PROJECT REPORT No. 196

A STUDY TO DETERMINE WHETHER ON-FLOOR AMBIENT DRYING SYSTEMS ARE CONDUCIVE TO THE FORMATION OF OCHRATOXIN A IN GRAIN

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by

K A SCUDAMORE[1] AND D R WILKIN[2]

[1] KAS Mycotoxins, 6 Fern Drive, Taplow, Maidenhead, Berkshire SL6 0JS
[2] 39 Denham Lane, Chalfont St. Peter, Gerrards Cross, Buckinghamshire SL9 0EP

SUMMARY

This is the final report of a nine month project which started in July 1998. The work was funded by a grant of £45,270 from HGCA (Project 1613).

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

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General introduction to the problem of ochratoxin A in cereals

Mycotoxins are toxic secondary metabolites produced by certain species of fungi and have a range of toxicological effects on man and animals. While many hundreds of such products have been identified, only 20 to 30 have been shown to be contaminants of human or animal food. One of these, ochratoxin A (OA) has been detected regularly in cereals grown in the UK. It is a bi-product of a fungus *Penicillium verrucosum* that can grow on the grain under certain conditions during storage. The occurrence of this toxin differs from some other mycotoxins as it is produced during storage rather than pre-harvest in the field.

Its toxicity to man and animals is not in doubt. The UK Committee on Carcinogenicity and the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment has advised that OA should be regarded as a possible genotoxic carcinogen. The Food Advisory Committee has recommended that OA in the food supply be reduced to the lowest technically achievable level.

A recent survey for OA in UK farm grain from the 1997 harvest has shown that in commercial stores receiving grain stored on farms for up to 9 months, OA was detected in 21% of the samples with 2.7% above 5 μg/kg. Comparison with earlier surveys shows that in spite of poor conditions prior to harvest, concentrations of OA were not significantly higher. Although it is well recognised

that OA is a problem of storage, it is clear that not all the factors involved in its formation are fully understood. The EC are currently discussing Regulation for OA in cereals and other products and the level at which a statutory limit is ultimately set will be of major significance to the grain industry. A possible maximum permissible level of 3-5 μg/kg is being discussed, a concentration range which is exceeded on, an infrequent, but regular basis in UK cereals.

In the UK, a significant percentage of the on-farm drying capacity is provided by on-floor or in-bin systems. The drying process is such that moisture is removed slowly by blowing air at close to ambient temperature through the grain. The bottom is dried first while moisture passes up through the bulk with the result that the upper layers may take 6 weeks or more to dry. Laboratory studies suggest that the conditions permitting fungal growth with the potential for OA formation might occur during this process.

Experiment design and methods

Thirty farms or commercial stores using slow drying systems were identified as possible experimental sites. In all cases grain was to be stored on drying floors and these would be used if drying was necessary. The trial sites all fell within main cereal growing regions on the eastern side of the country, from the South coast to the Humber and west to Wiltshire. Information from trade sources suggested that on-floor drying systems were uncommon in more northerly and westerly parts of the country. Farmers were contacted prior to harvest and agreed to allow use of their premises as an experimental site. Studies were carried out at 24 of the sites.

Collection of samples

Sampling systems were set up in 16 bulks of wheat and 8 barley whereby samples could be taken from permanent points within the bulks to give a horizontal and vertical distribution in one plane. A series of 9x25 mm i.d. plastic tubes were inserted into the grain at the time the first sample was collected. These were inserted as three groups of three, to depths of 3 m, 1.5 m and 0.5 m per group. At most sites the tubes were arranged linearly across the store so that replicate samples would be removed from grain harvested at about the same time and were left in position throughout the trial except at two sites where grain stirrers were used. The tubes were closed with stoppers to prevent channelling of air during drying.

A vacuum sampler was used to draw grain from each tube and composite samples of about 5 kg were obtained by combining the samples from the three tubes at the same depth. This grain was then thoroughly mixed before 1 kg was taken for laboratory examination. Three to 5 visits were made to

most stores to collect samples. The first sample was collected as far as possible before drying and immediately after harvest, the second was taken some weeks later and a final one after drying had been completed. In some stores grain was relatively dry at harvest, or dried quickly or was sold at short notice. For these, only two sets of samples were collected. At three sites where the initial moisture content of the grain was high and drying slow, further samples were collected. Samples were placed in self-seal plastic bags that were clearly labelled. After collection, the samples were held in an insulated container and usually taken to the laboratory within 3 days of collection. If this was not possible, samples were placed at 15°C until they could be delivered. All samples were held at the laboratory at -20°C except when being examined.

Temperature and moisture were measured throughout the study at each sampling point using commercial probes and moisture meters (Protimeter plc). Some information was collected from the farmer at the time of the first visit regarding the drying system used and the method of management.

Examination of samples

Samples were well mixed and finely ground before examination for OA and/or fungi, taking care to avoid cross contamination with moulds. Analysis for OA was carried out using an HPLC method sensitive to 0.1 μg/kg OA. Selected samples taken were examined for the fungi present at each sampling time and some were examined further to establish the presence or absence of *Penicillium verrucosum.*

RESULTS

Seven farms were sampled on only two occasions as at three of these the grain was relatively dry or had been dried very quickly and on a further four farms the grain was sold at short notice. At three sites, where the grain was harvested late and wet, additional samples were taken during the prolonged drying period required.

A review of temperature and moisture content measurements suggests that there were three typical situations. One was when moist grain was harvested and dried rapidly to safe storage conditions within 2 to 4 weeks. A second occurred when grain required drying but the reduction in moisture was slow partly because of sparing use of the ventilation system. The third occurred when grain was harvested wet (e.g. at more than 20% moisture) and took a considerable time to dry to a safe moisture content. At one farm one duct of the drying system

was blocked inhibiting ventilation andthis coincided with the sampling points. However the farmer sold all the grain, including the damp portion, after four weeks.

Observations on the drying systems and their operation:

In general, farmers understood the basic principals of operating floor drying systems; the stores were filled to the correct level and the grain was levelled after filling. However, a common problem was that the farmer did not have sufficient time to manage the drying process so that fans were not always turned on or sufficient sampling done during drying to confirm progress and to identify problems. Automatically controlled drier systems, which were widely used, did not always seem to give entirely satisfactory results. These deficiencies might have been far more important if the harvest had been wetter. Serious difficulties were experienced with drying on three farms principally because of the increase in ambient air humidity as autumn approached. No visible mould was noticed during sampling despite grain on several farms remaining above 18% moisture content for a considerable time.

During the Project more than 240 samples were collected. A total of 108 samples were analysed for OA. Eight samples from 4 bulks of grain were found to contain small amounts of OA although only one exceeded 5 μg/kg, (see Table 1). The moisture contents (MC) and temperatures given in the table are those at the time when the samples were removed. Although these concentrations are low they are quite similar to results obtained in several previous surveys of UK stored grain. OA was produced in one sample within 26 days of harvest. The data also show that OA can be produced after prolonged storage under cool damp conditions.

The species and amounts of fungi present were determined in 109 Samples. At the first sampling shortly after harvest, field fungi in which *Alternaria, Fusarium* and *Cladosporium* species were the predominant genera occurred in 76%,70% and 61% of samples respectively. *Penicillium* species were only found in 3 (9%) of these samples and this further confirms that OA is only likely to be a post harvest, storage problem. On most farms the numbers of field species declined during storage while *Penicillium* increased, except where grain had already dried quickly to a level inhibiting mould growth. However, in some of the wetter grain samples, field species continued to increase for a considerable time after harvest and this may have inhibited the development of storage fungi such as *Penicillium* and *Aspergillus*.

Table 1: Ochratoxin A detected during grain storage

Site	OA, µg/kg	Time after harvest, days	Position in grain	MC, %	Temp., °	*Penicillium* Detected
M5	0.3	80	Middle	16.7	7.7	Yes
M5	0.1	133	Middle	17.0	8.3	Yes
M5A	1.2	38	Middle	18.7	7.5	Yes
M5A	0.2	131	Top	20.3	5.5	Yes
NL6A	5.9	26	Top	22.0	10.0	Yes
NL6A	1.1	26	Middle	19.9	11.6	Yes
NL6A	0.2	53	Middle	17.0	3.4	No
W1*	0.2	End	All	19.0*	17.3*	Not tested

*= bulk sample provided by the farmer, temperature and moisture content after 18 days storage

Figure 1 shows the decrease of fungi during drying of wheat in samples taken from the middle of the bulk. Conditions on this farm might be considered as the ideal for drying cereals safely. Wheat, initially above 16.5%, dried gradually to under 15% while grain temperature dropped sharply from nearly 30°C at harvest to about 10°C after 3 months. The total fungal count consisted entirely of field fungi and decreased steadily to a low level. No *Penicillium* moulds were detected which was consistent with low moisture content and falling temperatures.

Results from a farm where problems with drying occurred because harvest was delayed until mid-September and the wheat was harvested above 22%, are shown in Figure 2. Here it required about 1 month before any sign of drying occurred in the upper layers of wheat and four months before this grain was dried to 17%. However temperatures fell during this period to below 10°C. Although initial mould infection was low, by one month a relatively low infection of *Penicillium* had developed and was accompanied by formation of OA in a concentration of 5.9 µg/kg. Fungal counts also increased up to about 3 months before subsequently declining again. The potential for a major problem with OA formation clearly existed but was probably avoided because grain temperatures were subsequently reduced to 5°C, at which toxin formation would be very slow. In the centre of this bulk, the wheat dried more rapidly and was down to 17% within 50 days although some *Penicillium* and small amounts of OA were detected during this period. However, despite this relatively low and

falling moisture content, by 4 months a high infection of *Penicillium* had built up. This was found to consist mainly of a xerophilic species, *P. brevicompactum,* which is not known as a mycotoxin producer although it can survive and grow at lower moistures. The grain at the bottom of the bulk typically dried more rapidly, mould development was much less and no OA was detected.

Of 30 samples of grain shown initially to contain *Penicillium* only 3 isolates were found to be *P. verrucosum* which needs relatively moist conditions to develop. However, these had been isolated from grain taken from 3 of the 4 farms where OA was detected and had been obtained from the upper part of grain bulks. None of these isolates formed OA in laboratory culture although it is well known that not all strains of a fungal species can be induced to produce mycotoxins under laboratory conditions or, alternatively, they may lose this ability when stored in the laboratory. None of the grain samples positive for OA were obtained from the bottom of stores.

DISCUSSION

Slow drying systems do not seem to carry an inherent risk of fungal growth and therefore the potential for mycotoxin production. Indeed, they have the advantage that blowing large volumes of air at close to ambient temperatures though the bulk of grain will tend to reduce its temperature. The use of slow drying systems when harvests are wet and/or late must carry some risk of mould growth before drying is completed. However, a bad harvest will also stretch the capacity of high temperature drying systems and may result in un-dried grain being left in heaps for some time, without the advantage of the cooling.

When slow drying systems are used it is important that enough time is devoted to their management. The most important aspect is the taking of temperature and moisture measurements from different parts of the bulk at different depths and comparing results over time. This management process becomes of critical importance if the intake moisture is above 18% and ambient conditions are less than ideal.

The aim of the drying process must be to minimise the time that any grain spends at moisture contents above 16% and at temperatures above 20°C. Once grain is below 16% moisture, cooling to below 10°C will ensure that toxin-producing moulds will not develop. It will also minimise any risk from insect pests.

Properly managed ambient air drying systems should normally be able to reduce damp grain to conditions safe for storage on most occasions within an acceptable time. At 20°C it is recommended that the target for this should be to reduce grain below 16% within 14 to 28 days maximum. This offers a considerable margin of safety as, even if the time required for drying is longer, a significant build up of the appropriate *Penicillium* species is also required.

OA only occurred in damp grain or when drying was slow. Failure to dry on 2 farms was due to the limited time when air blowers could be used because the ambient air was frequently at too high an ERH and the available heat source was not suitable to produce sufficiently dry air. The cooling effect of the drying air and the dominance of field fungal species may have helped to prevent development of high levels of OA.

In the management of stored grain, the inability to reduce moisture to below about 16% in a relatively short time should alert the farmer to the potential for a serious problem, especially if the grain temperature remains above 15°C. The top layers of grain will be at greatest risk because they are always the last to dry. OA formation depends on a number of factors and high moisture alone will not necessarily result in mycotoxin formation but it does provide a clear indication of risk. The presence of visible mould, whilst undesirable, is no reliable indicator of the presence of OA. Indeed there was no visible mould in any of the samples in which OA was detected.

The need to avoid even low-level contamination of cereals with OA is now demanded by many sectors of the Food and Feed Industries and is very likely to be backed by regulation setting a maximum permissible level for OA in cereals traded within the EC. At the moment there is no rapid, cheap and reliable test for OA or even a quick test for the mould *P. verrucosum* which could be used by farm staff although this may change in time. However, even if suitable tests were available both the fungus and OA are distributed so unevenly that pockets of contamination could easily escape detection. Therefore, control of moisture content and grain temperature in such a way as to eliminate any risk of OA production must remain the principal defence against farm-stored grain becoming contaminated with mould and/or OA. The lack of continuity in detecting OA in consecutive samples from the same points in a bulk, highlights the lack of knowledge about the distribution of the toxin.

CONCLUSIONS AND RECOMMENDATIONS

- Wet grain at harvest should be dried as soon as possible to below 16%.
- The drying time will depend on the temperature as well as moisture but with grain at about 20°C drying to 16% should be achieved within 14 days.
- Cooling the grain during drying should extend the time available to dry.
- To achieve effective drying, systems must be well maintained and operated effectively.
- Management must include the assessment of moisture content and temperature at sufficient points to ensure that the entire system is working correctly.
- The upper layers will remain wettest for longest but must be dried to below 16% within a reasonable time span.
- Absence of visible mould is no guarantee of freedom from OA.
- Additional drying may be required during storage if moisture content increases above 16%, for example if the surface layer re-adsorbs moisture.
- Where grain is regularly harvested at more than 20%, consideration should be given to the installation of systems such as a de-humidifier to allow greater flexibility in operating the system.
- Safe and effective storage must remain the best means of meeting the demands of grain users and future legislation.

