulation was increased by 25%, as was done with the analysis for Year 1, to improve agreement in the time before the surface of the bed began to dry. The resulting time course of surface moisture is a better representation of the experiment from which to calculate development of risk of spoilage. Figures 24 to 27 show moisture content versus time for experiment (points) and from simulation (lines) for depths of 0 and 0.5m with 125% measured airflow.

Figure 24. Predicted and measured moisture contents at the surface and a depth of 0.5 m for Bin 3 with 125% measured airflow.
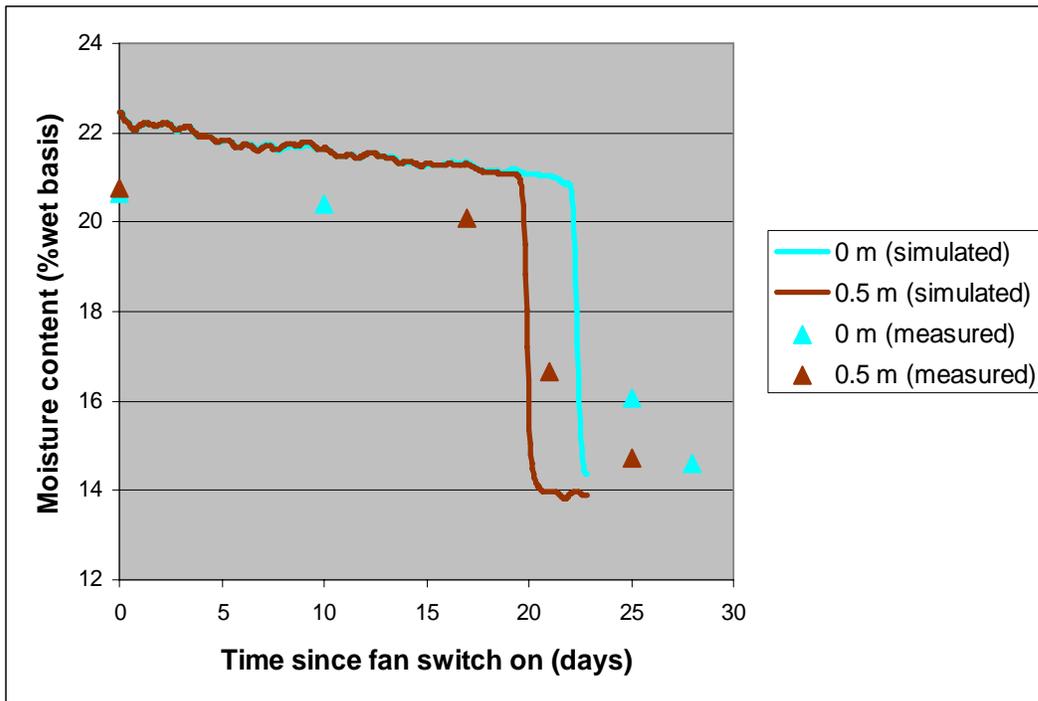
Figure 25. Predicted and measured moisture contents at the surface and a depth of 0.5 m for Bin 4 with 125% measured airflow.

