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Defining and managing risks to safety and quality during food and feed grain storage

by

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1. ABSTRACT

A key area of importance in protecting the grain supply chain from farm to consumer is good grain storage. Hazards in grain storage include fungi that can produce mycotoxins, and stored product insects and mites. All of these have the potential to reduce quality below acceptable standards for food hygiene. The overall objective of this project was to establish best practice for the UK storage industry, based on a hazard analysis and critical control point (HACCP) approach, to identify and prevent or control the risks associated with grain storage.

Key findings from the project are:

- *Penicillium verrucosum* is found frequently in UK grain stores. Conveyer systems and combine harvesters may harbour high levels of *Penicillium verrucosum.*
- Hygiene measures, in general, reduce the amount of inoculum present.
- *Penicillium verrucosum* is able to develop in the upper grain surface in the winter months, but a broader survey is required to determine the extent of this.
- Resistance to pirimiphos-methyl in *Oryzaephilus surinamensis* and *Acarus siro* populations is widespread. For *O. surinamensis* this may not result in a control failure, but control failure is possible for *A. siro* populations. Maintaining the correct physical conditions in the store is therefore important.
- Population growth models have been produced for two insect species on wheat and barley and three mite species on wheat, barley and oilseed rape. These models are likely to be the best that are currently available.
- The setting of the differential thermostat was the most important element in determining whether cooling was successful and for how many hours the fan had to be run. A 3°C diffstat setting was significantly more successful than a setting of 5°C. The current airflow recommended for cooling, 10 m^3/(h.tonne wet matter), was found to be optimal for cooling.
- Interpretation of insect trap catch is a very difficult area and complex models would be needed to fully elucidate this. However, the findings reinforced the need for monitoring for insect presence both at the surface and just below the surface.
- The ability of insects and mites to vector *P. verrucosum* illustrates the interactions between the various hazards likely to be encountered in UK stores and the importance of monitoring and establishing thresholds for control actions to reduce the risk.

The findings from this project and the HACCP approach developed were used for the production of a new Grain Storage Guide. The new guide, based on an HACCP approach, was launched in October 2011 with 30,000 copies produced. It is available electronically on the HGCA website (www.hgca.com) together with a more detailed description of the HACCP approach.

2. SUMMARY

Whilst much is known about good storage practice there are a number of fundamental areas in which we have insufficient knowledge to properly assess the risk in order to fully protect the supply chain. Best practice for cereal storage is based on the HGCA Grain Storage Guide (second edition), but this is not defined by robust risk assessment, is organised by individual threats and hence does not provide quantitative guidance for good control of hazards. Application of a Hazard Analysis Critical Control Points (HACCP) approach to the grain storage process, together with consultations across the industry, highlighted gaps in knowledge for the assessment of risk at all stages of the grain storage process. The overall objective of this project was to establish best practice for the UK storage industry, based on an HACCP approach, and including evaluation of current and possible future scenarios, to identify and prevent or control the risks associated with grain storage. This was achieved through four key objectives:

- Objective 1: Minimise the risks from mycotoxin-forming storage fungi – the output of this objective defined best practice with regard to storage mycotoxins.
- Objective 2: Minimise the risks from storage arthropods – the output of this objective defined best practice to prevent and control arthropod infestation.
- Objective 3: Define the interaction of presence of ochratoxin A (OTA), presence of arthropods and physical parameters – the output of this objective defined action thresholds for best practice for the combined risk of mycotoxins and arthropods.
- Objective 4: Validate and demonstrate effectiveness of approach – the output from this objective was a scientifically validated guide to best practice for the UK cereal storage industry with clearly identified risks, thresholds and actions.

2.1. Objective 1: To minimise the risks from mycotoxin-forming storage fungi.

The presence of *Penicillium verrucosum* in UK stores was established through sampling of different areas of a store to determine the areas that harbour *P. verrucosum* and could as a result contribute to grain contamination. In addition, a number of grain samples were collected in the field and additional samples taken during transfer to the store to see whether it was possible to determine at what stage in the process contamination of grain by *P. verrucosum* occurred.