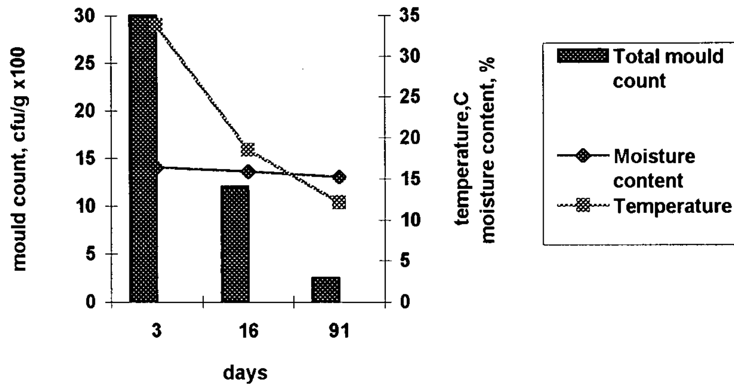

Figure 1: Change in mould count, moisture content and temperature in stored wheat during ambient air drying, -a model situation

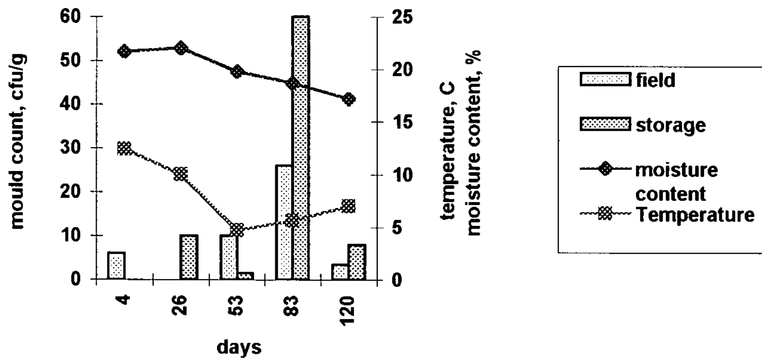

Figure 2: Change in mould count for field and storage fungi, moisture content and temperature in stored wheat during ambient air drying, upper part of grain bulk -a problem drying situation

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FULL REPORT

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Abstract

Mould developing in grain has always been one of the risks associated with its storage. However, the commercial significance of this problem has become more acute with the discovery that some species of mould may produce toxic mycotoxins during their growth such as ochratoxin A. This toxin is produced by a species of *Penicillium* mould that develops only during storage.

Surveys of UK grain have shown that ochratoxin A is found in about 10% of samples tested and sometimes occurs at levels that exceed the likely EU tolerance level of 3-5ppb. The reason for these occurrences is not completely understood as the mould that produces the toxin will only grow at moisture contents above 16%. One possibility is that the conditions found during on-floor drying might be suitable for mould growth and toxin development. Therefore, the HGCA funded an investigation into mould and toxin development during on-floor drying. The work was commissioned in June 1998 and required the assessment of grain stored and dried on farm during the 1998 harvest, with the results being available before the following harvest.

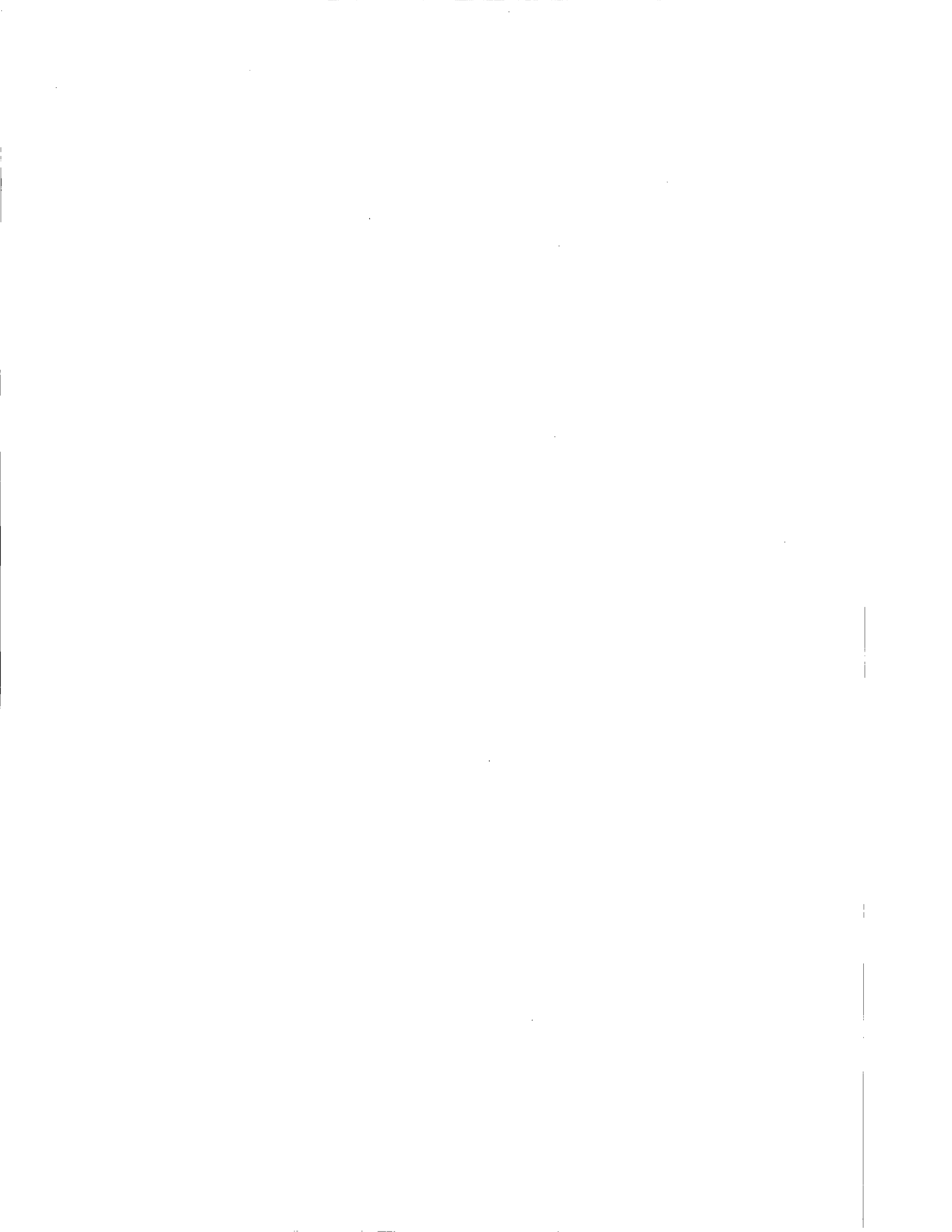
24 farms spread from the South Coast to North East Lincolnshire were assessed, all using on-floor drying systems. Samples of grain were collected from set points in each grain bulk immediately after harvest and then at intervals during drying and storage. The moisture content and temperature of the grain at the sample points was recorded and a number of selected samples were analysed for ochratoxin A and checked for mould flora.

240 samples were collected and out of 108 analysed, ochratoxin A was detected in only 8 on 4 of the farms, mostly at a low level. The highest level detected was 5.9 ppb. The mould associated with ochratoxin A formation was found in samples from 3 of these farms. In every case where mould increased and toxin was detected, the moisture content of the grain was above about 19% and drying was slow. These results show that on-floor drying does not carry an inherent risk of the development of ochratoxin A but when grain enters storage at a moisture content of 18% or more and drying is too slow, there is a serious risk that mould will develop and toxins may be produced.

Immediately after harvest grain was infected with a range of field fungi but these usually died out over time. In some cases, when the moisture content remained above 16 - 17%, a range of storage fungi replace the field species. At higher moistures, these sometimes included a species of *Penicillium* known to produce ochratoxin A. The rate at which mould grows in stored grain is affected by temperature as well as moisture. During on-floor drying grain temperatures generally fell and this cooling extended the drying/storage period before any toxin was detected.

The results from this work validate limited laboratory studies done to explore the conditions under which ochratoxin A can be produced in stored grain. This has allowed a risk assessment system to be developed that should provide effective advice to farmers, enabling them to minimise risks of mould or mycotoxins developing in their grain during on-floor drying and storage

This work could not have been completed without the assistance and co-operation of the farmers involved and sectors of the grain trade.



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Glossary of terms

A_w	Water activity
BCR	Community Bureau of Reference
CABI	Commonwealth Agricultural Bureau International- Bioscience
ERH	Equilibrium relative humidity
FAPAS	Food Analysis Performance Assessment Scheme
HPLC	High Performance Liquid Chromatography
IA	Immunoaffinity*
MAFF	Ministry of Agriculture, Fisheries and Food
mc	Moisture content
nm	Nanometre
NAMAS	National Accreditation of Measurement and Sampling
OA	Ochratoxin A
ppb	Parts per billion = µg/kg
RHM	Rank Hovis MacDougall Technology

* = This term relates to the affinity that an antibody has towards the compound used to raise that antibody in an animal species.

Objectives

1. To determine whether on-floor drying can lead to the formation of ochratoxin A.

2. To determine the conditions which favour ochratoxin A development.

3. To offer advice on how to avoid or minimize contamination.

General introduction

Mycotoxins are toxic secondary metabolites produced by certain species of fungi. They have a range of diverse chemical and physical properties and toxicological effects on man and animals. While many hundreds of such products have been identified, only 20 to 30 have been shown to be contaminants of human or animal food (Watson 1985).

Ochratoxin A (OA) is one mycotoxin that has been detected regularly in cereals grown in the UK and other European countries (Speijers and van Egmond 1993). It is a bi-product from the metabolism of a fungus that can grow on the grain under certain conditions during storage. Current knowledge suggests that only one species of *Penicillium* ('*P. verrucosum*') is capable of producing OA (Frisvad and Lund 1993). The occurrence of this mycotoxin differs from some other mycotoxins as it is produced during storage rather than pre-harvest in the field. This has been confirmed by a number of surveys (e.g. MAFF 1994, 1995a, Scudamore 1993).

The toxicity to man and animals of OA is not in doubt. The UK Committee on Carcinogenicity and the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment has advised that OA should be regarded as a possible genotoxic carcinogen. The Food Advisory Committee has recommended that OA in the food supply be reduced to the lowest technically achievable level (ANON 1993). MAFF aims to ensure that food is safe and wholesome for the consumer and one facet of the Ministry's research and regulatory strategy is designed to ensure that the levels of additives, contaminants and natural toxicants do not pose an unacceptable risk to human health. A survey of the blood and urine of adult volunteers in the UK has recently been completed and shows almost universal occurrence of OA (MAFF 1999b). The OA concentrations were related to the food intake of the participants for a one month period.

MAFF have supported a comprehensive investigation on OA to determine its frequency of occurrence and levels in cereals, coffee and retail products (MAFF 1994, 1995a, b, 1996a, b), and also to examine its persistence during processing including flour milling, bread preparation (Osborne *et al.* 1993) and extrusion processing. Surveys of stored cereals have suggested that about 2% of the grain tested can contain concentrations that exceed 5 µg/kg; the maximum that seems likely to be permitted by future legislation. A further survey for OA in farm grain from the 1997 harvest has recently been completed (Scudamore *et al.* 1999). This showed that in samples collected at commercial stores receiving grain stored on farms for up to 9 months, OA was detected in 21% of the samples analysed using a method sensitive to 0.1 µg/kg. Barley was more frequently contaminated than wheat and contained the highest mean concentration of OA. Oats was least contaminated. Comparison with surveys in 1994 and 1996 indicated that in spite of the worse conditions prior to harvest, concentrations of OA were not significantly higher (MAFF 1999a).

Work has been done to examine the physical parameters in grain that might permit the growth of *P. verrucosum* and production of OA. A laboratory study has suggested that the lower limit for moisture content of grain below which OA production is unlikely is about 16.5%, given a temperature of 15 - 20°C (Hetmanski 1997). However, rapid production was possible at 20% moisture and 20°C. More correctly, mould growth and toxin production should be assessed on equilibrium relative humidity (ERH) or water activity (A_w). However results suggest that under normal conditions of storage, based on current commercial practices and trading standards (maximum mc of 14 - 16%), little if any grain should be held in UK stores under conditions suitable for the production of OA. One possible explanation for this anomaly could be related to the drying processes used in this country.

There are two alternative drying systems open to farmers: rapid, high-temperature drying or slower, in-bin or on-floor drying systems. In hot air drying, grain is dried rapidly by a stream of hot air. However, sometimes when persistent wet weather occurs at harvest time, the amount of grain received may exceed the capacity of grain drying facilities. At such times cereals may be held for days or even several weeks before drying can be done. Alternatively, drying can be done with in-bin- or on-floor drying systems that use large volumes of relatively cool air and may take several weeks or even months to dry grain. Even when drying proceeds rapidly, the upper layers of grain may stay at a relatively high moisture content until the drying process is almost completed. It is just possible that this approach to drying may, inadvertently, create ideal conditions for the development of the fungus that produces OA.

A significant percentage (about 60%) of the on-farm drying capacity is provided by on-floor or in-bin systems (Prickett, 1988). The drying process is such that moisture is removed slowly by blowing air at close to ambient temperature through the grain. The bottom is dried first while moisture passes up through the bulk with the result that the upper layers may take 6 weeks or more to dry. Considering results of laboratory studies on ochratoxin formation, it seems likely that the conditions permitting fungal growth and the potential for OA formation might occur during this process. However, the presence or absence of *P. verrucosum* and the exact time/temperature/moisture combinations is of critical importance. In addition, fungi such as species of *Fusarium*, *Alternaria* and *Cladosporium* may colonise cereal seed in the field. Christensen and Kauffman 1969 classified the fungi which invade grain into 2 groups, field and storage. After harvest, field fungi gradually die out in storage and, given suitable conditions, are usually are outgrown by storage fungi such as *Penicillium* and *Aspergillus*. Little is known about the effect that this competition has on OA formation.

MAFF is funding a 3 year Research Project starting April 1999 that is looking at the factors responsible for the occurrence of OA in grain and a small part of this work will include a pilot scale laboratory study on the effect of drying regimes. However, it is likely to be approximately 4 years before results are available. The work here differs from the MAFF-funded study and is entirely complementary to its objectives.

Experiment design

Target sites

A number of farms or commercial stores using slow drying systems were identified for use as experimental sites. The trial sites all fell within main cereal growing regions and, in consequence, were concentrated on the eastern side of the country, south of the Humber. Information from trade sources suggested that on-floor drying systems were uncommon in more northerly and westerly parts of the country.