Figure 26. Predicted and measured moisture contents at the surface and a depth of 0.5 m for Bin 5 with 125% measured airflow.
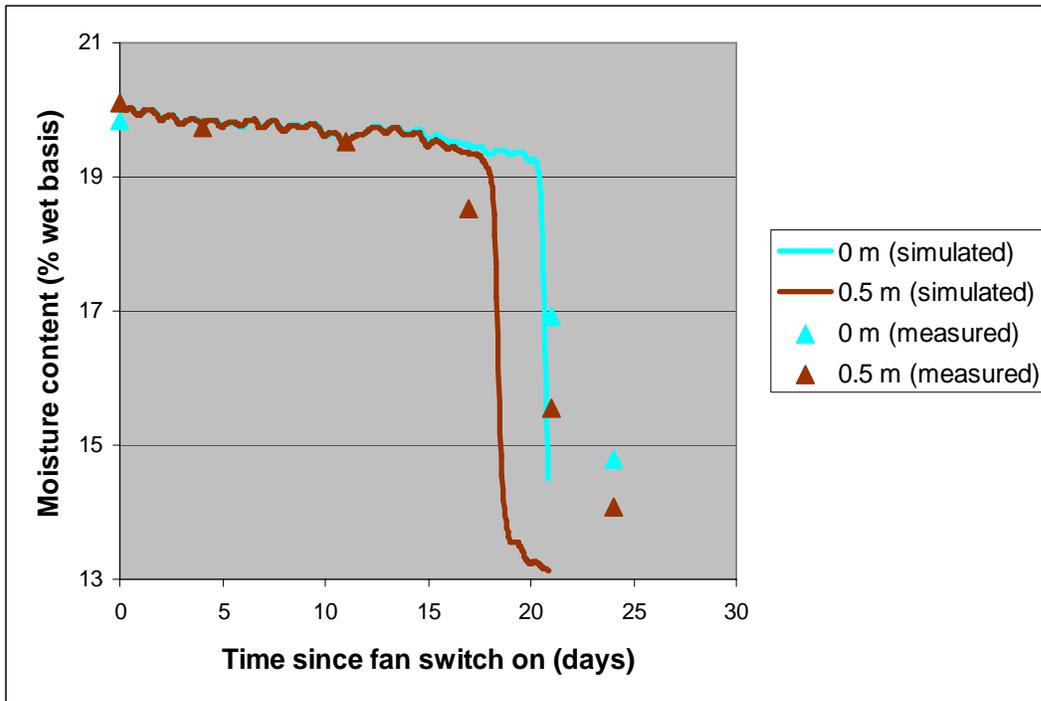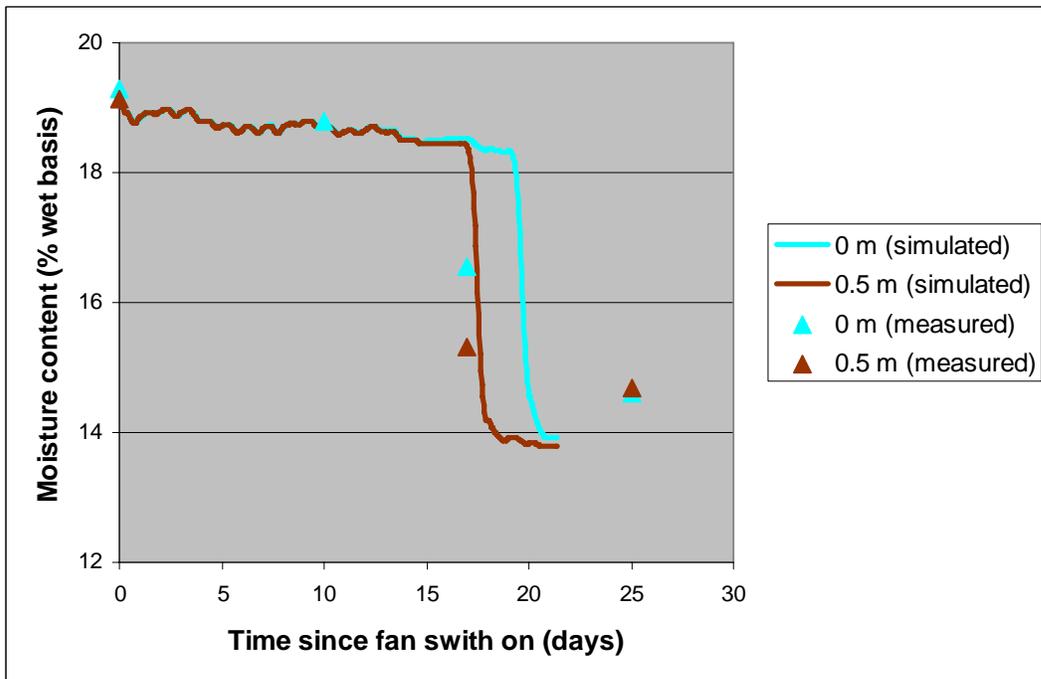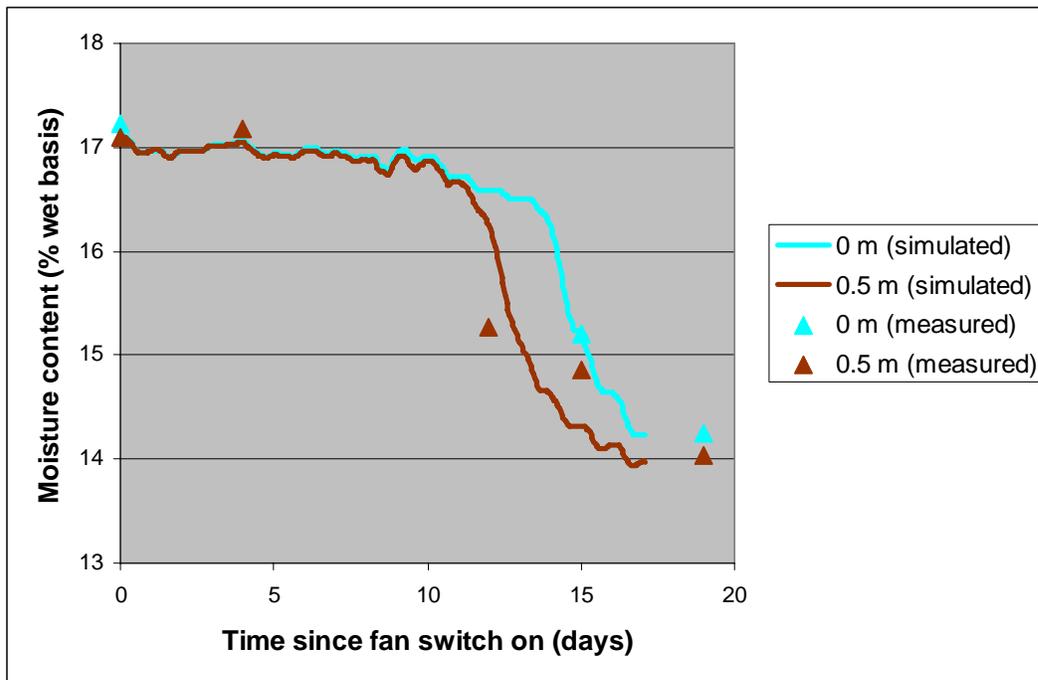
Figure 27. Predicted and measured moisture contents at the surface and a depth of 0.5 m for Bin 6 with 125% measured airflow.

