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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

3. TECHNICAL DETAIL

3.1. Introduction

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. The main current threat in storage is from ochratoxin A (OTA) produced by *Penicillium verrucosum*. Insects and mites are also hazards and can further create hazards by acting as vectors or causative agents in producing conditions in which fungal growth can occur. All of these have the potential to reduce quality below acceptable standards for food hygiene.

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. Legislation on feed hygiene (EC No. 183/2005) requires the implementation of control procedures based on the principles of Hazard Analysis Critical Control Points (HACCP). This is an analytical tool for the systematic assessment of all steps in a process, which permits development of management strategies to control those process steps that present a risk to the product. Application of an 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 major areas identified by industry requiring extensive research were: how to reduce the risk of formation of mycotoxins, in particular OTA; the limits for arthropod infestation that could be deemed as acceptable; the role of arthropods as vectors in transmitting microorganisms and strategies for deployment of monitors to assess risk and prove due diligence.

The industry must also be in a position to respond to future changes which may be legislative or caused by environmental conditions, such as climate change. For example, consumer pressure for reduction in the use of chemical control agents combined with changes in legislation for pesticide approval could limit the availability of products for control, and may also increase pressure on the development of resistant populations. Alternative control strategies, such as those based on drying and cooling may be affected by climate change or influenced by future increased energy costs.

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: To minimise the risks from mycotoxin-forming storage fungi – the output of this objective defined best practice with regard to storage mycotoxins.
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- 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.

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

The main threat from the storage mycotoxin ochratoxin A (OTA) in the UK is when the grain in store is damp. In the light of EU limits, the growth requirements for OTA have been defined and monitoring instruments and a model of OTA production have been produced. However, the quantity of toxin-producing fungi present is important for their subsequent growth and this has yet to be established in UK grain stores. The effectiveness of measures for reducing this initial inoculum has likewise yet to be established. Cereal growers often find it necessary to manage harvest backlogs by ventilating damp grain at low-volume cooling rates, and the risk in doing this with regard to OTA production is also unknown. Finally, it has not been established whether the uptake of moisture at the grain surface that occurs even in very dry bulks is sufficient to permit mould development and OTA production.

3.2.1. Step 1.1 Origins of inoculum

It has previously been established that *Penicillium verrucosum* (the fungi responsible for ochratoxin A production in UK grain) does not originate in the field. However, it was still not clear at which stage during processing grain was most likely to be contaminated by *P. verrucosum*.

The aim of this section of the project was, therefore, to establish, through sampling of different areas of a store (intake pits, conveyers, floors, bins etc.), areas that harboured *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.

1.1.1 Occurrence of P. verrucosum in the store

Materials and methods

Sampling

Debris/dust samples were collected over a 2-year period (2006 and 2007) from various locations in commercial and farm grain stores. Samples were collected during the period when the store had been emptied and was ready to receive the next harvest's grain. The areas sampled included intake pits, conveyers, floors, bins and air filtration systems. Ideally, 100–200g of debris was collected from each location and sent to the laboratory for analysis of the fungal content.

Mould counts

For each sample, 40 g of debris was weighed directly into a stomacher bag and 360ml of 0.1% buffered peptone water (BPW (Unipath CM509) 1g; distilled water 1L) was added. The sample was soaked in the peptone water for 30 minutes, stomached for 1 minute and then serially diluted to 10^{-4}. For each dilution, two DG18 agar plates (commercial dehydrated formulation (Unipath CM729) 31.5g; glycerol 220mL; chloramphenicol 100mg (2 vials); distilled water 1L) were labelled and 0.1 ml of the appropriate suspension spread plated onto the agar surface; DG18 is a semi-selective agar for the identification of *Penicillium verrucosum* (Figure 1a and b). The DG18 plates were incubated at 25°C for 10 days after which any colonies were identified and counted. Counts were expressed as colony forming units (cfu) per gram of debris.