Sampling

The aim was to collect samples from each site on 3 occasions during drying although the exact timing was not considered critical. A sampling system was established at each site whereby samples

were extracted from set permanent points within the bulks. The aim was to obtain samples from the grain bulk in a manner that allowed comparisons to be made between successive sampling occasions.

Analysis for ochratoxin A

Analysis for OA was carried out by Rank Hovis MacDougall Technology (RHM) using an HPLC method (Appendix 1) previously approved and used for MAFF surveillance. This method was sensitive to 0.1 µg/kg OA. Appropriate validation and recovery tests were done on spiked samples as part of the analytical procedure.

Examination for moulds

Selected samples taken were examined at the Commonwealth Agricultural Bureau Institute Bioscience (CABI) for the fungal genera present at each sampling time (Appendix 2). Some samples, selected on the basis of initial results, were examined further, to establish the presence or absence of *P. verrucosum* (note: the differentiation of *Penicillium* species related to *P. verrucosum* is difficult and requires particular expertise and culture media). Because of these taxonomic problems, selected samples were examined further for their ability to produce OA.

Methods

Selection of experimental sites

Lists of farms known to use slow drying facilities were obtained from several agricultural merchants, seed companies and co-operatives. Individual farmers were contacted prior to harvest and their agreement was sought to use their premises as an experimental site. The aim when selecting farms was to cover a wide geographic area but also to group several farms within an area to give replication and to simplify the collection of samples.

The project protocol called for an assessment to be made at up to 20 farms to increase the chance of including some stores with really damp grain. However, in order to cover likely problems and to allow some redundancy, a list of about 30 farms was drawn up initially. These were spread from Southern Kent to Wiltshire and to North-East Lincolnshire. In all cases grain was to be stored on drying floors that would be used if drying was necessary. Two of the farms had in-bin drying systems but safety constraints made it impossible to sample grain in these bins in an effective manner. Therefore, the work was confined to on-floor drying systems.

Collection of samples

Sampling systems were set up at 24 of the 30 sites initially listed of which 16 contained wheat and eight barley. Six sites were discarded for reasons such as the grain being very dry at harvest or sufficient alternative sites being available in the same region.

The system of collecting samples was designed to give a horizontal and vertical distribution in one plane. A series of 9x25 mm i.d. plastic tubes were inserted into the grain when the first sample was collected. The tubes were inserted as three groups of three, to depths of 3 m, 1.5 m and 0.5 m per group. In general the groups of tubes were arranged linearly across the store so that replicate samples would be removed from grain harvested at about the same time. In almost every case, these tubes were left in position throughout the trial to ensure that successive samples were collected from the exactly the same point. The only exceptions were at two sites where grain stirrers were used. When tubes were left in place, they were closed with stoppers to prevent channelling of air during drying. The selection of sample points was assisted by use of a Protimeter Grain Probe (Protimeter PLC, Marlow, Bucks) that gave very rapid indications of moisture content within the bulk.

When samples were collected, a vacuum sampler was used to draw grain from each tube at the same depth for about 5 seconds. Composite samples of about 5 kg were obtained by combining the samples from the three tubes at the same depth. This grain was then thoroughly mixed before 1 kg was taken for laboratory analysis.

Three visits were made to most stores to collect samples. The first sample was collected as far as possible before drying and immediately after harvest, the second was taken some weeks later depending on the conditions and the final sample was to be taken after drying had been completed and after a period of storage. However, there were some exceptions. In some stores grain was relatively dry at harvest or dried quickly. In others grain was sold at short notice. In these cases only two sets of samples were collected. At three sites where the initial moisture content of the grain was high and drying slower, extra series of samples were collected as these were of particular relevance to this study. Four sets were taken at one and 5 sets in the other two. All 24 sites were visited shortly after harvest had started and the store had been only partly filled with grain. Therefore, initial samples were usually taken between 0 and 10 days of the grain being harvested, although one bulk of grain had been in store for 30 days before sampling could be carried out. In some cases drying had started before the first sample was collected but mostly first samples were obtained before significant drying had occurred.

On-site collection of information

Some information was collected from the farmer at the time of the first visit regarding the drying system used and the method of management. No attempt was made to influence the drying strategy although, if a direct question was asked, this was answered to the best ability of the sampler.

As each composite sample was collected (from 0.5, 1.5 or 3 m depth), the moisture content of a sub-sample taken from the mixed composite was measured with a Protimeter P4900 moisture meter. The meter was calibrated by the manufacturer immediately before the start of the project and was rechecked in the middle of the sampling phase and at the end of the work. The built-in self-check system was also used at intervals. Three replicate assessments of moisture were done on each sample and the mean recorded.

The temperature of the grain at the three depths was recorded using Protimeter temperature probes. Two probes were attached permanently to the 3 and 1.5 m tubes at the centre of the three sets of sample tubes. Another probe was inserted to a depth of 0.5 m on each occasion that samples were taken. This was always left in place for at least 15 minutes before a temperature reading was taken,

Transport of samples

Samples for analysis were placed in self-seal plastic bags that were clearly labelled. After collection, the samples were held in an insulated container and delivered to RHM for storage, processing and analysis. Most samples were delivered within 3 days of collection but if this was not possible, samples were placed at -15°C and held at that temperature until they could be taken to RHM. They were then transported in a frozen condition. All samples were held by the RHM laboratory at -20°C except when being examined. When mould analysis was required, ground, frozen samples were transported directly to CABI. It is recognised that prolonged freezing may reduce the viability of some mould spores but it was considered that this would not seriously interfere with the aims of this study.

Examination of samples

Samples were well mixed and finely ground before examination. To avoid cross contamination with moulds, external parts of the mill were washed with alcohol before a sample of sterilised grain was milled to remove any non-sterile grain remnants from the equipment. The experimental sample was then ground, thoroughly mixed and 50g transferred to a sterile polypropylene bottle and refrozen for transport to CABI. This sample was used for mould examination. A sample was then removed from the remainder for OA analysis. Details of the

analytical method for determination of OA are given in Appendix 1 and for mould identification and quantification in Appendix 2.

Results and observations

Collection of samples and on-site information

The system for collecting, handling and delivering samples worked smoothly without problem.

Seven farms were sampled on only two occasions. At three of these the grain was relatively dry or had been dried very quickly to moisture levels which would not support significant mould growth. At a further four farms the grain was sold at short notice before a third set of samples could be collected. At least three samples were collected from the remaining 17 farms included in the assessment. At three sites, where the grain was harvested late and wet, additional samples were taken during prolonged drying.

The temperatures and moisture content profiles for each farm are shown in Appendix 3. These show that there were three typical situations and one exception. In the first group, moist grain was harvested and was dried rapidly to safe storage conditions within 2 to 4 weeks. Typical of this were farms NL1, NL5, NL6, K1, O1 and O2. On farms from the second group, grain required some drying but the reduction in moisture was slow, because of sparing use of the drying system e.g. NL2A, NL3, NL4, NL6, H2, M2, M4 and M4A. The final group included farms where grain was harvested wet and took a considerable time to dry to a safe moisture e.g. NL6A, M5, and M5A. The exception was W1 where one duct of the drying system had blocked and this coincided with the sampling points. The farmer ultimately sold all the grain including the damp portion after four weeks.

Observations on the drying systems and their operation:

In general, all the farmers understood the basic principals of operating on-floor drying systems. In almost every case, stores were filled correctly to the level prescribed by the manufacturer and the grain was levelled after filling. Where this was not done the grain was relatively dry so that only small amounts of moisture had to be removed.

One common problem was that the farmer did not have sufficient time to manage the drying process. Fans were not always turned on and insufficient sampling was done during drying to confirm progress and to identify problems. Automatically controlled drier systems, which were

widely used, did not always seem to give entirely satisfactory results. These deficiencies might have been far more important if the harvest had been wetter. Serious difficulties were experienced with drying on farms M5, M5A and NL6A, principally because of the increase in ambient air humidity as autumn approached.

No development of visible mould was seen at any site despite grain on several farms remaining above 18% moisture content for a considerable time. Specific checks were not made for other storage problems but no insect infestation was seen or detected by the farmer and a surface mite infestation was noticed at only one site.

Selection of samples for mycotoxin and mould assessment

During the Project more than 240 samples were collected but financial constraints limited the number that could be analysed for OA or mould species. Therefore, certain characteristics were used to select a range of samples for assessment, although all were preserved in the freezer until the end of the work in case further analyses were necessary. At first, assessments were directed towards those samples that appeared to have the highest possibility of the development of fungi or OA: samples with a moisture content of more than 16%. At a later stage, a wider range of samples was checked to confirm that selection system was appropriate.

Table 1: Ochratoxin A detected during grain storage

Site	OA, µg/kg	Time after harvest, days	Position in grain	MC, %	Temp, °C	*Penicillium* Detected
M5	0.3	80	Middle	16.7	7.7	Yes
M5	0.1	133	Middle	17.0	8.3	Yes
M5A	1.2	38	Middle	18.7	7.5	Yes
M5A	0.2	131	Top	20.3	5.5	Yes
NL6A	5.9	26	Top	22.0	10.0	Yes
NL6A	1.1	26	Middle	19.9	11.6	Yes
NL6A	0.2	53	Middle	17.0	3.4	No
W1*	0.2	End	All	19.0*	17.3*	Not tested

*= bulk sample provided by the farmer, temperature and moisture content after 18 days storage

Determination of ochratoxin A

A total of 108 samples were analysed for OA. Recovery of OA from spiked samples was between 74% and 98%, within the range expected for the method and all results were corrected for recovery. Only 8 samples from 4 bulks of grain were found to contain small amounts of OA, Table 1. These were from farms M5 (2 samples), M5A (2), NL6A (3) and W1 (1). Of these, only 3 samples contained more than 1 μg/kg. The moisture contents and temperatures given in the table are those at the time when the samples were collected.

Although the concentrations of OA are low they are quite similar to results obtained in several surveys of UK stored grain. Only one exceeded 5 μg/kg, which could be adopted in the future as the maximum permissible level for OA in cereals. This study confirms that OA can be produced quite early during storage (26 days after harvest) if conditions favour mould development. It also shows that OA can be produced after prolonged storage under cool damp conditions.

Determination of mould genus and mould count

The approach was used as described for OA to select samples for fungal assessment.

The range of genera and species of fungi present and an estimate of the level of contamination were determined in 109 Samples. Complete data are given in Appendix 4. As would be expected, samples taken during the first sampling shortly after harvest, contained predominately field fungi consisting of a mixture of *Alternaria, Fusarium* and *Cladosporium* species. In most cases grain from the middle depth only was analysed from the first set of samples as changes due to storage would not have taken effect. When samples from other depths were analysed shortly after harvest (eg farms NL2, M2 and O2) results were quite similar to those from the middle.

A total of 33 samples were examined for moulds shortly after harvest and *Alternaria* were found in 25 (76%), *Cladosporium* 23 (70%) and *Fusarium* in 20 (61%). The average mould counts were for *Cladosporium, Alternaria* and *Fusarium* respectively, 9.2, 2.3 and 1.0 x10^3 colony forming units /g (see Figure 1). These results are similar to those reported from numerous other studies. One difference in this relatively small number of farms was that *Fusarium* was present less often and in significantly lower average amounts than were the other two principal field genera.

In the set of samples taken shortly after harvest, *Penicillium* species were only found in 3 (9%) which supports the conclusion made elsewhere that OA is only normally likely to be a post harvest, storage problem. However, at site K1 the sampling point 0.5 m below the grain surface contained an initial level of 21 $x10^3$ colony forming units/g of *Penicillium* considerably outnumbering 3 $x10^3$ colony forming units/g of *Cladosporium*. The corresponding point 0.5m from the bottom of the grain contained no *Penicillium* but did contain the expected mixture of field fungi. This wheat came directly from the field with no intermediate storage which raises the question as to what was the source of infection. The moisture of grain near the surface was 18.7% although this was dried successively to 14.2% within a month. One possibility is that this grain was some of the first to be harvested and may have been contaminated with residues in the combine tank or grain conveying system.

Another interesting observation was that initial samples from 3 of the sites housing the wettest grain contained very low or no significant mould growth in laboratory culture. This is surprising and no explanation can be offered although the sample numbers involved were very low.

Figure 2 shows the decrease of field fungi during drying of wheat stored on farm M4 in samples taken from the middle of the bulk. While the situation on each farm is unique, the sequence of events on this farm approached what might be considered as ideal for drying cereals safely. Wheat, initially at about 16.5%, dried gradually to under 15% while grain temperature dropped sharply from nearly 30°C at harvest to about 10°C after 3 months. The total fungal count consisted entirely of field fungi and decreased steadily to a low level. No *Penicillium* moulds were detected which was consistent with low moisture content and falling temperatures. Assuming this situation applied throughout the grain bulk, safe storage and freedom from OA was assured.

Another situation is illustrated in Figure 3. The moisture content of the grain near the bottom of the store NL2 although initially at a safe moisture content, increased slowly over the 6 months storage to a level where moulds could start to grow. This was probably caused by damp air seeping into the grain via the ducting. After the expected decline in field fungi, a significant although low infection of *Penicillium* started to develop.

The data for site M5A are shown in Figures 4-6. These give the results for the upper, middle and bottom depths over the 4-month period during which monitoring took place. Wheat was harvested at the end of the first week in September and was initially between 19 and 21% moisture, being slightly wetter at the bottom. The bottom of the bulk was dried to below 17% within 6 weeks but there was no drying in the middle and top layers. Later, the top layer slowly increased in moisture as water appeared to be translocated upwards through the grain and was still above 20% at the final measurement (see Figure 4). Initial mould counts were low but by 38 days both field and storage moulds had increased, although *Penicillium* species were the most common. After that time field species continued to increase but *Penicillium* rose only slightly and then declined. *Penicillium* species not detected after 4 months. Despite prolonged storage at high moisture content, only a trace level of OA was detected at the final sampling. The middle position followed a similar pattern except that, as some decrease in moisture content was achieved, less field fungi grew and the *Penicillium* species survived to the end of the study. A level of 1.2 µg/kg OA was found after 38 days but was not detected again at this position. Drying of the lower layers of wheat was much more successful and moisture content was reduced below 17% within 38 days of harvest (see Figure 6). Field fungi developed initially but subsequently declined while only a trace amount of *Penicillium* was detected around 82 days. No OA was detected which was consistent with the lower moisture content and the low amount of *Penicillium*.