The data from year 1 showed that the air emerging from the simulated bed was less saturated than that measured. Figure 23 shows that this was again the case in year 2 for Bin 5. Simulations for Bins 4 and 6 showed a similar result although Bin 3, the bin with the highest moisture content, was well simulated.

This lower humidity shows why drying took longer in the simulation than in practice. Figure 23 shows that the r.h. of the exhaust air was higher than measured. The lower the initial moisture content of the bin the greater was the difference between simulated and measured r.h. At the end of drying, the simulated moisture tended to be lower than measured. The r.h. sensors had been calibrated at high humidity and the moisture contents were measured by standard oven method so both sets of values can be relied on. Hence the equilibrium relationship used in the simulation between equilibrium moisture and equilibrium r.h. may not be as good as possible.

2. Biodeterioration

Physical conditions close to those measured must emerge from the simulation in order to be able to test the biodeterioration model via the spoilage data measured in the experiment. Otherwise any error may arise from shortcomings of temperature and moisture prediction rather than shortcomings of the biodeterioration model. The time

for the development of the spoilage fungi in the simulation was appropriate when drying with 125% measured airflow so this was used in the following evaluation. The biodeterioration model calculates progress towards spoilage using temperature and equilibrium r.h. at each layer in the grain bed for each time step. If the equilibrium r.h. were lower in the simulation than in the experiment, predicted safe storage time would be longer. (This is provided the r.h. was below the optimal r.h. for growth of *P. verrucosum*, which is the case here.)

As described above, no OTA was detected in Bins 5 or 6. In Bin 4, with initial moisture content of 20%, OTA was found in only 1 sample in 36 samples analysed. No OTA was detected in the sample taken using the statutory method from bin 4.

In Bin 3, with the highest initial moisture content, nominally 22%, there was significant OTA. The sample with the highest level, at 3.1ppb, was under the EU food limit of 5ppb. Although no samples exceeded the EC regulatory limit, this result shows that the safe life of the wheat had been exceeded.

The main difference between the experiments of Year 1 and Year 2 was the addition of inoculation as a factor, with three levels. No influence of the inoculation treatments on measured OTA levels could be seen in the results.

3. Predictions of the Jonsson model

Once the temperature and moisture in the grain bed has been calculated by the simulation, the model of Jonsson (Olsen *et al.* 2004) is then used to calculate the spoilage index as drying proceeded. Once this index, which starts at zero and increases over time at a rate dependent on grain moisture and temperature, exceeds unity, the safe life is considered to have expired and the risk of OTA is then significant. The spoilage index approach does not predict the <u>level</u> of OTA, simply the time after which the presence of OTA becomes a possibility. However, it is true to say that the more the index exceeds unity, the more likely it is that OTA would be present, and for values of spoilage index of 2 or more, the presence of OTA might be expected. For the predicted result to agree with the OTA data at the end of drying, the spoilage index calculated using the Jonsson model would be less than unity for Bins 5 and 6, perhaps close to unity in Bin 4 (given the presence of OA in one sample), and greater than unity for Bin 3.

Table 15 shows for the four bins the maximum spoilage index calculated by the simulation anywhere in the bed of wheat (it is normal for this to be found close to the surface because the grain there remains moist longest), at the single OTA sampling times for Bins 5 and 6, and at both sampling times for Bins 3 and 4.

Table 15. Spoilage index values, calculated using the Jonsson model in the Storedry simulation, for four bins and both sampling occasions. Drying was simulated with 125% of the experimentally measured airflow.

	Bin 3	Bin 4	Bin 5	Bin 6
Spoilage index at first sampling time	4.38	2.13	1.58	0.45
Spoilage index at second sampling time	4.48	2.14	n/a	n/a

4. Comparison of experimental and simulated biodeterioration

While the predicted spoilage of Bin 6 is well below unity, in line with experimental results, it is clear that the Jonsson model predicted spoilage index values well in excess of unity in all the other bins whereas the experiment produced a significant number of positive OTA samples only in Bin 3. For Bin 5, the spoilage index was 1.6 but no experimental samples showed detectable OTA, so the Jonsson model must be conservative by at least 1.6. For Bin 4, the spoilage index was 2.14, *i.e.* more than twice the safe life had expired by the end of the experimental run. Only one sample of OTA was detected and this has been discounted for reasons explained above. This implies that the Jonsson model must be conservative by at least 2.1. For Bin 3, with 12 samples out of 18 having detectable OA on the first sampling, the spoilage index was 4.38, *i.e.* the safe life had been exceeded by four times. Although only low amounts of OA were produced, the consistent presence of OTA shows that the bin had exceeded its safe storage time. Therefore the Jonsson model is not more conservative than 4.4.