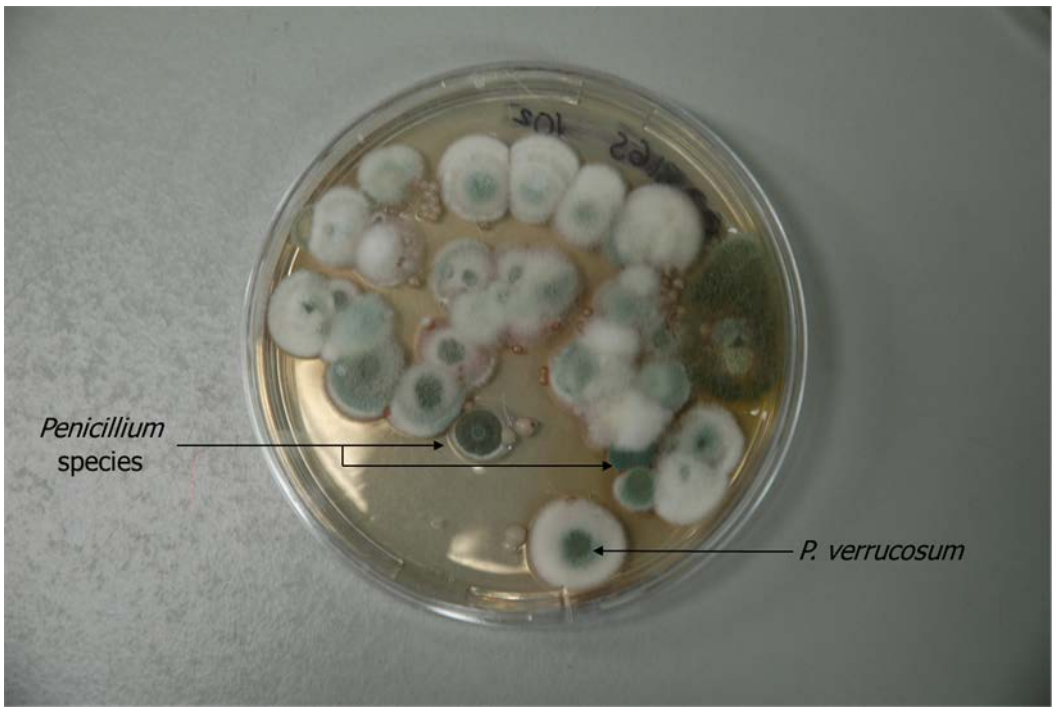
OTA analysis

The amount of OTA present in the sample was determined by HPLC using the method of Scudamore and Macdonald (1998). The sample was extracted with a mixture of acetonitrile:water (60:40, v/v) for 3–5 minutes using an Ultra Turrax blender. The extract was filtered and a portion (5ml) diluted with phosphate buffered saline (PBS). An aliquot of the diluted extract was cleaned up by immunoaffinity column and analysed by HPLC with fluorescence detection.

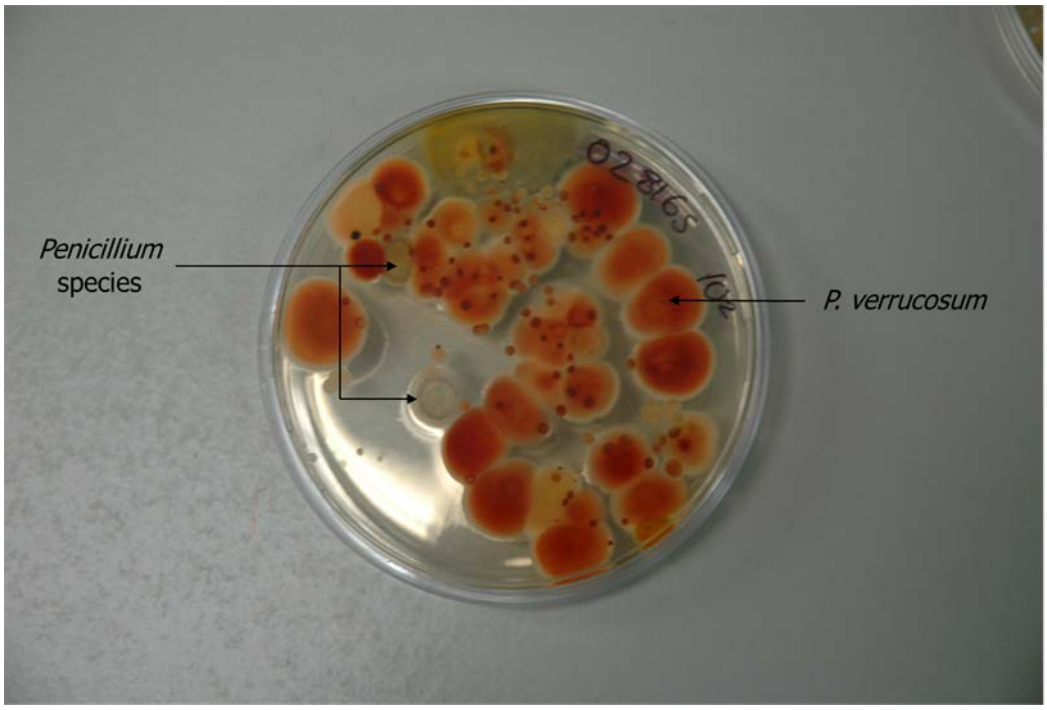
Results and discussion

A total of 46 debris/dust samples were collected from 19 different premises, with locations sampled representing five broad categories:

1) Floor stores/floors/bins
2) Elevators/conveyors
3) Outlet spouts/tops of conveyors
4) Aeration systems/air filters
5) Others – separators, walls etc.