Figures 7-9 show similar data for site NL6A at which the harvest was delayed until mid-September and wheat was harvested at above 22% moisture. It was about 1 month before any sign of drying occurred in the upper layers of wheat (Figure 7) although temperatures fell during this period to below 10°C. Four months of drying and storage were required before the top layer was dried to 17%. As at site M5A, initial mould infection was low. However, by one month a relatively low infection of *Penicillium* had developed accompanied by a level of 5.9 µg/kg of OA, the maximum value detected in this study. The potential for a major problem with OA formation clearly existed but was probably avoided because grain temperatures were subsequently reduced to 5°C, at which toxin formation would be very slow. In the centre of this bulk (Figure 8) the wheat dried more rapidly and was down to 17% within 50 days although some *Penicillium* and small amounts of OA were detected during this period. Despite the relatively low and falling moisture content, by 4 months a high infection of *Penicillium* had built up. However, this was found to be a xerophilic species, *P. brevicompactum,* which is not known as a mycotoxin producer although it can survive and grow at lower moistures. The increase in fungi at this time may have been accentuated by a rise in grain temperature. The

grain at the bottom of the bulk typically dried more rapidly and mould development was much less and no OA was detected, Figure 9. However a low infection of *P. brevicompactum* had developed by 3 months.

Table 2: *Penicillium* species identified from selected grain samples (S= surface sample, T= 0.5 m from surface, M= mid-height, B= 0.5 m above bottom of grain)

Farm reference	Storage time, days	Position	Species found	Total *P.* count, cfu/g, x10^3
NL1	14	T	*P.aurantiogriseum*	1.0
NL2	170	B	*P. brevicompactum, P. hordei*	2.0
NL3	135	M	*P. brevicompactum*	0.2
NL4	73	M	*P. cyclopium, P. brevicompactum*	1.1
NL6A	26	T	*P. aurantiogriseum*	1.0
	26	M	*P. chrysogenum, P.griseofulvin*	0.5
	83	T	*P. brevicompactum, P.cyclopium*	6.0
	83	M	*P. chrysogenum, P.griseofulvin*	0.8
	83	B	*P. brevicompactum, P. chrysogenum*	0.2
	120	T	*P.aurantiogriseum, P. verrucosum*	0.8
	120	M	*P. hordei*	32.0
K1	2	T	*P. cyclopium*	21.0
	32	T	*P. verrucosum*	2.0
M4A	33	T	*P.aurantiogriseum, P. brevicompactum, P. expansum, P. verrucosum,*	0.3
	84	M	*P. citrinin*	0.1
M5	15	M	*P. brevicompactum, P. hordei*	2.0
	15	B	*P. brevicompactum*	0.25
	41	M	*P. brevicompactum, P. cyclopium*	0.3
	80	T	*P. brevicompactum*	5.0
	80	M	*P. brevicompactum*	2.3
	133	S	*P. brevicompactum*	2.9
	133	T	*P.aurantiogriseum, P. brevicompactum*	5.0
	133	M	*P. chrysogenum, P. cyclopium, P. roquefortii*	0.4
M5A	38	T	*P. brevicompactum, P. cyclopium*	0.5
	38	M	*P. chrysogenum*	0.2
	82	T	*P. brevicompactum*	1.4
	82	M	*P. brevicompactum*	2.0
	82	B	*P. brevicompactum*	0.15
	131	S	*P. aurantiogriseum*	
	131	M	*P. hordei*	1.5

Identification of Penicillium species

Thirty samples of grain shown initially to contain *Penicillium* from 9 of the farms were examined to identify the species of *Penicillium* present. The results are given in Table 2. This yielded 46 isolates representing 10 different species. Those occurring most frequently were *P.*

brevicompactum (17 occasions), *P. aurantiogriseum* (6), *P. cyclopium* (5), *P. chrysogenum* (5) and *P. hordei* (4). *P. verrucosum* the species considered responsible for the formation of ochratoxin A was isolated from three farms, K1, NL6A and M4A. None was found on farm M5/5A although small amounts of OA were isolated from the grain on this farm on several occasions

When *P. brevicompactum* occurred, it was quite evenly distributed between the sampling positions while in contrast *P. aurantiogriseum* and *P. verrucosum* were only isolated in samples from the upper layers of grain. This is consistent with the growing characteristics of the fungi and with the operation of ambient air drying which ensures that the bottom layers dry first. *P. brevicompactum* is a xerophilic mould capable of growth at water activities of about 0.78 at 25°C while *P. aurantiogriseum* and *P. verrucosum* require higher water activities for growth that are more likely in the upper layers of grain. No OA was found in samples near the bottom of any of the grain bulks.

Ability of Penicillium fungi to produce OA

A selection of *Penicillium* strains were examined to check for their ability to form OA in culture. Twenty three strains mostly with morphology similar to that for *P.verrucosum* and *P. aurantiogriseum* were examined. Two samples from a laboratory culture collection were grown up under identical conditions and run alongside the field samples. These controls produced both OA and citrinin. No OA was found in any of the field samples, including the 3 identified as *P. verrucosum*.

Discussion

The 1998 harvest was relatively dry so that much grain came from the fields at moisture contents of 16% or less. Despite the dry harvest grain at the majority of sites included in this work needed some drying. When used, the drying systems mostly seemed to remove moisture in an effective manner. However, it appeared that, as the bottom of a bulk was dried, moisture moved up and grain at the surface could become wetter. This could pose a risk of fungal growth if for any reason the drying process had to be suspended before the drying front had reached the surface.

When grain was harvested late so that the ambient temperature was falling, drying appeared to become more difficult and more easily disrupted by wet weather. However, at the farms where wet grain had been put into store late any attempt at drying caused a rapid reduction in grain

temperatures even if little moisture was removed. Under these difficult conditions, fans were run for extended periods and, even though this achieved little drying, grain temperatures were reduced to below 10°C by the second month of storage, thus slowing any fungal growth.

Most farms used in this work represented separate, individual units. Different varieties and species of grain were harvested on different dates and drying strategies varied. Despite this, the assessment of moulds shows that the same groups of species of field and storage fungi were found in every case. This is consistent with other work done throughout the world on the flora of fungi in stored grain. It seems reasonable to assume that at harvest all grain is contaminated to some extent with these groups of fungi and that this distribution is sufficiently homogeneous to provide a common start point at harvest. Standard mould plating techniques, as used in this work, in which the fungal colonies are counted and identified on plates of the highest dilution typically supporting 5-50 colonies, favours the detection of the predominant moulds. Hence the presence of a very few *Penicillium* or *Aspergillus* can easily be masked by a large number of colonies of field fungi or *vice versa*. The high incidence of field fungi and failure to detect storage fungi at harvest is not an indication of their total absence. The critical factors controlling any subsequent change in the make-up and level of fungal contamination are the physical conditions within the grain.

Surprisingly little is known about the exact conditions that trigger the production of OA in grain. In temperate climates, many *Penicillium* species have been reported as forming OA. However, Frisvad and Lund (1993) have suggested that these studies were bedevilled by mis-identification of *Penicillium* isolates and that only one species, *P. verrucosum* should be considered as the principle OA producer.

A recent review addressed the subject of the formation of OA under marginal conditions (Hetmanski 1996). This showed that most of the published studies concentrated on the conditions that maximise OA formation while only a few report experimental studies carried out under marginal conditions likely to occur during drying and storage in the UK. In the review, three studies were cited where OA formation was studied in the laboratory by inoculating wheat and barley with *P. verrucosum*. Harwig and Chen (1974) found formation at 22% and 18% moisture content on inoculated ground wheat while Muller and Boley (1992) produced a similar effect in whole wheat grains at 20 and 18%. Only one relevant study was reported for barley, Northolt *et al.* (1979). In this work no OA was formed on ground barley at 14.4% although it was formed at 17.9 and 23.6%. Abramson *et al.*, (1980, 1982, 1983, 1984,

1987, 1990 and 1992), reported a series of field studies on Canadian stored wheat and barley. With a much less sensitive detection method, OA was formed in varieties of wheat at 19% moisture content and in one occasion in durum wheat at 15%. Regrettably little information was given for intermediate moistures. For barley, the lowest moisture content where OA was reported was 18%.

Subsequent to the review, Hetmanski (1997) carried out a laboratory study in which OA formation in wheat and barley was assessed over the range 14 to 22% moisture content. Composite samples were produced by mixing 10 different wheat and 10 different barley samples. These were stored under controlled conditions of temperature (in the range 10-20°C) and ERH. OA formation was followed both in un-inoculated samples and after inoculation with *P. verrucosum*. No OA was formed in un-inoculated wheat or barley except on one occasion in barley when the moisture exceeded 21%. With inoculated wheat, OA was formed within 8 weeks at 17.3% moisture content at both 15° and 20°C although in larger amounts at 20°C. For barley some sporadic formation occurred at 16.3% within a few weeks. Figure 10 shows the relationship between OA formation, temperature, moisture content and time for inoculated wheat. The conclusion from this study was that, given time, OA could be formed at moistures of more than 16.5% and temperatures of 15°C. These findings appear to be supported by the results from this work where OA was never found in grain with a moisture content of less than 16.5%. However, the data from the laboratory experiments suggested that a more widespread formation of OA might have been expected at one or two sites although this would have depended on the presence of OA-producing *Penicillium* species. In the event none of the *Penicillium* species found in the samples collected during this work produced OA in the laboratory although 3 isolates were identified as *P. verrucosum*. However, it is common for some strains of a toxin-producing species to be non-toxigenic. Alternatively, as isolates were stored frozen for some time, the ability of the strains to form OA may have been lost. This problem is recognised by mycologists. As formation of OA is slow below 10°C, the rapid fall in grain temperature to between 5° and 7°C in less than 6 weeks at the sites where drying was slow, may provide the explanation why more OA was not found. Another possible explanation is that the predominance of field fungi could have suppressed the development of *Penicillium* moulds and OA production. However, further studies would be needed to confirm this.

Given the limited knowledge available about the production of OA, it is hardy surprising that even less is known about the specific distribution of the toxin across a bulk or between grains. Therefore, it is impossible to explain the apparent lack of consistency in the detection of OA

over time during this work. When OA was found before the final sample collection, subsequent samples from the same sampling point either showed reduced levels or none. The toxin is very stable so breakdown cannot be offered as an explanation. It is possible that the act of withdrawing grain from a sample point disrupted the growth of mould and limited further toxin production. However, an alternative suggestion is that toxin production occurs on a micro scale, with just a few grains at any point being contaminated. Once again more work seems to be justified.

The results of this study suggest that the distribution of mycotoxins within a grain bulk may be extremely heterogeneous and the detection of OA at individual points is not indicative of the mean concentration within a bulk but merely confirms that there is a risk. However, within a floor-dried bulk of grain, the temperature and moisture content is likely to be stratified in the vertical plane because any cooling and drying effects start at the bottom and move up. Sometimes, drying strategies may require that some of air ducts are closed to concentrate airflow to a particular section of grain or mechanical failure can result in no airflow at some points. Except in these situations, the sampling system used in this work was justified and provided a general picture of changes in temperature and moisture content that occurred within the bulks during the period of assessment. Unfortunately, any destructive sampling method must cause some disturbance around the sampling point. The extent to which removal of grain may have changed local airflow patterns or disrupted fungal growth remain the subject of speculation. However, finding of OA at any point would seem to indicate that any part of the bulk having the same temperature/moisture content combination as the sample position, was at risk from mycotoxin formation. Unfortunately, the sampling schedule used was not designed to test the validity of proposed legislative sampling regulations for OA in stored grain but the results do raise some serious questions about its use in practice and highlights the need for more research..

Risk assessment

The laboratory studies referred to earlier, do not provide enough data to fully predict the risk during storage, although they do confirm that OA would be unlikely to occur in grain dried quickly to or stored below 16%, regardless of temperature. Grain above 18% at between 10° and 25°C would be at some risk from OA formation within one month. The risk will increase with higher moisture content, higher temperatures and extended storage. Using these limited data, it is possible to group the sites sampled during this study into potential risk categories based on the temperature and moisture content of the grain and the period of storage (see Table 3)

Table 3: The predicted risk of formation of ochratoxin A in grain bulks studied

Table 3: The predicted risk of formation of ochratoxin A in grain bulks studied

Rating	Sites			
High risk	M5*	M5A*	NL6A*	W1*
Moderate risk	NL6	M4A		
No/low risk	H1	H2	K1**	K2
	K4	M1	M2	M3
	M4	NL1	NL2	NL2A
	NL3	NL4	NL5	O1
	O2	W2		

* ochratoxin A detected in at least one sample

** initial grain presented a potential problem but grain was dried quickly

Despite reservations about the laboratory data used to develop these predictions, OA was only detected at sites classed as high risk and none was detected on any other farm. Of the 4 farms where OA was detected, the wheat on farms M5, M5A, and NL6A was harvested wet and relatively late on in the season. The barley at site W1 was harvested at above 18% moisture content in early August and then stored without drying or cooling because of technical problems, although it was sold relatively quickly. In the case of the two sites classed as moderate risk, NL6 and M4A, some of the grain remained above 17% moisture content for at least 1 month and at temperatures in the range 10-22°C. However no OA was detected at the designated sampling points. No OA was detected in any of the low risk stores.

From the above, it is possible to suggest on-farm drying strategies must be geared towards a rapid reduction of moisture to 16%. The exact time for drying will depend on the starting moisture content and temperature of the grain. Any attempt at drying will tend to reduce the grain temperature and this must at least slow the development of harmful moulds. However, cooling grain at moistures above 16% is not a substitute for drying if risks from OA are to be avoided but it will allow the drying period to be extended.

Conclusions and Recommendations

Slow drying systems do not seem to carry an inherent risk of fungal growth and therefore the potential for mycotoxin production. Indeed, they have the advantage that blowing large volumes of air at close to ambient temperatures though the bulk of grain will tend to reduce its temperature. The use of slow drying systems when harvests are wet and/or late must carry some risk of mould growth

before drying is completed. However, a bad harvest will also stretch the capacity of a high temperature drying systems and may result in un-dried grain being left in heaps for some time, without the advantage of the cooling.

When slow drying systems are used it is important that enough time is devoted to their management. The most important aspect is the collecting of samples from different parts of the bulk at different depths and comparing results over time. This management process is of critical importance if the intake moisture is above 18% and ambient conditions are less than ideal.

The aim of the drying process must be to minimise the time that any grain spends at moisture contents above 16% and at temperatures above 20°C. Once grain is at or below 16% moisture, cooling to below 10°C will ensure that toxin-producing moulds will not develop. It will also minimise any risk from insect pests. However, further drying is needed to avoid the risk of mite infestations.