Overall this comparison confirms the overall result of the Year 1 experiment, that the spoilage model of Jonsson is conservative. From the Year 1 data, the Jonsson model was shown to be conservative by a factor of at least 2.2 and possibly up to 6. From the data currently available from the Year 2 experiment, the upper value of this range has been reduced to 4.4 and the lower value confirmed.

The discrepancy between measured and simulated exhaust r.h. was noted earlier. Had the simulated r.h been perfectly calculated, the spoilage index values in Table 14 would have been higher, so the Jonsson model must be a little more conservative than the previous paragraph considered.

It should be noted that the experiment used continuous ventilation. Practical drying systems generally use intermittent ventilation, turning the fan off in conditions unsuitable for drying. It may be that the constant presence of a drying airflow in some way inhibits the rate of development of the mould or of the OTA. An experiment to check the conclusions hold for intermittent ventilation would be advisable.

For comparison with the results from the Jonsson model, the spoilage index was also calculated using an old but widely used model of spoilage (Fraser and Muir, 1981, based on their own data and that of Kreyger, 1972) in which the safe storage life was based on visible mould and loss of viability. Using this model, the simulations predict that the upper layers of Bins 3 and 4 would experience such spoilage. However, there was no visible moulding on any of the mould samples sent for analysis.

As a way of comparing the results from the present work with other studies, two studies are of particular relevance. Lindblad *et al.* (2004) develop a model for wheat that predicts the probability of OTA exceeding the regulatory limit of 5 ppb as a function of the number of colony forming units of *P. verrucosum* and the moisture content of the wheat. The samples from which this model was developed were inoculated and ventilated with air at controlled temperature and relative humidity in the apparatus used by Jonsson to develop his model. The Lindblad model shows that at 21%, lower than the moisture content of Bin 3, the risk of OTA being more than 5 ppb was 80%. At 23%, the moisture content at the surface of Bin 3, the risk was almost 100%. This work, which appears to differ from the present experiment basically only in scale, therefore indicates that less OTA was present in the present experiment than expected from Lindblad's work. This was either because there was less fungal growth than expected or that the fungi that did develop produced less OTA than in Lindblad's experiment. Of course the Lindblad work was used the same small scale apparatus as Jonsson, whereas the purpose of the present work was to provide a test on a commercial scale. But, whereas the drying behaviour at full scale would differ markedly from that in a small scale situation, the influences on fungal

development would be micro scale and so such differences in scale would not be expected to matter.

The second study, by Olsen *et al.* (2004), indicates that the present experiment had less fungal growth than expected from previous work, but it does not eliminate the question of toxin production of the fungi. Though Olsen does not fully report results, a graph (their Fig 15, p208) shows the evolution with time of colony count of *P. verrucosum* at 21% moisture content and 20 $^{\circ}$C for four levels of inoculation (similar to conditions in Bin 3 though somewhat less favourable conditions for growth of *P. verrucosum*). Inoculation levels of 1000, 100 and 0 spores/g, used in the present experiment, are shown. Based on this graph, the 11 day $\log_{10}$ count of Bin 3 at 1000 spores/g inoculation level was 5.5 and would be rising very rapidly. Shortly after this time, the $\log_{10}$ count at 1000 spores/g inoculation level would reach levels that, by Lindblad's results, would certainly give OTA in excess of 5 ppb. However in the present experiment, even after 22 days, the colony count only reached a $\log_{10}$ count of 5.4. Therefore it appears that there was much less fungal growth than expected from Olsen's study. It is noted that the work reported by Olsen also made use of the Jonsson small scale respirometer equipment.

The comparison indicates two possibilities.
(a) Some aspect(s) of the practical scale experiment was/were such that the growth rate of *P. verrucosum* in the commercial scale work reported here was much slower than in the small scale apparatus used by Jonsson and Lindblad *et al*.
or, (b) The strain of *P. verrucosum* used in Lindblad's experiment was inherently much faster growing than the strain used in the present work.

Two differences may be highlighted between the large scale of the present experiment and that of Olsen and of Lindblad but the significance of each cannot be assessed here. The continuous ventilation of the grain bed with sufficient air for drying may in some way inhibit fungal growth rate in a way that the very low ventilation rate in the test tubes of wheat in the Jonsson apparatus did not. The distribution of the inoculum would have been more uniform in the small scale experiment of Lindblad *et al*, simply because it was easy to mix thoroughly at such small scale.

Given that the Jonsson model is now shown to be conservative by a factor of at least 2, does this mean that the current drying recommendations are adequate to avoid

OTA? In an earlier report to HGCA (Bruce *et al*. 2006), five drying strategies were simulated at 4 initial moisture contents at five locations in England and the results analysed. Two spoilage models were used, Jonsson and Fraser & Muir based on Kreyger, and the success or otherwise of the strategies was calculated. Approximately one third of the runs that dried successfully when using the Fraser & Muir spoilage model were not successful when the Jonsson model was used, and it was concluded that there was a significant risk that dryers operated to keep within the criterion of 'no visible mould' of Kreyger would nonetheless be at risk of OTA according to Jonsson.