Penicillium verrucosum was present in at least one sample in 68% of the stores tested (19 premises in total), with 67% of all samples received containing *P. verrucosum*. Samples were obtained from areas within 1) Floor stores/floors/bins 2) Elevators/conveyors 3) Outlet spouts/tops of conveyors 4) Aeration systems/air filters 5) Others – separators, wall etc. *Penicillium*

verrucosum was detected in all five of the broad categories outlined with the highest levels found in samples taken from store elevators/conveyors.

As part of general storage procedures various hygiene measures are used within the fabric of the building and on equipment prior to grain entering the store. Investigation of how different hygiene methods affect levels of *P. verrucosum* was achieved in two ways, firstly using the controlled conditions of a trial in a flat store in the experimental grain storage facility at The Food and Environment Research Agency (Fera) and secondly through sampling farm and commercial stores pre- and post-cleaning.

The trial carried out in the Fera store showed that the level of *P. verrucosum* in a store can be effectively reduced through sweeping and that levels could be further reduced by the application of a disinfectant (in this case Sorgene 5). The disinfectant used in this trial was not effective when trialled without a sweeping pre-treatment potentially due to organic matter on the store floor inactivating the peroxygen active ingredients.

For the farm and commercial stores, sweeping was the main form of cleaning used at all sites where both pre- and post-cleaning samples were taken. Overall, cleaning gave a reduction in *P. verrucosum* with the count reducing from $8.7x10^4$ cfu/ml pre-clean to $1.5x10^4$ cfu/ml post-clean. However, the *P. verrucosum* count was only reduced on 6 of the 11 sites and was actually found to increase on the other 5 sites. The counts of other storage moulds followed a similar pattern to the counts for *P. verrucosum*, with cleaning the store reducing the levels of a number of other toxin producing species such as *Aspergillus ochraceus, A. niger* and *A. flavus*. The areas which appeared to benefit most from cleaning were floors, elevators and combine harvesters.

Laboratory and farm scale experiments were conducted on the extent to which moisture translocation and uptake of atmospheric moisture during the winter make contributions to the fungal and mycotoxin load. Samples were initially obtained from 20 stores. Second samples were obtained from 13 of these stores and a third sample from 10 stores. Moisture contents increased between the first and third samplings in six out of the 10 stores. The moisture content in the surface layer of grain in some of the stores reached levels that would permit growth of *P. verrucosum*. Due to the different times at which the samples were taken in each of the stores, interpretation and analysis of the results is difficult. A greater number of stores and a more rigorous sampling program would be needed to provide robust evidence, which increases in the moisture content in the surface layer of grain permits growth of *P. verrucosum.* However, the results indicate that the moisture content in the surface layer does reach levels that would permit growth of the fungus, if it is present.

The risk associated with extending the safe storage period by aeration of damp grain held in temporary storage was also assessed. This was a laboratory study, which monitored the levels of *P. verrucosum* and ochratoxin A in grain stored for different periods of time at two different temperatures, 15°C and 30°C and three different moisture contents, 18, 20 and 22%. The temperatures represent those likely to be found in ventilated bulks (15°C) and in newly harvested or heating grain (30°C).

The moisture content of the grain decreased over the duration of the experiment and this was particularly the case for samples held at 30°C. This needs to be considered when interpreting the results. Levels of *P. verrucosum* increased by a factor of $10^2 – 10^3$ above the initial inoculum level in all treatments with grain at 22% m.c. at both 15°C and 30°C. High levels of OTA were found in samples at 30°C, 22% m.c. after 8 days. It is likely that these levels could have risen further if the moisture content of the grain had remained at 22% throughout the experiment. In general, the results show that although *P. verrucosum* grows and may produce OTA at 15°C, this happens more slowly than at 30°C. The results were compared to a previous model for OTA production, which has been validated by drying experiments in HGCA project 3133, where it was concluded that a safety factor of 2.0 was justified. At 30°C, 18, 20 and 22% m.c., the data from the laboratory test is compatible with this model. However, at 15°C, the data show that OTA production occurred at times far shorter than the model predicts. For example, if we examine the result at 15°C, 22% m.c., OTA production occurred in <4 days, whereas the model predicts 52 days. The main difference between the laboratory experiment reported here, and the experiments used to develop the model and the drying experiments in HGCA project 3133, is that the latter two used an airflow, but there was no airflow in the laboratory experiment. This could indicate that periods either pre-drying or during bulk drying when grain is unventilated may pose a greater risk that was previously considered.