Properly managed ambient air drying systems should normally be able to dry damp grain to a suitable moisture content safe for storage on most occasions within an acceptable time. At 20°C it is recommended that the target for this should be to reduce grain to 16% within about 14 days. This offers a considerable margin of safety as cooling associated with drying will extend the time before OA will be produced. This is because a significant build up of the appropriate *Penicillium* species is required and the rate of growth is dependent on temperature as well as moisture.

OA only occurred in damp grain or when drying was slow. Failure to dry on 2 farms was because the ambient air was frequently at too high an ERH and the available heat source was not suitable to produce sufficiently dry air. Despite these difficulties, no high or widespread development of OA was detected. The cooling effect of the 'drying' air and the dominance of field fungal species may have helped to prevent this.

In the management of stored grain, the inability to reduce moisture to below about 16% in a relatively short time should alert the farmer to the potential for a serious problem, especially if the grain temperature remains above about 15C. The top layers of grain will be at greatest risk because they are the last to dry. OA formation depends on a number of factors and high moisture alone will not necessarily result in mycotoxin formation but it does provide a clear indication of risk. The presence of visible mould, whilst undesirable, is no reliable indicator of

the presence of OA. Indeed there was no visible mould in any of the samples collected during this work, including those in which OA was detected.

The need to avoid even low-level contamination of cereals with OA is now demanded by many sectors of the Food and Feed Industries and is very likely to be backed by regulation setting a maximum permissible level for OA in cereals traded within the EC. At the moment there is no rapid, cheap and reliable test for OA or even a quick test for the mould *P. verrucosum* which could be used by farm staff although this may change in time. Therefore, control of moisture content and grain temperature in such a way as to eliminate any risk of OA production must remain the principal defence against allowing farm-stored grain to become contaminated with mould and/or OA. The lack of continuity in detecting OA in consecutive samples from the same points in a bulk, highlights the lack of knowledge on the distribution of the toxin. It also demonstrates the need for experimental work to validate proposed definitive sampling methods for OA in grain.

In summary, wet grain at harvest should be dried as soon as possible to 16%. The allowable time for drying will depend on the grain temperature during the drying process but at about 20°C drying to 16% should be achieved within 14 days. Cooling the grain during drying should extend the time available to dry. In order to achieve effective drying, low-temperature on-floor drying systems must be well maintained and operated effectively. Management during drying must include the collection and assessment of sufficient samples to ensure that the entire system is working correctly. When calculating drying times and risk of OA, it must be born in mind that the top will remain wettest for longest. Occasional additional drying may be required during storage if moisture contest increases above 16%, for example if the surface layer re-adsorbs moisture. Also at one farm the bottom layers of grain became wetter during storage, presumable because of damp air diffusing in though the ducting. Where on-floor drying systems are used under marginal conditions on a regular basis, for example grain being harvested at more than 20%, consideration should be given to the installation of a de-humidifier to allow greater flexibility in operating the system.

Acknowledgement

The authors would like to thank all the farmers who assisted in this study by allowing the use of their grain and stores. We would also like to thank Banks Southern Ltd, Lillico Ltd, Nickerson Seeds and Viking Cereals for their help in locating sites. Finally, we wish to thank Protimeter plc for the loan of equipment and calibration services.

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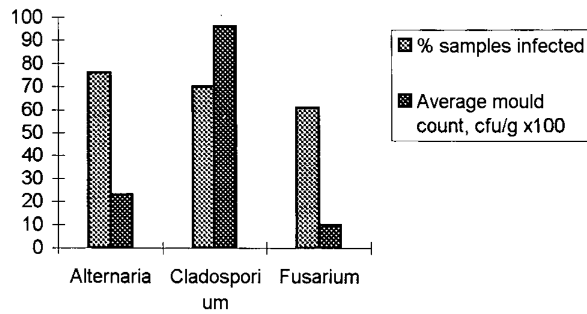


Figure 1: % of samples infected by field fungi farm NL6A, and average mould counts at harvest on 24 farms

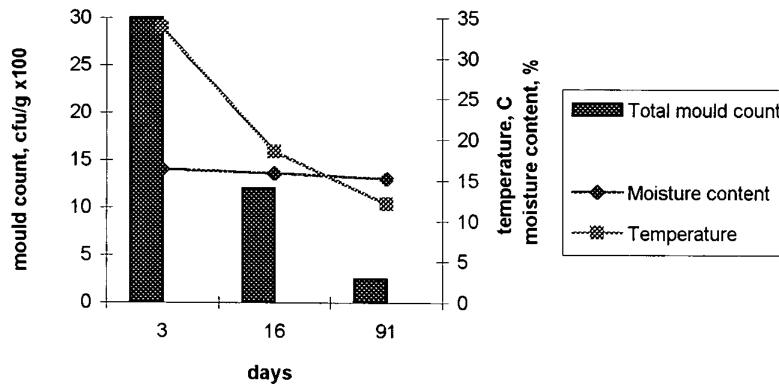


Figure 2: Change in total mould count, moisture content and temperature, farm M4, wheat, centre position

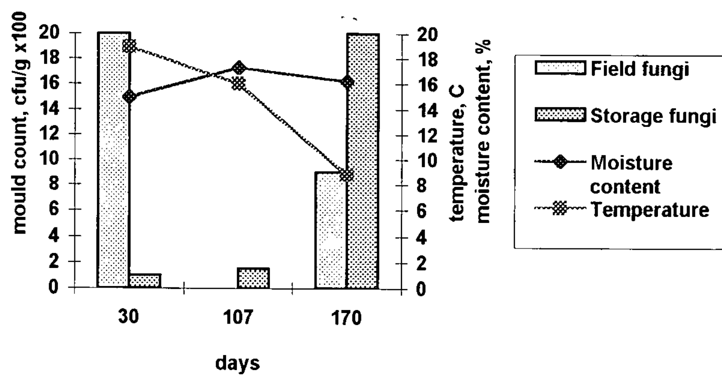


Figure 3: Change in mould flora, moisture content and temperature, farm NL2, barley, bottom position

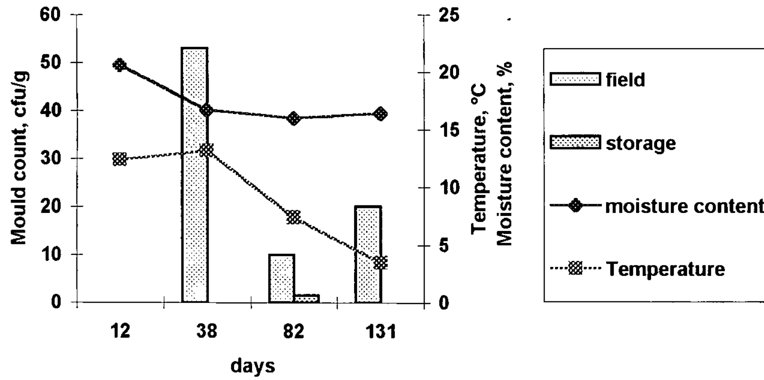


Figure 4: Change in mould flora, moisture content and temperature, farm M5A, wheat, bottom position

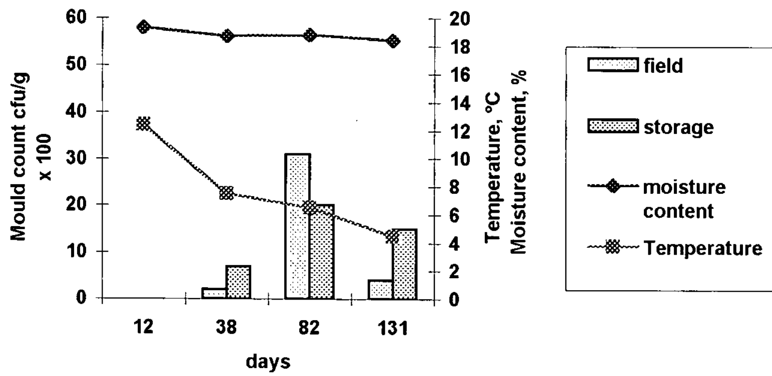


Figure 5: Change in mould flora, moisture content and temperature, farm M5A, wheat, centre position

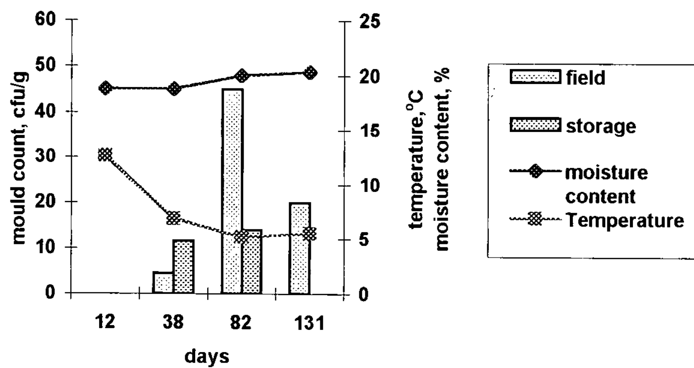


Figure 6: Change in mould flora, moisture content and temperature, farm M5A, wheat, top position

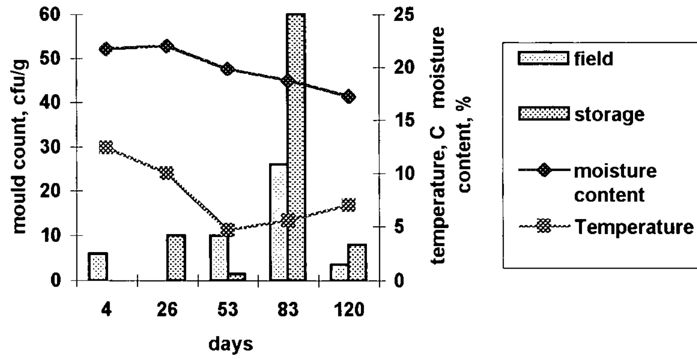


Figure 7: Change in mould flora, moisture content and temperature, farm NL6A, wheat, top position

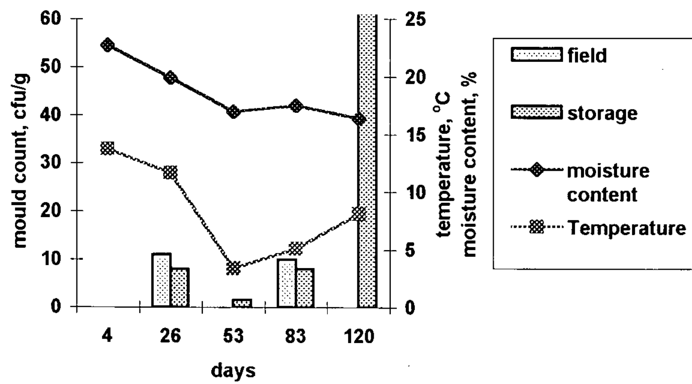


Figure 8: Change in mould flora, moisture content and temperature, farm NL6A, wheat, centre position

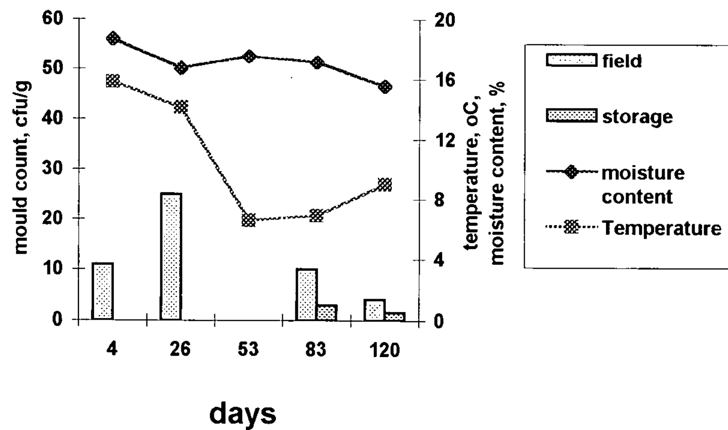


Figure 9: Change in mould flora, moisture content and temperature, farm NL6A, wheat, bottom position

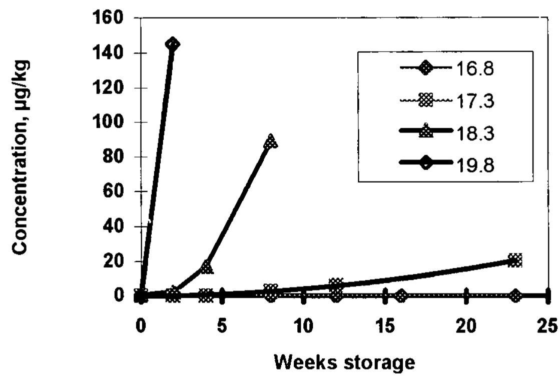


Figure 10: Formation of ochratoxin A in wheat at different grain moisture contents, %, at 20°C

Appendix 1 METHODS *Analysis for ochratoxin A*

Determination of ochratoxin A was carried out by RHM Technology, who is NAMAS accredited for mycotoxin analysis including ochratoxin A in cereals. The quantity of sample received from farms was usually approximately 1kg.. The whole sample was then ground and thoroughly homogenised. As most samples were to be examined subsequently for fungi, the grinder was flushed out with sterile grain between each grinding and washed with ethanol. A 50 g aliquot was transferred to a sterile pot and stored until required at -20°C. If not examined immediately, the rest of each ground sample was also stored at -20°C. A 25 g sub-sample was taken for analysis

All analyses were conducted with spiked samples, i. e. to each cereal type on each day, a known amount of ochratoxin A was added prior to extraction, clean-up and HPLC determination. One spike was included for every batch of 1-5 analytical samples. These results were used to assess recovery and all reported results were corrected using the values obtained. Values between 70 and 110 % were considered acceptable. Spiking level was equivalent to 2 µg/kg. Limit of detection was 0.1 µg/kg with limit of determination 0.2 µg/kg. The limit of detection is defined as 3 times the electronic baseline noise and the limit of determination as 6 times baseline noise. The lowest point on the calibration curve was equivalent to 0.2 µg/kg of ochratoxin A. After analysis samples were retained and stored at -20°C.

Extraction and clean-up.

A 25 g portion of sample was extracted with a mixture of 250 ml chloroform and 25 ml of 0.1M orthophosphoric acid by shaking for 30 minutes. The extract was filtered and a 50 ml volume of the extract reduced to dryness and 1ml of toluene added.