These runs were re-examined using as the measure of success that the spoilage index calculated using the Jonsson model should be less than 2, instead of less than 1, when the moisture targets were met. This was compared with runs in which the criterion for success was a spoilage index of less than 1 using the Fraser & Muir model. Of the 3980 simulations, 3337 dried within the time limit of which 47% were spoiled with the Fraser & Muir model and a spoilage index ≥1 while 42% were spoiled with the Jonsson model and a spoilage index ≥2. From this evidence we can conclude that the Jonsson model does not imply greater difficulty in drying than the established 'visible mould or significant drop in viability' criteria of Fraser & Muir. However, the criterion of Kreyger and of Fraser & Muir is based on the appearance of mould, so if mould is visible the fungi must have got well into the rapid growth stage. The OTA model aims to impose a limit so that the fungi do not get into this phase. So the OTA model would be expected in principle to be more demanding.

CONCLUSIONS

Scientific conclusions

The experiment used moisture contents and airflows that allowed OTA development in the wettest treatment but prevented it in the lower moisture content treatments. The experiment produced a good set of data, appropriate for use in validating both the physical and biodeterioration elements of the simulation model. Checks on the internal consistency of the data gave satisfactory results.

1. Neither the presence or the amount of OTA, or the counts of fungal colonies of *P. verrucosum* showed any significant relationship to level of inoculum.

2. The simulation, 'Storedry', calculated well the general drying behaviour, final moisture content and time for the wettest layers, near the surface of the bed, to dry (Figs 10 to 13). But because the simulated drying zone was much

narrower than in practice, a better estimate of time above the moisture content suitable for fungal growth was obtained by increasing the measured airflow by 25%. This allowed the biodeterioration model to be correctly tested. The exhaust air in the simulation was less saturated than in the experiment. The relationship between wheat equilibrium moisture content and air relative humidity is responsible for this aspect of the simulation, so its accuracy at high relative humidity needs to be reviewed and improved.

3. The new model of safe time before OTA production may start was too conservative. For continuous ventilation drying, the data indicated a safe storage time between 2.1 and 4.3 times longer than predicted by the model. It should be noted that the new model has not been tested in intermittent ventilation, as used by most farm drying systems.

4. The criterion for successful drying, that spoilage index should not exceed 2, was applied to earlier simulation runs and results were compared with simulations using the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability. It was found that fewer conditions spoiled using the Jonsson model, with a spoilage index ≥2, than using the Fraser & Muir model. Based on this, the new model with the higher spoilage index was no more demanding than the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability.

5. The use of continuous rather than intermittent ventilation may be a reason why the Jonsson model was found to be conservative, though there is no mechanism that would readily explain this.

Industrial conclusions

1. When drying by continuous ventilation, the risk of OTA appears no greater than the risk of visible mould or significant loss of viability.
2. For drying by continuous ventilation, the time for the drying front to pass through the bed does not need to be reduced. Hence, recommendations for bed depth and airflow rate in The Grain Storage Guide remain appropriate for continuous ventilation.
3. Drying rate does not need to depend on the amount of inoculum present.
4. Regulatory sampling for OTA, which involves taking 100 samples from the bulk to be tested, may be more rigorous and time consuming than is necessary.

Results from the present study suggest that similar results are given when a smaller number of samples are taken from, and near to, the surface, which are the areas most at risk from OTA. These samples may be all that is necessary to provide a satisfactory test.

RECOMMENDATIONS

1. Although this experiment used continuous ventilation, most practical drying strategies use intermittent ventilation of the grain bed to conserve energy. The risk of OTA in grain dried using intermittent airflow should be evaluated, in order to check whether the conservativeness of the Jonsson model applies to such drying treatments.
2. Prediction of exhaust humidity by Storedry should be improved. To this end, the equilibrium relationship used in Storedry should be reviewed at high end of its range.

ACKNOWLEDGEMENTS

REFERENCES

Armitage, D.M. and Wildey, K.N. 2003. The Grain Storage Guide. 36pp, HGCA, London.

Air Control Industries 2006. Centrifugal fans MR280.
http://www.aircontrolindustries.com/pdf%20june%2007/Centrifugal%20Fans(pdfs)/MR280.pdf

Bruce D.M., Jonsson N. and Armitage D.M. 2006. Practical strategies for minimising the production of Ochratoxin A in damp cereals. Project Report No. 399, HGCA, London.

Commission Directive (EC) No. 2002/26/EC 2002. Methods of sampling for official control of the levels of Ochratoxin A in certain foodstuffs. Official Journal of the European Union. L75/38.

Commission Regulation (EC) No. 1881/2006 2006 Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. L36, 4/5.