(a)



(b)

Figure 1. Growth of *Penicillium verrucosum* on DG18, a) upper and b) lower surface view

The vast majority of the samples came from categories 1 and 2, with 20 and 14 samples, respectively; 3, 4 and 5 samples were received for categories 3, 4 and 5, respectively. Colony counts, as determined by growth on the semi-selective media DG18 were made (Table 1). Two groups of fungi/mould were identified in nearly all samples. The first group were those commonly found in the field, which were mostly the sooty mould producing species *Cladosporium* and

Alternaria (Figure 2a). The second group was made up of the ‘storage moulds’ and was mostly comprised of *Penicillium* species, *Aspergillus* species (Figure 2b), *Eurotium* species (the sexual stage of a number of *Aspergillus* species, Figure 2c) and *Wallemia* species; species within all these genera are known to produce mycotoxins of one type or another. *Aspergillus flavus*, a known aflatoxin producer, was found at two premises. *Aspergillus ochraceus* was not found in any samples during this study.

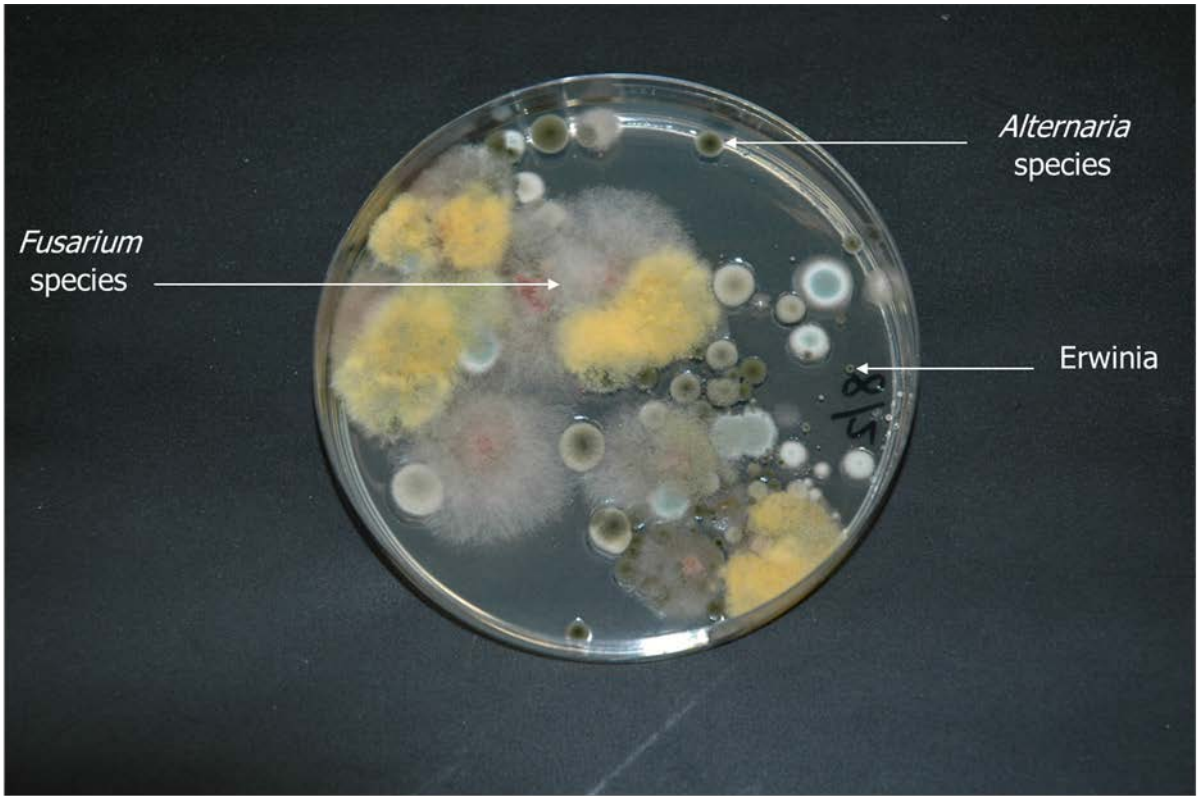


Figure 2a. Growth of field fungi on DG18.