A silica Sep-Pak cartridge was pre-washed with 10ml of toluene and the sample added to the cartridge in the 1 ml of toluene. The cartridge was then washed with 10 ml of toluene followed by 10 ml of chloroform : methanol (97:3) and ochratoxin A then eluted with 10 ml of toluene : acetic acid (90:10). This fraction was then evaporated to dryness, transferred to a vial and made up in 0.5ml of the HPLC mobile phase.

HPLC determination of ochratoxin A.

HPLC equipment consisted of a Gilson 307 pump, Gilson 231/401 auto-injector and a Perkin Elmer LC 240 fluorescence detector with excitation wavelength set at 333 nm and emission wavelength at 470 nm. The column used was a Spherisorb ODS 2 (25 cm x 3.2 mm i.d.) with elution solvent of 0.1% orthophosphoric acid : acetonitrile (50:50) at 0.5 ml/minute flow rate. Sample volumes of 20 µl were injected.

HPLC method validation and quality control

This method has been used in several collaborative and Intercomparison trials and the results obtained were as follows:

FAPAS series XVII, June 1997, Z-score 0.0 (mean value for ochratoxin A, 5.45)

FAPAS series XVII, June 1998, Z-score 0.1 (mean value 9.3)

BCR certification of reference material, 6.9 µg/kg (mean value 8.2)

BCR Intercomparison, November 1993, 6.9 µg/kg (mean value 7.5)

BCR intercomparison, October 1991, 15.9 µg/kg (mean value 13.2)

On-going control of the method was monitored using an in-house naturally contaminated reference material (3.5 µg/kg) which was then spiked at 2 µg/kg. Typical recoveries were between 90 and 98% with coefficient of variability between 3% and 5% for 10 replicates.

Appendix 2 METHODS *Examination for moulds*

Mould counts and species identification were carried out at the Commonwealth Agricultural Bureau International (CABI) Egham. Frozen ground cereal samples were transported in a cold box from RHM to CABI and stored until required. A few of the initial samples were subdivided at RHM and matched ground and whole cereal transferred. The whole portions were ground at CABI in a hammer mill and the mould counts and species of the matched portions compared to check that no major difference occurred. As results were acceptable, all subsequent samples were ground by RHM and these were used for mould counts and identification.

Total mould count to genus level

A 1 g portion of sample was added to 9 ml peptone water and 0.05% Tween and serial dilutions down to 10^3 prepared. For each dilution, 0.1 ml of solution was spread plated onto malt agar plates containing antibiotics, in duplicate. These were then incubated at 25°C for 6 days. Identification to genus and counts were then made.

For some samples, direct plating was carried out. To do this, ground sample (0.5 g) was sprinkled directly onto the agar medium. These plates were then incubated at 25°C for 6 days and assessed as above.

Identification of Penicillium species with special attention to P. verrucosum

From 1g of each sample a dilution range of 10^0 to 10^3 was prepared in peptone water and 0.05% Tween solution. From each dilution factor, 0.1 ml was spread plated in duplicate onto malt agar plus antibiotics (MA+AB) and dichloron rose bengal yeast sucrose agar (DRYS). The plates were incubated at 25°C for 7 days and fungal counts were then made. All *Penicillium* species were further subcultured onto diagnostic medium (Czapek Dox agar) using a 3 point inoculation technique, incubated at 25°C for 7 days and identified to species level.

Penicillia are identified to species level using a combination of macro- and micro-morphological characters (Pitt 1980). Macro-morphological characters include growth rate on standard media, colony texture (floccose, fasciculate), colony colour, presence of droplets on colony surface, soluble pigmentation in the agar medium, colour of colony reverse and smell. Micro-morphological characters (those viewed using microscopy) are based on the level of branching (number and arrangement of branches in the penicillus), surface texture of the stipe

(stalk), shape of the conidiogenous cell (phialide), and shape and ornamentation of the conidia (spores).

Testing for ochratoxin A production

Selected *Penicillium* isolates were grown up on yeast extract sucrose agar. Small plugs of the agar taken from the growing mould using a method described by Paterson and Bridge 1994. These plugs were pressed onto silica TLC plates and these were then developed with toluene acetic acid. The plates were viewed under UV light at 354 nm.

Two IMI culture strains of *P. verrucosum* known to produce OA (and citrinin) were included as controls.

Fungi isolated

Field species found

Acremonium	*Alternaria*
Aureobasidium	*Botrytis*
Cladosporium	*Epicoccum*
Fusarium	*Mucor*
Trichoderma	*Verticillium*

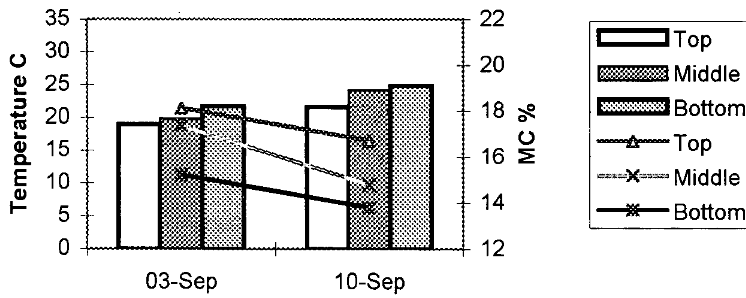
Storage species found

Penicillium
Aspergillus

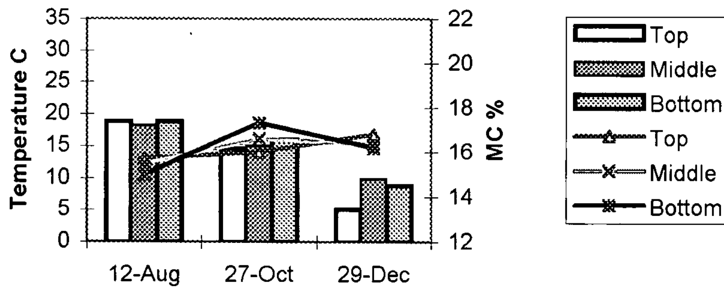
Appendix 3

Temperature and moisture content records from farms for each sampling visit. Temperatures measured with Protimeter probes and moisture with Protimeter P900 moisture meter.
Temperatures shown as histograms
Moisture shown as lines

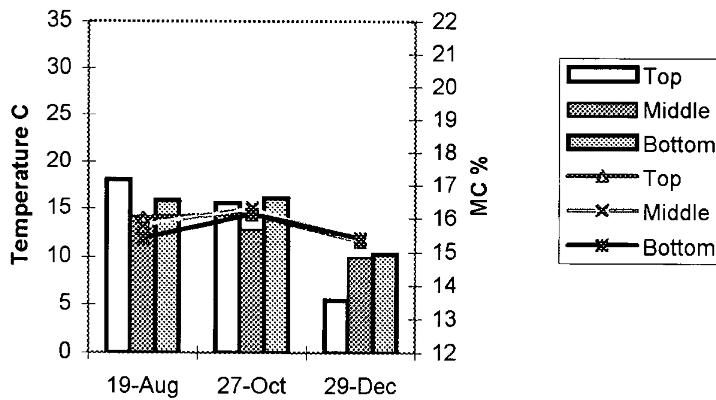
NL1 (Wheat)



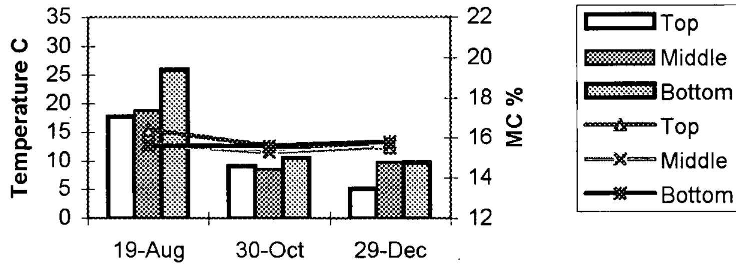
NL2 (Barley)



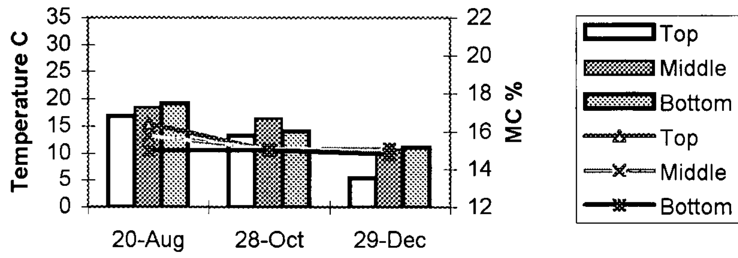
NL2A (Wheat)



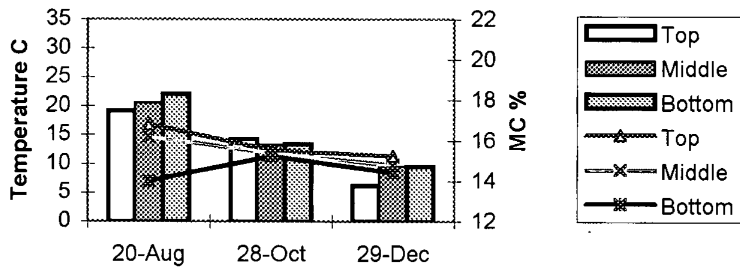
NL3 (Wheat)



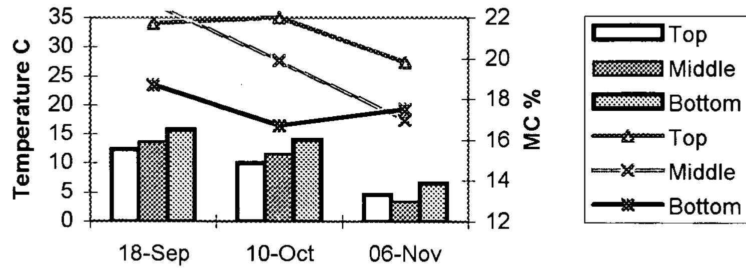
NL4 (Wheat)



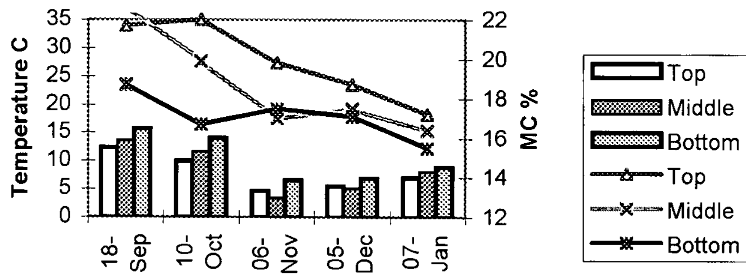
NL5 (Wheat)



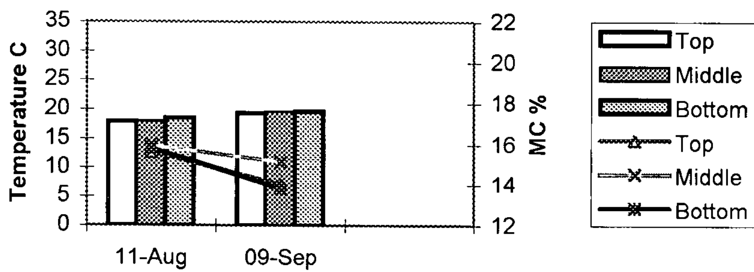
NL6 (Wheat)



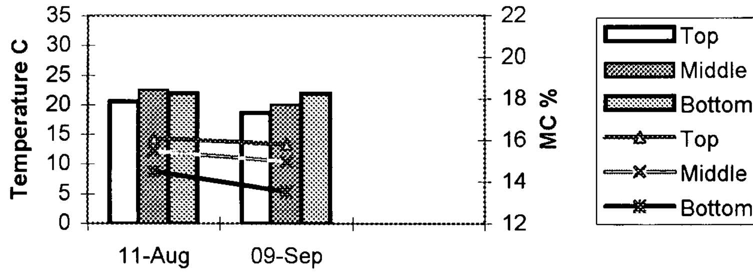
NL6A (Wheat)



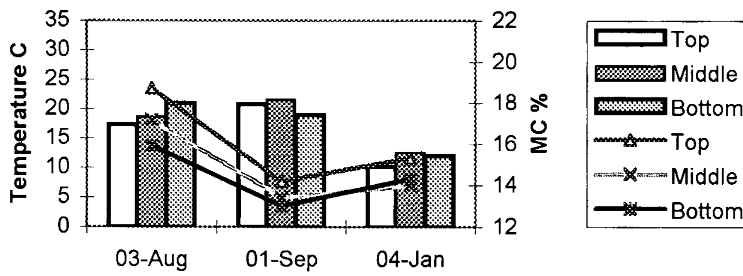
H1 (Barley)



H2 (Barley)



K1 (Wheat)



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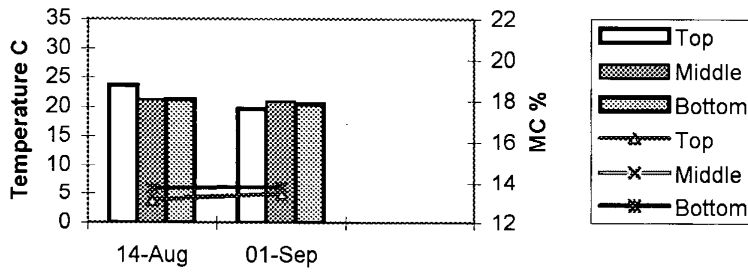

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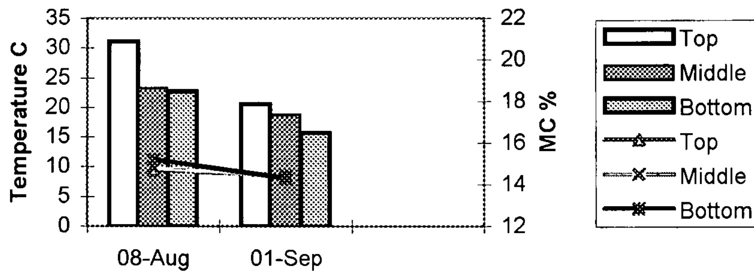