Fraser, B.M.; Muir, W.E. (1981) Airflow requirements for drying grain with ambient and solar-heated air in Canada. Trans ASAE, 208-210.

Frisvad, J.C. and Filtenborg, O., 1989. Terverticilate penicillia: chemotaxonomy and mycotoxin production. Mycologia, 81, 837-861.

Henderson, S. 1987. A mean moisture content - relative humidity relationship for nine varieties of wheat. Journal of Stored products research, 23, 143-147.

Kreyger, J. (1972) Drying and storing grains, seeds and pulses in temperate climates Publikatie 205. Institute for Storage and Processing of Agricultural Produce (IBVL), Netherlands.

Lindblad, M; Johnsson, P.; Jonsson, N.; Lindqvist, R. and Olsen, M. (2004) Predicting noncompliant levels of ochratoxin A in cereal grain from *Penicillium verrucosum* counts. J Appl Microbiol 97, 609-616.

MAFF 1999 A survey of human exposure to ochratoxin A. Food Safety Directorate, Food Surveillance Information Sheet No. 172, April 1999.

Northholt, M.D. and Bullerman, L.B. 1982. Prevention of mould growth and toxin formation through control of environmental conditions. Journal of Food Protection, 45, 519-526.

Olsen M., Jonsson, N. *et al.* (2004) Prevention of Ochratoxin A in Cereals - OTA PREV. Final report of Project No. QLK1-CT-1999-00433, Quality of Life and Management of Living Resources.

SCOOP 2002 Assessment of dietary intake of ochratoxin A by the population of EU member states. http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf.

Sharman M., MacDonald S. and Gilbert J. 1992. Automated liquid chromatographic determination of ochratoxin A in cereals and animal products using immunoaffinity column clean up. Journal of Chromatography, 603, 285-9.

Scudamore, K.A., Patel, S. and Breeze, V. 1999. Surveillance of stored grain from the 1997 harvest in the United Kingdom for ochratoxin A. Food Additives and contaminants, 16, 281-290.

Scudamore, K.A. and Wilkin, D.R. 1999. A study to determine whether on-floor ambient drying systems are conducive to the formation of ochratoxin A in grain. Project Report No. 196, HGCA, London.

APPENDIX 1.

Analysis of experimental data for Year 1.

Start and end of run for the four bins.

Bin 3 Run proper started Day 228 (16/8) 15:40, ends Day 251 (8/9) at 16:30

Bin 4 Run started Day 223 (11/8) at 20:40, ends Day 254 (11/9) at 18:10.

Bin 5 Run started on Day 220 (8/8) at 16:50, ends at Day 250 (7/9) at 15:30

Bin 6 Run started Day 223 (11/8) at 17:50, ends Day 255 (12/9) at 16:20

Grain parameters

Calculate **the initial effective bed depth** using the ventilated portion of the volume of grain in the bin. Duct area calculated as $0.087m^2$ from measurements. Length of ducts = 3m less a small amount at end, say 0.2m at each end, = 2.6m. So volume of 2 ducts per bin = $0.45m^3$. Grain below duct is assumed to form a horizontal surface level with lower edges of duct. Volume of grain in tapering section of bin down to bottom edge of duct = $2.30m^3$. Ventilated volume of bin is depth of 3*3m part of bin plus $2.30m^3$. Parallel sided part of bin is 4m high.

For bin 3, grain initial level is 0.25 below top, so volume of cuboidal part is 9*(4-0.25) = $33.75m^3$. Total volume of grain is 33.75+2.30 = 36.05 m^3. For a grain bed area of $9m^2$, effective depth of bed is 4.01m.

For bin 4, grain initial level is 0.32 below top, so volume of cuboidal part is 9*(4-0.32) = $33.12m^3$. Total volume of grain is 33.12+2.30 = $35.42m^3$. For a grain bed area of $9m^2$, effective depth of bed is 3.94m.

For bin 5, grain initial level is 0.33 below top, so volume of cuboidal part is 9*(4-0.33) = $33.03m^3$. Total volume of grain is 33.03+2.30 = $35.33m^3$. For a grain bed area of $9m^2$, effective depth of bed is 3.93m.

For bin 6, grain initial level is 0.35 below top, so volume of cuboidal part is 9*(4-0.35) = $32.85m^3$. Total volume of grain is 32.85+2.30 = $35.15m^3$. For a grain bed area of $9m^2$, effective depth of bed is 3.91m.

Calculate **initial grain mc**.

There were no samples taken as the grain was loaded in the bins, so data was only available for the top 2.5m from sampling. A weighting was calculated to assign a weight to each mc value proportional to the mass of grain below that sampling level. This had little effect on the initial mc because it was substantially uniform but had a significant effect on the final mc.

Weighted average for bin 3 is 20.05 %wb.

Weighted average for bin 4 is 23.22 %wb.

Weighted average for bin 5 is 19.94 %wb.

Weighted average for bin 6 is 23.83 %wb.

Calculate **final grain mc**.

Weighted average for bin 3 is 14.94 %wb.

Weighted average for bin 4 is 14.45 %wb.