2.2. Objective 2: To assess and minimise the risks from the occurrence of arthropods in stored grain

Storage insects and mites invade freshly-harvested grain from residues and harbourages within the grain store where they subsist between harvests. There is anecdotal evidence for an apparent change in pest status of certain species, but there has been no survey of their occurrence in UK stores since 1987–9. A limited information gathering exercise was undertaken to establish the insect and mite species present and their abundance in both empty stores and stored grain bulks. The involvement of partners representing different areas of the grain storage industry ensured that a variety of representative storage and processing premises were used. Primary beetle pests (those that cause serious and damaging infestation) i.e. *Oryzaephilus, Cryptolestes, Sitophilus, Tribolium* and *Rhyzopertha* were found in 18.3% of the samples. Secondary beetle pests (those associated with mould or poor hygiene) i.e. *Ahasverus* and Ptinidae were found in 7.9% of the samples. Ptinids were found in 5.4% of the samples. Psocids were found in 34.6% of the samples.

The number of mites found in traps from a single premise ranged from one mite to in excess of 2500. Representative mites were taken from the traps for identification to species level. The most commonly occurring species was *Acarus siro,* which was present at 13 of the 23 premises. This was followed by *Tyrophagus palmarum* at 12 premises, *Lepidoglyphus destructor* at 8, *Tyrophagus longior* at 7, *T. putrescentiae* at 3, *Acarus gracilis* at 3, *Glycyphagus domesticus* at 2 and *T. similis* at 1 premise.

The resistance status of stored product pests in the UK has not been established for over 10 years, and with changing pesticide use, considerable changes are possible. We report here on the results of resistance testing of field populations of the saw-toothed grain beetle *Oryzaephilus surinamensis* and the flour mite *Acarus siro*. Sixteen populations of *O. surinamensis* were collected from different stores and tested with the discriminating dose of pirimiphos methyl (0.12% pirimiphos methyl in total solvent mixture). The survivors of four of these populations were bred and tested again. All populations, except one, showed resistance/tolerance to the discriminating dose of pirimiphos methyl. For the four populations where the progeny of survivors were tested, there were a greater number of survivors than for the parental population, which confirms resistance. One population was tested on wheat treated with the field dose of pirimiphos methyl. After 7 days, there was 22% survival of adult *O. surinamensis*. After 8 weeks, there was no progeny present, indicating 100% inhibition of the population. This demonstrated that although this population shows a high level of resistance to the discrimination dose, this would not result in a control failure in the field.

Fifteen populations of *Acarus siro* were collected from different stores, and tested with the discriminating dose of pirimiphos methyl (0.11%, equivalent to 15µg/cm^2). The survivors of thirteen of these populations were bred up and then tested again. Ten (66.7%) of the populations collected were classed as resistant and three (20%) as tolerant. Two populations did not have their resistance status confirmed as the percentage kill in the initial population was akin to the percentage kill seen in the control (susceptible) population. One population was tested using the recommended field dose of pirimiphos methyl to determine if the level of resistance could result in a control failure. The percentage inhibition of this population was less than half that of the susceptible laboratory strain. A large proportion of the mites survived the treatment indicating that this level of resistance could result in a control failure.

This limited study has shown that resistant *O. surinamensis* and *A. siro* populations are widespread in the UK. It would be of benefit to examine the resistance status of other insect and mite species and of other active ingredients that are currently approved. In light of the limited number of actives that are currently approved for use in stores, the potential for control failures to chemical treatments and mechanisms that can be used to prevent build up of populations that are multi-resistant requires examination.