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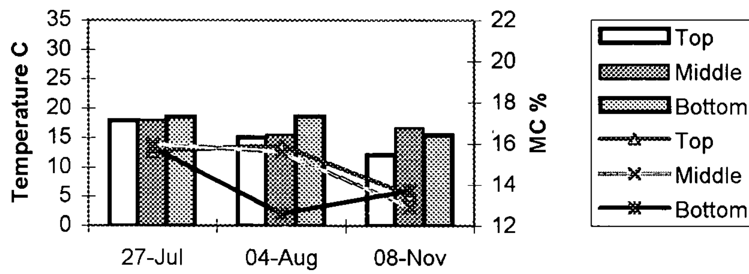

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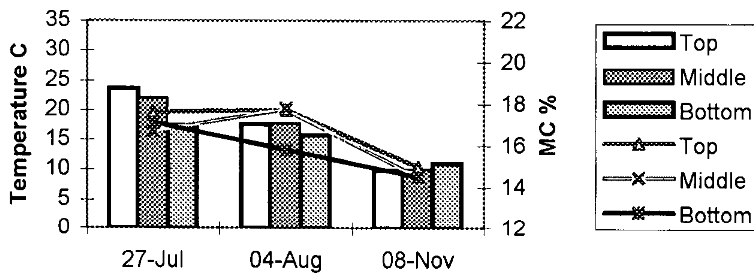

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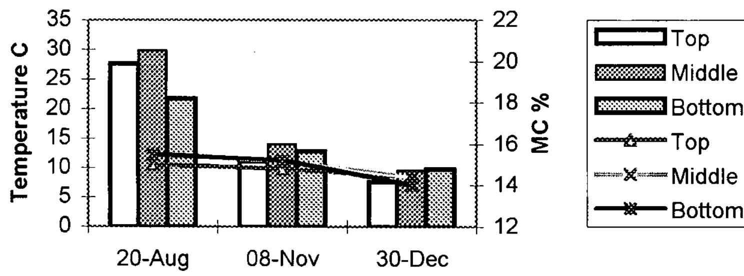

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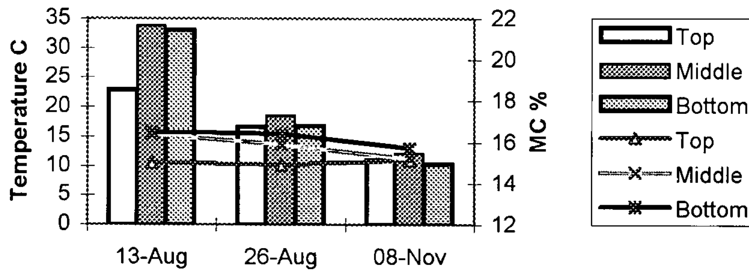
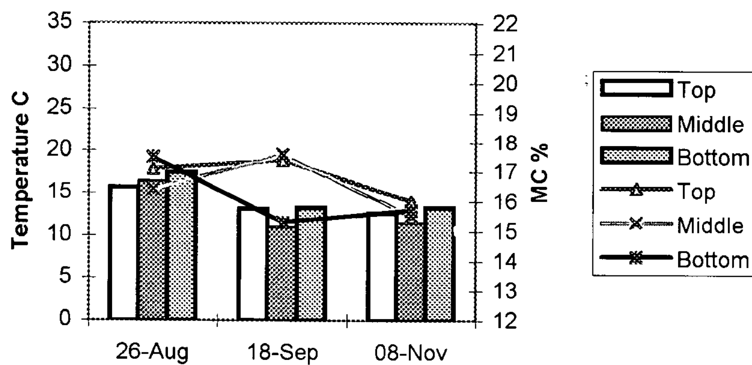


M4A (Wheat)



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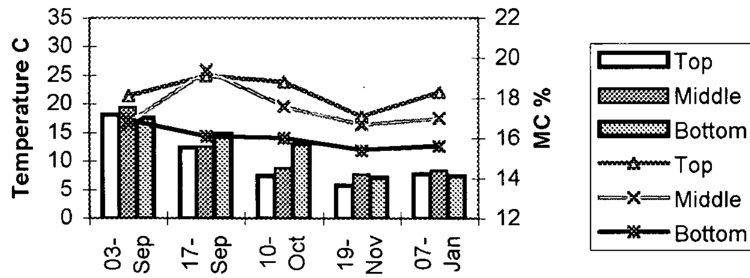
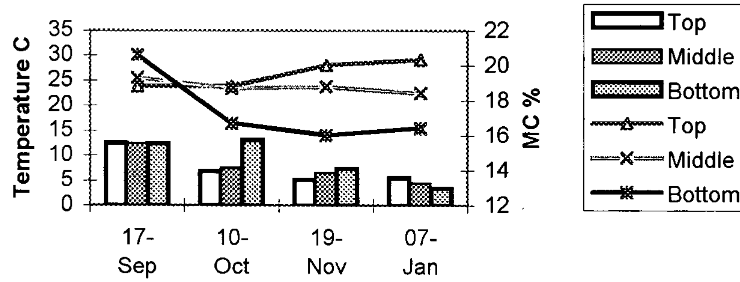


M5A (Wheat)



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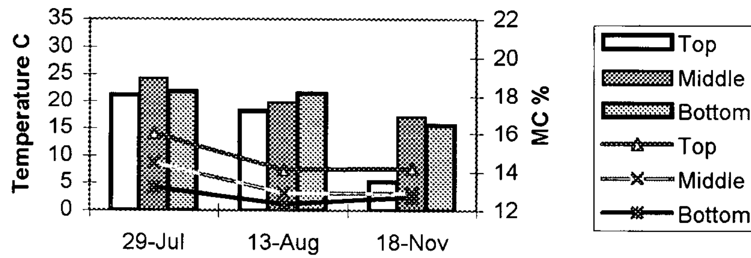
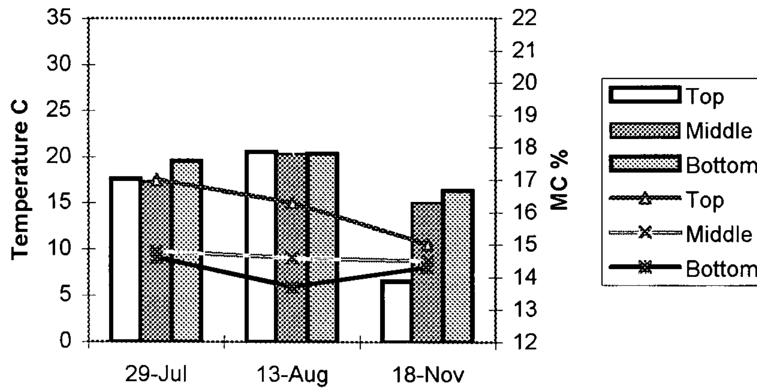


O2 (Wheat)



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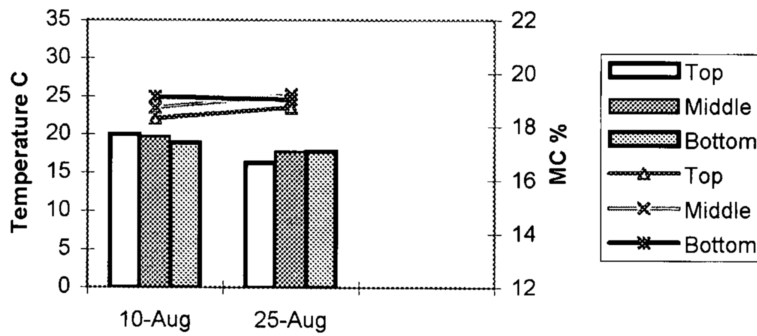
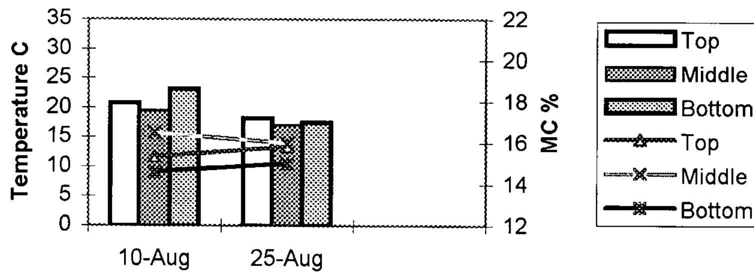


W2 (Wheat)



DATA SUMMARY SHEET FARM: NL1 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	3 September			10 September			none		
Harvest+days	7			14					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
							GRAIN SOLD		
Temp	19.0	19.8	21.7	21.6	24.1	24.9			
MC	18.1	17.4	15.2	16.7	14.8	13.8			
Cladosporium				2.5×10^2	3.0×10^3				
Alternaria	1.2×10^2			1.0×10^2	1.5×10^2				
Fusarium				1.0×10^2					
Acremonium	1.0×10^2			1.0×10^3	2.6×10^3				
Verticillium					5.0×10^2				
Botrytis									
Epicoccum					1.0×10^2				
Mucor									
Trichoderma									
Aureobasidium									
Penicillium				1.0×10^3					
Aspergillus									
Bacteria	1.0×10^4			3.9×10^5					
Yeasts	1.0×10^3			9.0×10^2					
OA	nd			nd	nd				

DATA SUMMARY SHEET FARM: NL2 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	12 August			27 October			29 December		
Harvest+days	30			107			170		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	18.9	18.2	18.9	14.6	15.0	16.0	5.1	9.9	8.8
MC	15.7	15.5	15.0	16.0	16.6	17.3	16.8	16.4	16.2
Cladosporium	3.0×10^2		8.0×10^2				1.5×10^2		
Alternaria	7.0×10^2		6.0×10^3				1.0×10^2		
Fusarium	3.0×10^2		3.0×10^2						
Acremonium								1.5×10^2	2.5×10^2
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									7×10^2
Penicillium			1.0×10^2			1.5×10^2		1.0×10^2	2.0×10^3
Aspergillus								1.0×10^2	
Bacteria						2.5×10^3			
Yeasts									
OA	nd		nd		nd	nd	nd	nd	nd

DATA SUMMARY SHEET FARM: NL2A WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	19 August			27 October			29 December		
Harvest+days	1			71			134		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	18.1	14.1	15.9	15.6	12.8	16.1	5.5	9.9	10.3
MC	16.0	15.8	15.4	16.1	16.3	16.1	15.3	15.3	15.4
Cladosporium		1.5×10^4			1.0×10^2			3.5×10^2	
Alternaria		1.3×10^4			2.0×10^2				
Fusarium		1.0×10^2							
Acremonium					4.0×10^2			5.0×10^2	
Verticillium									
Botrytis								1.0×10^2	
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium					1.5×10^2			1.0×10^2	
Aspergillus									
Yeasts		N@10^2							
OA		nd			nd			nd	

DATA SUMMARY SHEET FARM: NL3 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	19 August			30 October			29 December		
Harvest+days	2			75			135		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	17.8	18.8	26.0	9.1	8.5	10.6	5.1	9.7	9.7
MC	16.4	15.7	15.6	15.6	15.3	15.6	15.5	15.6	15.8
Cladosporium		6.0×10^3			2.5×10^2		1.5×10^2	1.5×10^2	
Alternaria		1.4×10^3					2.0×10^2	2.0×10^2	
Fusarium		2.0×10^2			1.0×10^2				
Acremonium									
Verticillium									
Botrytis					1.0×10^2				
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium							1.0×10^2	2.0×10^2	
Aspergillus									
Yeasts		N@10^2					5.0×10^2	1.2×10^3	
OA		nd			nd			nd	

DATA SUMMARY SHEET FARM: NL4 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	20 August			28 October			29 December		
Harvest+days	3			73			135		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	16.9	18.4	19.2	13.3	16.4	14.1	5.5	10.7	11.2
MC	16.4	15.8	15.0	15.0	15.1	15.0	15.1	15.1	14.8
Cladosporium		5.0×10^3			3.5×10^2			1.0×10^2	
Alternaria		8.0×10^3			6.0×10^2			2.5×10^2	
Fusarium		3.0×10^2							
Acremonium					2.0×10^2			4.0×10^2	
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium		1.0×10^3			1.1×10^3				
Aspergillus									
Bacteria									
Yeasts									
OA		nd			nd			nd	

DATA SUMMARY SHEET FARM: NL5 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	20 August			28 October			29 December		
Harvest+days	10			80			141		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	19.2	20.5	22.1	14.3	13.2	13.4	6.2	9.6	9.6
MC	16.8	16.2	14.0	15.5	15.4	15.2	15.2	14.8	14.4
Cladosporium		1.5×10^4							
Alternaria		6.0×10^3						1.0×10^2	
Fusarium								3.0×10^2	
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium					1.0×10^2				
Aspergillus									
Bacteria									
Yeasts		N@10^3							
OA		nd			nd			nd	

DATA SUMMARY SHEET FARM:NL6 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	19 August			17 September			6 November		
Harvest+days	1			30			80		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	23.6	22.0	17.4	16.6	20.1	19.1	9.7	12.5	11.6
MC	17.5	16.3	16.2	17.3	16.5	15.6	15.9	15.3	15.0
Cladosporium		1.0×10^4			1.5×10^3			6.0×10^2	
Alternaria		3.0×10^3						4.5×10^2	
Fusarium		N@10^2			1.1×10^3			2.0×10^2	
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium				2.5×10^2				2.0×10^2	
Aspergillus									
Bacteria									
Yeasts					3.1×10^3				
OA		nd		nd	nd	nd		nd	

DATA SUMMARY SHEET FARM:NL6A WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	18 September			10 October			6 November		
Harvest+days	4			26			53		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	12.4	13.7	15.8	10.0	11.6	14.1	4.7	3.4	6.6
MC	21.7	22.7	18.7	22.0	19.9	16.7	19.8	17.0	17.5
		no growth							
Cladosporium						3.0×10^2			
Alternaria			7.0×10^2			4.0×10^2	4.0×10^2		
Fusarium	2.5×10^2						1.0×10^2		
Acremonium	3.5×10^2		3.0×10^2		1.0×10^3	1.5×10^3	5.0×10^2		
Verticillium			1.0×10^2			1.5×10^2			
Botrytis									
Epicoccum						1.50×10^2			
Mucor									
Trichoderma					1.0×10^2				
Aureobasidium									
Penicillium				1.0×10^3	5.0×10^2		1.5×10^2	1.5×10^2	
Aspergillus					3.0×10^2				
Bacteria						2.3×10^4		2.5×10^3	
Yeasts									
OA	nd	nd	nd	5.9	1.1	nd	nd	0.2	nd