Weighted average for bin 5 is 14.89 %wb.

Weighted average for bin 6 is 14.04 %wb.

This is the appropriate value to use for comparison with the simulation, which bases its completion on a true average mc of all layers. Graphical comparison during drying of simulated average mc with measured, even a weighted measurement, is problematical because in the early stages, no drying is seen in samples but drying is seen in simulation.

Note **final surface grain mc**.

Bin 3 is 16.56 %wb.

Bin 4 is 15.66 %wb.

Bin 5 is 15.00 %wb.

Bin 6 is 16.13 %wb.

Calculate **mass of ventilated grain in the bin**. No samples were taken for bulk density. Assume density is 0.780 t/m^3 or 780 kg/m^3 at storage mc, i.e. at the end of the experiment.

For Bin 3, the depth of grain below the top at end of drying was 0.5 m, so grain volume was 33.8m^3. So at 0.780t/m^3, final mass was 26.4t. Average mc at end was

14.94%wb, so bin contained 22.46t dry matter. Dry matter density at end was therefore 0.664t/m^3.

For Bin 4, the depth of grain below the top at this stage was 0.78m, so grain volume was 31.28m^3. So final mass was 24.4t. Average mc at end was 14.45%wb, so bin contained 20.87t dry matter. Dry matter density at end was therefore 0.667t/m^3.

For Bin 5, the depth of grain below the top at this stage was 0.55m, so grain volume was 33.35m^3. So final mass was 26.0t. Average mc at end was 14.89%wb, so bin contained 22.14t dry matter. Dry matter density at end was therefore 0.663t/m^3.

For Bin 6, the depth of grain below the top at this stage was 0.78m, so grain volume was 29.21m^3. So final mass was 22.78t. Average mc at end was 14.07%wb, so bin contained 19.58t dry matter. Dry matter density at end was therefore 0.670t/m^3.

Calculate **initial grain temperature.**
Bin 3 t/cs 3-7, 18-22, 65-69 and 80-84 are in the grain, recorded by Logger 1. Readings from Logger 3 were not used here. Average grain temp was 25.9 C, calc from values from 3-7, 18-22 on day 228 at 15:30 and 15:40.

Bin 4 t/cs 54-58, 44-48 are in the grain, recorded by Logger 2. Readings from Logger 3 were not used here. Average grain temp was 17.1°C, calc from values from at day 223, 20:30 and 20:40.

Bin 5 t/cs 8-12, 13-17, 70-74, 75-79 are in the grain, recorded by Logger 1. Readings from Logger 3 were not used here. Average grain temp was 24.7°C, calc from values from 8-12, 13-17 on day 220 at 16:40 and 16:50.

Bin 6 t/cs 34-38, 49-53 are in the grain, recorded by Logger 2. Readings from Logger 3 were not used here. Average grain temp was 17.1°C, calc from values from at day 223, 17:40 and 17:50.

Air parameters
Calculate **airflow.**
Bin 3. Averaging all the values for flow into fan gives 29.4m^3/min. Averaging readings of flow at surface gives 33.7m^3/min. There appears to be no reason why one should

be more accurate than the other, so the best estimate of airflow is the average of both, i.e. 31.6m^3/min. Converting using 22.46t dm for Bin 3, get 0.0234 m^3/s/t dm.

Bin 4. Fan flow 32.2, surface flow 33.7m^3/min. Average 32.95m^3/min. Converting using 20.87t dm, get 0.0263m^3/s/t dm.

Bin 5. Fan flow 29.8, surface flow 32.2m^3/min. Average 31.0m^3/min. Converting using 22.14t dm, get 0.0233m^3/s/tdm.

Bin 6. Fan flow 31.6, surface flow 31.8m^3/min. Average 31.7m^3/min. Converting using 19.58 t dm, get 0.0270m^3/s/t dm.
Bed shrinkage:-
Bin 3, 0.27m
Bin 4, 0.46m
Bin 5, 0.22m
Bin 6, 0.43m

Analysis of experimental data Year 2

0. Start and end details of four bins.
Bin 3 Run proper started Day 214 (2/8) 17:00, fan switched off on Day 247 (4/9) at 14:30, last moisture samples taken on 31/8, Day 243, 15:00. Length of run for moisture comparison = 694h.
Bin 4 Run started Day 236 (24/8) at 12:15, ends Day 261 (18/9) at 12:10, last moisture samples taken on 17/9, Day 260, 15:00. Length of run for moisture comparison = 579h.
Bin 5 Run started on Day 214 (2/8) at 17:00, ends at Day 247 (4/9) at 14:30, last moisture samples taken on 28/8, Day 240, 15:00. Length of run for moisture comparison = 622h.
Bin 6 Run started Day 227 (15/8) at 18:55, ends Day 253 (10/9) at 12:00, last moisture samples taken on 4/9, Day 247, 15:00. Length of run for moisture comparison = 476h.

1. File of weather data prepared from logged records of T and RH.

2. Grain parameters

Initial effective bed depth: -

For bin 3, effective depth of bed is 3.81m.