Data and models for population growth of two storage insect species and three storage mite species at a range of temperatures and moisture contents on wheat, barley and oilseed were determined. This data is needed to define the potential risks of the presence of storage arthropods.

This study examined population growth of *Oryzaephilus surinamensis* and *Sitophilus granarius* on wheat and barley at eight temperatures (12.5°C, 15°C, 17.5°C, 20°C, 22.5°C, 25°C, 27.5°C and 30°C) and two relative humidities (60% and 70%). Population growth of two species of mites (*Acarus siro* and *Tyrophagus putrescentiae*) on three commodities (wheat, barley and oilseed rape) and at four temperatures (10ºC, 15ºC, 20ºC and 25ºC) and three relative humidities (65%, 75% and 85%) was also examined. Laboratory tests were carried out to generate data, which were then used to construct population growth models. The model of *Tyrophagus putrescentiae* and the insect models for *Oryzaephilus surinamensis* and *Sitophilus granarius* are all probably the best available as they are built from a complete and coherent data set for two or three grains; wheat, barley and oilseed rape.

Current best practice recommends that stores are thoroughly cleaned prior to the introduction of the grain. However, there is no information available on the effectiveness of the hygiene measures that are currently used. A limited study was undertaken by farmers and store keepers employing their usual methods for store cleaning to determine the effect on the presence of insects and mites in the store. The results indicate that primary insects and storage mites can be difficult to eradicate from premises by the hygiene measures as undertaken by storekeepers and farmers. The most effective hygiene measure could not be ascertained from this study due to the small sample size. Ideally, a controlled experiment should be undertaken to examine the effectiveness of the various hygiene measures. It is also important to establish the role of resistance, if any, in the presence of primary insect pests and storage mites after pesticide treatment.

In principle, ultraviolet C (UVC) radiation may provide an effective means of combating pest infestations associated with the structure of a building and may serve as a potential new hygiene measure. The aim of these laboratory experiments was to assess the potential of using UVC against major stored product pests. The effect of UVC on egg hatch and laying was determined for two species of beetle and mite pests. The effect on mycotoxin-producing fungi was also evaluated. The experiments demonstrated that UVC is effective at reducing egg hatch in storage pests and spore germination of *P. verrucosum*. However, the doses required to elicit the responses varied greatly with species. The absence of food during treatment had a significantly greater effect on egg hatch than when food was present, indicating that the food protected the eggs from the effects of the UVC. This demonstrates the limited penetrative ability of UVC through substrates and suggests that the treatment would be less effective if food particles, dust and debris were present and also if pests were present within crack and crevices. Practical applications of UVC within a storage environment may, therefore, lie in the treatment of structural and equipment surfaces, such as conveyor systems, as an additional hygiene measure. The costs and safety implications of using UVC should also be considered.

One of the key methods of preventing and minimising the impacts of insect and mite infestation is the use of high and low volume aeration for drying and cooling respectively. Modifications to the simulation program 'Storedry' were implemented to allow it to run simulations of cooling such that various strategies and options for controlling insect pests could be explored. The model for population growth of *O. surinamensis* developed in this project was incorporated to enable the change in concentration of *O. surinamensis* to be predicted over time at each depth in the bed, based on initial concentration and on the temperature and relative humidity at each layer. Once the insect model had been incorporated and tested, Storedry was used to explore the likely time course of insect concentration under a range of initial conditions and of strategies for control of the cooling air. In Phase 1, the risk posed by insects in a typical cooling situation using historic weather records was explored. In Phase 2, a wide ranging examination of the effect of various parameters and strategies was done, again using historic weather records. In Phase 3, the likely effect of climate change on the efficacy of insect control by cooling with ambient air was explored.