DATA SUMMARY SHEET FARM:NL6A WHEAT

	Sampling date 4			Sampling date 5			Sampling date 6		
Date	5 December			11 January 1999					
Harvest+days	83			120					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	5.6	5.1	6.9	7.0	8.1	9.0			
MC	18.7	17.5	17.1	17.2	16.4	15.5			
Cladosporium	1.5×10^3		1.6×10^3						
Alternaria	4.0×10^2	3.0×10^2	3.0×10^2	2.0×10^2		3.0×10^2			
Fusarium	3.0×10^2	2.0×10^2	1.0×10^2			1.0×10^2			
Acremonium	4.0×10^2		4.0×10^2	1.5×10^2					
Verticillium		5.0×10^2	3.0×10^2						
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium	6.0×10^3	8.0×10^2	2.0×10^2	8.0×10^2	3.2×10^4	1.5×10^2			
Aspergillus			1.0×10^2						
Bacteria									
Yeasts				7.0×10^2					
OA	nd	nd	nd	nd	nd	nd			

DATA SUMMARY SHEET FARM: K1 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	3 August			1 September			4 January		
Harvest+days	2			32			155		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	17.3	18.6	21.0	20.8	21.5	19.0	10.2	12.6	12.0
MC	18.7	17.2	15.9	14.2	13.4	13.0	15.3	14.0	14.3
Cladosporium	3.0×10^3		1.2×10^4	2.2×10^4					
Alternaria			2.0×10^3						
Fusarium			1.0×10^3	2.0×10^3					
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor				3.0×10^2					
Trichoderma									
Aureobasidium									
Penicillium	2.1×10^4			2.0×10^3					
Aspergillus									
Bacteria									
Yeasts	N@10^2		4.5×10^6	4.3×10^6					
OA	nd			nd					

DATA SUMMARY SHEET FARM: K2 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	14 August			1 September			none		
Harvest+days	2			20					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	23.7	21.2	21.4	19.7	20.9	20.5			
MC	13.1	13.7	13.7	13.4	13.8	13.8	GRAIN SOLD		
Cladosporium		2.9×10^4							
Alternaria		6.0×10^3							
Fusarium		2.0×10^3							
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Yeasts		$N@10^3$							
OA		nd							

DATA SUMMARY SHEET FARM: K4 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	8 August			1 September			none		
Harvest+days	3			27					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	31.1	23.2	22.7	20.6	18.8	15.7			
MC	14.7	14.8	15.2	14.4	14.4	14.3			
Cladosporium		8.0×10^3							
Alternaria		4.0×10^2							
Fusarium		2.0×10^2							
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria									
Yeasts		N@10^2							
OA		nd							

DATA SUMMARY SHEET FARM: H1 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	11 August			9 September					
Harvest+days	5			34					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	18.0	18.0	18.6	19.4	19.6	19.7			
MC	15.6	16.0	15.8	14.0	15.1	13.8	GRAIN SOLD		
Cladosporium		1.8×10^4			1.5×10^2				
Alternaria		2.0×10^3			1.0×10^2				
Fusarium		1.0×10^2			1.0×10^2				
Acremonium					4.0×10^2				
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria					N@x10^1				
Yeasts		N@10^3			9.0×10^2				
OA		nd			nd				

DATA SUMMARY SHEET FARM: H2 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	11 August			9 September					
Harvest+days	4			33					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	20.6	22.5	22.0	18.7	20.0	21.9			
MC	16.1	15.5	14.5	15.8	15.0	13.5	GRAIN SOLD		
Cladosporium		9.0×10^3							
Alternaria		2.0×10^2							
Fusarium		2.0×10^3			7.0×10^2				
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria					6.0×10^5				
Yeasts		2.0×10^5							
OA		nd			nd				

DATA SUMMARY SHEET FARM: M1 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	27 July			4 August			8 November		
Harvest+days	3			11			76		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	18.0	18.0	18.6	15.1	15.5	18.7	12.1	16.6	15.5
MC	15.6	16.0	15.8	15.9	15.6	12.6	13.5	12.9	13.7
Cladosporium		$4.0x10^3$						$2.5\ x10^2$	
Alternaria		$1.0x10^2$			$1.0\ x10^2$			$3.0\ x10^2$	
Fusarium		$2.0x10^2$							
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria								$1.1\ x10^4$	
Yeasts		$1.0x10^5$							
OA		nd			nd				

DATA SUMMARY SHEET FARM: M2 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	27 July			4 August			8 November		
Harvest+days	2			10			100		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	17.6	16.7	17.1	17.7	17.8	15.8	9.9	10.0	11.1
MC	15.8	16.0	15.5	15.1	15.7	17.4	15.0	14.5	14.5
Cladosporium	5.0×10^3		1.4×10^4			7.0×10^3			
Alternaria	2.0×10^2		2.0×10^2			2.0×10^3	1.5×10^2		
Fusarium	1.0×10^2					5.0×10^3			
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria									
Yeasts	N@10^3		N@10^3			N@10^3			1.2×10^2
OA	nd					nd		nd	nd

DATA SUMMARY SHEET FARM: M3 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	20 August			8 November			30 December		
Harvest+days	5			85			137		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	27.7	29.8	21.7	11.1	14.0	12.9	7.7	9.5	9.8
MC	15.0	15.5	15.5	14.8	15.2	15.2	14.4	14.4	14.0
Cladosporium		1.1×10^4			4.0×10^2			1.0×10^2	
Alternaria		4.0×10^3			2.0×10^2				
Fusarium		1.0×10^3			2.0×10^2			2.5×10^2	
Acremonium					1.0×10^2				
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium								1.0×10^2	
Aspergillus									
Bacteria									
Yeasts		N@10^2							
OA					nd				

DATA SUMMARY SHEET FARM: M4 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	13 August			26 August			8 November		
Harvest+days	3			16			90		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	22.9	33.8	33.0	16.6	18.5	16.8	11.1	12.1	10.4
MC	15.0	16.4	16.5	14.9	15.9	16.4	15.1	15.2	15.7
Cladosporium		2.0×10^3			6.0×10^2				
Alternaria		7.0×10^2			4.0×10^2				
Fusarium		3.0×10^2			1.0×10^2			1.0×10^2	
Acremonium								1.5×10^2	
Verticillium									
Botrytis									
Epicoccum					1.0×10^2				
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria					N@10^3			1.0×10^5	
Yeasts		2×10^5							
OA		nd			nd			nd	

DATA SUMMARY SHEET FARM: M4A WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	26 August			18 September			8 November		
Harvest+days	10			33			84		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	15.7	16.4	17.5	13.2	11.0	13.3	12.7	11.5	13.3
MC	17.1	16.4	17.5	17.4	17.6	15.3	16.0	15.5	15.7
							GRAIN SOLD		
Cladosporium	5.0×10^2			1.1×10^3			2.0×10^2	6.0×10^2	1.0×10^2
Alternaria	4.0×10^2			2.0×10^2			2.0×10^2	2.5×10^2	1.0×10^2
Fusarium				1.0×10^2					
Acremonium				3.0×10^2			1.0×10^2		1.0×10^2
Verticillium									
Botrytis				1.0×10^2					
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium				3.0×10^2				1.0×10^2	1.5×10^2
Aspergillus	1.0×10^3								
Bacteria	4.1×10^3			1.0×10^5			2.2×10^4	5.0×10^4	
Yeasts	5.0×10^2								
OA	nd			nd	nd	nd	nd	nd	nd

DATA SUMMARY SHEET FARM: M5 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	3 September			17 September			10 October		
Harvest+days	1			15			41		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	18.1	19.4	17.6	12.4	12.5	14.9	7.5	8.8	13.1
MC	18.1	16.7	16.9	19.1	19.4	16.1	18.8	17.6	16.0
Cladosporium				8.0×10^2	1.0×10^3	2.5×10^2			1.0×10^2
Alternaria	1.0×10^2			4.0×10^2	1.0×10^2			1.0×10^2	2.0×10^2
Fusarium									
Acremonium				1.1×10^3		3.3×10^3	3.0×10^2	3.0×10^2	1.5×10^2
Verticillium				7.0×10^2	2.8×10^3	6.0×10^2			
Botrytis									
Epicoccum					1.5×10^2	1.0×10^2			
Mucor									
Trichoderma									
Aureobasidium									
Penicillium					2.0×10^3	2.5×10^2		3.0×10^2	2.0×10^2
Aspergillus							2.0×10^3		
Bacteria	2.3×10^2			3.0×10^3		3.5×10^5			1.5×10^6
Yeasts									
OA	nd			nd	nd	nd	nd	nd	nd

DATA SUMMARY SHEET FARM: M5 WHEAT

	Sampling date 4			Sampling date 5			Sampling date 6		
Date	19 November			11 January 1999					
Harvest+days	80			133					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	5.8	7.7	7.2	7.8	8.3	7.5			
MC	17.1	16.7	15.4	18.3	17.0	15.6			
								M4 surface	
Cladosporium	8.0×10^2		4.0×10^3			1.0×10^3		1.0×10^2	
Alternaria		1.5×10^2		1.0×10^2	1.0×10^2			1.0×10^2	
Fusarium									
Acremonium	2.0×10^3	1.0×10^2	2.7×10^3	2.0×10^2		1.5×10^3			
Verticillium	7.0×10^2	4.0×10^2							
Botrytis									
Epicoccum	1.0×10^2								
Mucor									
Trichoderma									
Aureobasidium									
Penicillium	5.0×10^3	2.3×10^3		5.0×10^3	4.0×10^2	1.0×10^2		2.9×10^3	
Aspergillus		1.5×10^2			1.0×10^2				
Bacteria	2.6×10^4	1.8×10^4							
Yeasts					7.0×10^2				
OA	nd	0.3	nd	nd	0.1	nd			

DATA SUMMARY SHEET FARM: M5A WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	17 September			10 October			23 November		
Harvest+days	12			38			82		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	12.6	12.4	12.4	6.9	7.5	13.2	5.2	6.5	7.4
MC	18.8	19.3	20.6	18.8	18.7	16.7	20.0	18.8	16.0
Cladosporium				1.0×10^2		5.5×10^2	2.5×10^3	1.3×10^3	4.0×10^2
Alternaria					1.0×10^2	3.5×10^3			
Fusarium								3.0×10^2	2.0×10^2
Acremonium					1.0×10^2	1.2×10^3			
Verticillium				3.5×10^2			2.0×10^3	1.5×10^3	3.0×10^2
Botrytis									
Epicoccum						1.0×10^2			1.0×10^2
Mucor									
Trichoderma									
Aureobasidium									
Penicillium				5.5×10^2	2.0×10^2		1.4×10^3	2.0×10^3	1.5×10^2
Aspergillus				6.0×10^2	5.0×10^2				
Bacteria		8.0×10^3		5.0×10^4	2.5×10^3	3.7×10^3	2.0×10^4	4.5×10^4	
Yeasts									
OA	nd	nd	nd	nd	1.2	nd	nd	nd	nd

DATA SUMMARY SHEET FARM: M5A WHEAT

	Sampling date 4			Sampling date 5			Sampling date 6		
Date	11 January 1999								
Harvest+days	131								
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	5.5	4.5	3.5						
MC	20.3	18.4	16.4						
Cladosporium		1.5×10^2	7.5×10^2						
Alternaria	1.0×10^3								
Fusarium			2.0×10^2						
Acremonium		1.5×10^2	4.0×10^2						
Verticillium			4.0×10^2						
Botrytis									
Epicoccum		1.0×10^2	1.5×10^2						
Mucor	1.0×10^3		1.0×10^2						
Trichoderma									
Aureobasidium									
Penicillium		1.5×10^3							
Aspergillus									
Bacteria									
Yeasts									
OA	0.2	nd	nd						

DATA SUMMARY SHEET FARM: O1 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	29 July			13 August			18 November		
Harvest+days	2			17			109		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	21.3	24.3	21.9	18.2	19.7	21.6	5.3	17.1	15.7
MC	16.0	14.5	13.2	14.1	12.9	12.3	14.2	12.9	12.7
Cladosporium		2.3×10^4		9.0×10^4					
Alternaria				4.0×10^3					
Fusarium		3.0×10^2							
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria									
Yeasts				N@10^3					
OA		nd		nd					

DATA SUMMARY SHEET FARM: O2 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	29 July			13 August			18 November		
Harvest+days	2			17			109		
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	17.6	17.3	19.6	20.6	20.3	20.4	6.6	15.0	16.4
MC	17.0	14.8	14.6	16.3	14.6	13.7	15.0	14.5	14.3
								surface	
Cladosporium	1.0×10^4		3.0×10^3	6.0×10^3			1.5×10^2		
Alternaria	6.0×10^2		2.0×10^3	6.0×10^3					
Fusarium			6.0×10^0	1.0×10^3					
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium								3.5×10^2	
Aspergillus									
Bacteria							1.5×10^3	2.5×10^3	
Yeasts	N@10^3		N@10^3	N@10^2					
OA	nd			nd			nd	nd	

DATA SUMMARY SHEET FARM: W1 BARLEY

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	10 August			25 August					
Harvest+days	3			18					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	20.0	19.7	18.9	16.3	17.7	17.8			
MC	18.3	18.7	19.1	18.7	19.2	19.0		GRAIN SOLD	
Cladosporium		$5.0 x10^3$		$1.4 x10^4$	$1.3 x10^4$				
Alternaria		$7.0 x10^3$		$8.0 x10^3$	$1.0 x10^4$				
Fusarium		$1.0 x10^3$		$2.0 x10^2$					
Acremonium									
Verticillium									
Botrytis									
Epicoccum									
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus								**end sample**	
Yeasts		$N@10^3$		$N@10^3$	$N@10^3$				
OA		nd		nd	nd			0.2	

DATA SUMMARY SHEET FARM: W2 WHEAT

	Sampling date 1			Sampling date 2			Sampling date 3		
Date	10 August			25 August					
Harvest+days	3			18					
Position	top	middle	bottom	top	middle	bottom	top	middle	bottom
Temp	20.8	19.5	23.2	18.3	17.0	17.6			
MC	15.3	16.5	14.6	15.8	16.0	15.0		GRAIN SOLD	
Cladosporium		1.8×10^4							
Alternaria		5.0×10^2			4.0×10^2				
Fusarium									
Acremonium		3.0×10^2			1.0×10^2				
Verticillium									
Botrytis									
Epicoccum					1.0×10^2				
Mucor									
Trichoderma									
Aureobasidium									
Penicillium									
Aspergillus									
Bacteria									
Yeasts		N@10^3							
OA		nd			nd				