For bin 4, effective depth of bed is 3.76m.

For bin 5, effective depth of bed is 3.99m.

For bin 6, effective depth of bed is 3.33m.

Initial grain mc: -

Bin 3 was 20.78%w.b. averaged from surface to 1.0m, and 23.31%w.b. averaged from 1.5 to 2.5m. Total volume of grain = $34.25m^3$. Assuming the moisture for the top three measurement points, 0, 0.5 and 1.0m extends to 1.25m deep from surface, it relates to a volume of $11.25m^3$. So $23.0m^3$ grain is assumed to be at the average mc of samples taken at 1.5-2.5m. So weighted by volume, the average mc is ((20.78*11.25) + (23.31*23.0)) / 34.25 = 22.48% w.b.

Simple average for bin 4 is 20.04%w.b.

Simple average for bin 5 is 19.13%w.b.

Simple average for bin 6 is 17.11%w.b.

Final grain mc: -

Average moisture at surface for bin 3 is 14.60%w.b. on 31/8. No later measurements available. The mc value on 31/8 at the surface is very close to that at 0.5m taken 3 days earlier (14.71% w.b.), so it can be taken as the bin final mc.

Average for bin 4 (at surface and 0.5m) is 14.44%w.b. on 17/9. No later measurements available.

Average for bin 5 (at surface and 0.5m) is 14.64%w.b. on 28/8. No later measurements available.

Average for bin 6 is 14.14%w.b. on 4/9. No later measurements available.

Note **final surface grain mc.**

Bin 3 is 14.60%wb. on 31/8

Bin 4 is 14.79%wb. on 17/9.

Bin 5 is 14.59%wb. on 28/8.

Bin 6 is 14.24%wb. on 4/9.

Mass of ventilated grain in the bin. No values are available for bulk density. Assume density is $0.780t/m^3$ or $780kg/m^3$ at storage mc, *i.e.* at the end of the experiment.

For Bin 3, the depth of grain below the top at end of drying was 0.81m, so grain volume was 31.01m^3. So, at 0.780t/m^3, final mass was 24.19t. Average mc at end was 14.60 %w.b., so bin contained 24.19*(1-0.1460) = 20.66t dry matter

For Bin 4, final mass was 24.26t. Average mc at end was 14.44%w.b., so bin contained 20.76t dry matter.

For Bin 5, the depth of grain below the top at end of drying was 0.59m, so grain volume was 32.99m^3. So, at 0.780t/m^3, final mass was 25.73t. Average mc at end was 14.64%w.b., so bin contained 21.96t dry matter.

For Bin 6, the depth of grain below the top at end of drying was 1.11m, so grain volume was 28.31m^3. So, at 0.780t/m^3, final mass was 22.08t. Average mc at end was 14.14%w.b., so bin contained 18.96t dry matter.

Initial grain temperature:-

For Bin 3, average grain temp was 24.3°C

For Bin 4, 20 average grain temp was 19.8°C

For Bin 5, average grain temp was 25.0°C.

For Bin 6, average grain temp was 20.9°C.

3. Air parameters

Calculate **airflow.**

Bin 3. Averaging readings of flow at surface gives 3.47m/min, which over 9m^2 gives 31.2m^3/min. As the bin held 20.66t dm, specific flow was 0.0252m^3/s/t dm.

Bin 4. 3.19m/min, which gives 28.71m^3/min. As the bin held 20.76t dm, specific flow was 0.0230m^3/s/tdm.

Bin 5. 3.30m/min, gives 29.70m^3/min. As the bin held 21.96t dm, specific flow was 0.0225m^3/s/tdm.

Bin 6. 3.66m/min, gives 32.94m^3/min. As the bin held 18.96t dm, specific flow was 0.0290m^3/s/t dm.

Exhaust air relative humidity:-

The following start from the first time when the reading was stable and continues to the end of the steady period.

For Bin 3, mean exhaust rh between 5.5d and 17.0d (steady period) was 93.4% at 16.7 C. Avg surface mc was 14.60% wb.

For Bin 4, mean exhaust rh between 2.5d and 14.5d (steady period) was 91.3% at 16.6 C. Avg surface mc was 14.79% wb.

For Bin 5, mean exhaust rh between 2.0d and 13.0d (steady period) was 89.3% at 17.8 C. Avg surface mc was 14.59% wb.

For Bin 6, mean exhaust rh between 1.5d and 7.5d (steady period) was 83.9% at 17.2 C. Avg surface mc was 14.24% wb.

Plenum temperature, and heating effect of the fan:-

Bin 3 temp rise was 3.7 C averaged over run, and quite consistent.

Bin 4 temp rise was 3.7 C averaged over run, and quite consistent.

Bin 5 temp rise was 3.5 C averaged over run, and quite consistent.

Bin 6 temp rise was 3.7 C averaged over run, and quite consistent.

Bed shrinkage:-

Bin 3, 0.36m

Bin 4, 0.30m

Bin 5, 0.32m

Bin 6, 0.18m