Simulation of cooling showed that the setting of the differential thermostat was the most important element in determining whether cooling was successful and for how many hours the fan had to be run. A 3°C diffstat setting was significantly more successful than a setting of 5°C. The airflow recommended in the HGCA Grain Storage Guide for cooling, 10 m^3/(h.tonne wet matter), was found to be optimal for cooling. Using 75% of this flow reduced cooling success and at 150% of this airflow 35% more energy per tonne was used than at the recommended airflow.

Control of *O. surinamensis* and *Acarus siro* was achieved using any of the cooling treatments simulated. The temperature at which they stopped multiplying was reached quickly enough for the concentration not to rise significantly above the initial value.

Two climate change scenarios and their controls were simulated at one location. Looking at the window 10–40 years ahead and at the 90 percentile change scenario ('very likely'), cooling to end December was successful in only 61% of the 200 years simulated, against 88% of years for the control scenario (baseline historic). Allowing cooling to continue to end February raised the success rate to 95% with a 9% increase in total fan hours being needed compared with control.

With the more severe 50 percentile scenario (‘as likely as not’), cooling success reduced from the control of 92% to only 38% by the end of December deadline. Extending the time to end February raised the success rate to 87% of years simulated and required 13.5% more fan time. In both scenarios, *O. surinamensis* was well controlled both in those years where the target was achieved and where it was not. It has not been tested whether control of other species of pest would be achieved.

2.3. Objective 3: To examine the interaction of mycotoxins, presence of arthropods and physical parameters

In this objective, the ability to accurately determine arthropod population through interpretation of trap catch, the development of accurate in-store moisture content assessment and the ability of arthropods to vector mycotoxin-forming fungi were assessed.

Methods for the detection of insect pests have been developed for use in both empty stores and in grain bulks. Currently, although these methods are more sensitive in detecting insects than the sampling methods used previously, it is not possible to relate the number of insects caught in a trap to the level of infestation. This study used both laboratory and large bin scale trials to determine whether such a model could be produced. The study focussed on two species, *O. surinamensis* and *S. granarius*, using pitfall cone (PC^{TM}) trap catches.

In the laboratory study, buried traps were a better predictor of population size than surface traps for *O. surinamensis*. There was a significant relationship between moisture content and trap catch. More insects were caught in buried traps at 17% m.c. than at 13% m.c. There was no significant relationship between temperature and trap catch. Surface traps were a better predictor of population size than buried traps for *S. granarius*. There was a significant relationship between moisture content and trap catch. More insects were caught in surface traps at 13% m.c. than at 17% m.c. Temperature was a better predictor than moisture content for buried traps but there was no significant relationship between temperature and trap catch.

In the pilot scale trial, a late harvest due to the weather resulted in mean temperatures recorded during the pilot scale study that were lower than the temperatures used in the laboratory. Temperatures in the grain store fell below 0ºC during a very cold period at the beginning of December 2008. Moisture content of the grain at the surface increased from 13% to approximately 15.5%. The total numbers of insects caught in traps decreased with time. There was considerable variation between bins in the numbers of insects caught. For both species, more insects were caught in surface than in buried traps. There was good agreement between laboratory and grain store data for *O. surinamensis* surface trap catches at both moisture contents, but fewer insects than predicted were caught in buried traps. Given that the temperature in the grain store was below 10ºC, fewer insects would have been expected in traps than were caught in the laboratory at 10ºC. More *S. granarius* were caught in surface traps in the grain store than was predicted from the laboratory data. Slightly fewer *S. granarius* were caught in buried traps than was predicted from the laboratory data.

In a second pilot scale trial, carried out with grain temperatures between 15°C and 20°C, there was very little agreement between numbers of insects trapped at each density in the laboratory and in the grain store. In almost every case, more insects were caught in the grain bins than in the laboratory. Slightly more *O. surinamensis* than predicted were caught in both surface and buried traps and slightly more *S. granarius* than predicted were caught in buried traps. Much greater numbers of *S. granarius* than predicted were caught in surface traps. Trap catches were very unevenly distributed across the bins.

In conclusion, from these studies it would appear that trapping cannot be used to give an estimate of population size in large bins of grain. More information about how insect populations move around in grain bulks would be necessary to integrate with this data in order to make such predictions.

The time for which grain can be stored safely is increased by reducing the temperature and moisture content as this reduces the risk of insect and mite development and mycotoxin formation. Monitoring of temperature and moisture content is, therefore, essential for safe storage and marketing of grain and oilseeds. Storecheck RH, an experimental computer controlled grain management system was installed in the Fera grain store by Robydome Ltd. The system was designed to cool the grain in a cost effective manner and to monitor temperature and humidity. The modification of a sensor that could measure humidity was evaluated. Humidity readings from the grain were more variable than expected and moisture contents calculated from probe readings using Integrated Grain Store Manager did not agree with moisture contents determined using the oven method (ISO 712). The probes were, therefore, modified in three different ways in an attempt to improve the humidity readings: 1. a new tip was designed to prevent contamination of the sensor by grain entering the tip; 2. the top of the probes was sealed with silicone to prevent the movement of air; 3. the bottom of the probes was sealed with silicone to prevent the movement of air. Sealed probes settled over the course of 4 days to give steady predictions close to the oven moisture content. This pilot scale trial has established that it is possible to modify existing humidity sensors to reliably predict moisture content of grain. This provides an option for the remote sensing of temperature and moisture content of the grain both in bulks and in bins. This information can be used to provide an early warning of potential risks to the stored grain.

Storage pests are often associated with fungi either because they feed on them or because they exist under similar conditions. They are acknowledged to be potential transmission sources for fungal infection and may, therefore, spread mycotoxin-producing fungi throughout stores. In this project, the natural fungal load carried externally and internally by storage insects and mites was determined. In addition, the ability of storage insects and mites to vector mycotoxin-producing fungi was evaluated. The results of these experiments indicated that not only do pests originating from storage facilities harbour mycotoxin-producing fungi on their external surfaces, but that they are also able to vector these fungi from contaminated to uncontaminated grain. Stored grain pests were found to acquire, retain and transmit micro-organisms within a column of wheat. Therefore, not only are stored grain pests important contaminants in post-harvest commodities affecting quality and value, but they are also potential vectors of micro-organisms and associated toxins, making their presence within a store increasingly important.

2.4. Objective 4: Validation of the integrated approach by using data from quantitative hazard analysis to produce new guidelines on best storage practice.

Based on the knowledge obtained from the previous objectives and existing knowledge, an HACCP approach was defined to determine realistic hazards and effective control measures in the storage process. It was planned that the approach would be validated at various commercial sites and comparisons made with existing practices. Unfortunately, it was not possible to engage sufficient sites for this exercise, and as the robustness of current recommendations had been demonstrated within this project, this comparison was not carried out, with resources focusing on other areas of the project. Data was, however, collected from some participants on current practices used.

Once the HACCP approach had been established, the Grain Storage Guide was revised, new information added and recommendations updated to produce a new guide to best practice for the UK industry. The findings from this project and the HACCP approach described above were used for the production of a new Grain Storage Guide. The new guide, based on an HACCP approach, was launched in October 2011 with 30,000 copies produced. It is available electronically on the HGCA website (www.HGCA.com) together with a more detailed description of the HACCP approach.

2.5. Conclusion and recommendations

This project has involved partners representing different sectors of the grain storage industry and has used a multi-disciplinary approach to establish best practice and provide written recommendations for the industry as a whole. This approach has ensured that the findings are scientifically valid, robust and fit for purpose. The research has examined both current and possible future scenarios. Whilst much has been established during the course of the project there are still some areas where improved knowledge would add to the recommendations that are provided. Some areas for future consideration for research are:

1. Establishing the extent of resistance to currently used pesticides for other insect and mite species
2. Determining the most effective hygiene measure in controlled experiments
3. Determination of the risk of OTA development in unaerated grain
4. Validation of the cooling models for control of insects in large scale trials
5. Development of models of insect movement in grain