Figure 2b.Growth of *Aspergillus* species on DG18

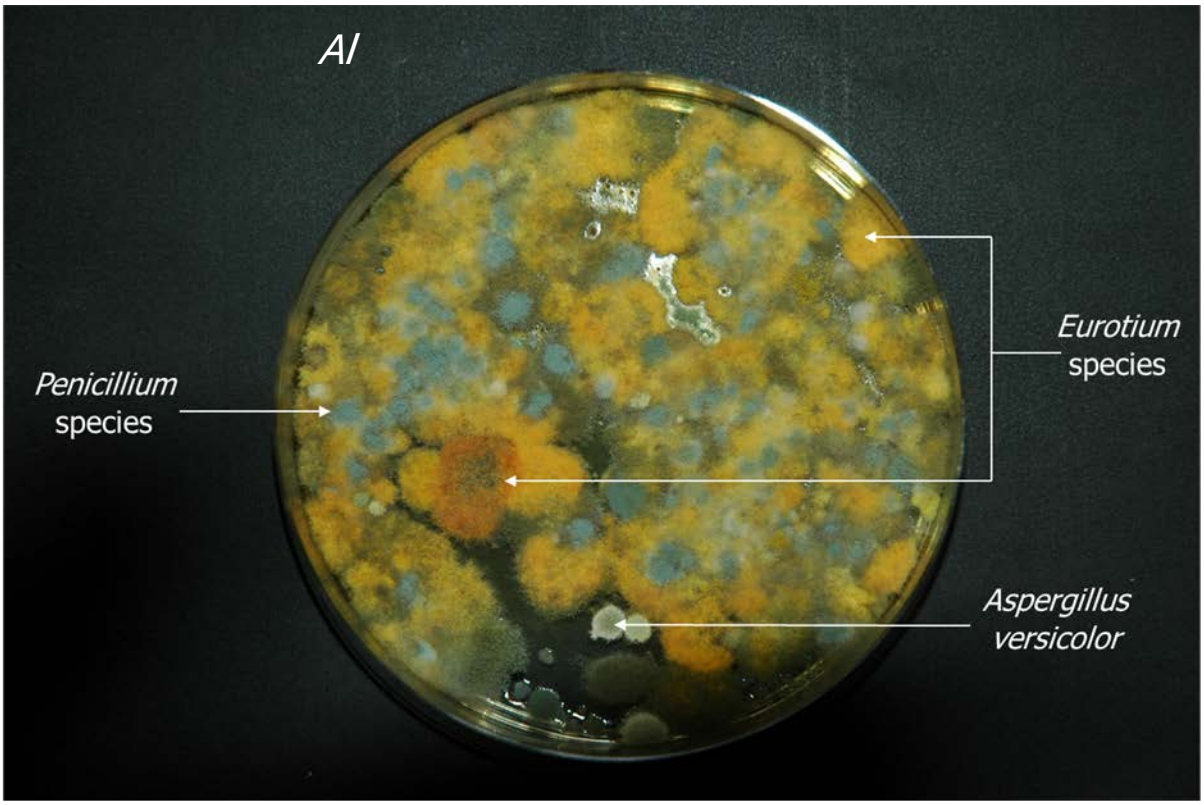


Figure 2c. *Eurotium* species on DG18

Table 1. Colony counts from debris/dust samples taken from commercial and on-farm grain stores during 2006 and 2007

Premises	Sample location	Colony counts on DG18 (colony forming units x 10^3 / g debris)													
		Field Fungi		Storage 'moulds'											
		Cladosporium spp.	*Alternaria* spp.	*Penicillium verrucosum*	Other *Penicillium* spp.	*Eurotium* spp.	*Aspergillus versicolor*	*A. flavus*	*A. candida*	*A. niger*	Other *Aspergillus* spp.	Absidia	Aureobasidium	*Wallemia* spp.	Yeasts
1	Floor	150	0	240	1,320	120	0	0	0	0	0	0	0	60	540
	Inside conveyor	0	0	3	330	6	0	0	0	0	0	0	0	42	321
	Intake pit	4,860	0	210	1,260	330	0	0	0	0	0	0	0	240	4,740
2	OP elevator floor	75	6	21	36	3	0	3	0	0	0	0	60	6	300
	Gravel floor, front	363	33	6	18	9	0	0	0	0	0	0	33	9	366
	Elevator 7	66	21	24	54	9	0	0	0	0	0	0	102	24	480
	Top floor	189	24	3	54	24	0	0	0	0	0	0	18	9	69
	Conveyor (C11/C150)	288	3	6	24	33	0	0	0	0	0	0	30	21	54
3	Shed 1, floor oust (aeration laterals)	0	0	0	120	30	0	0	120	0	0	0	0	0	TNTC
	Elevator E, boot dust	90	0	540	3,660	390	30	0	0	0	0	0	0	300	5,430
	Dust box	30	0	840	3,000	390	0	0	0	0	0	0	0	870	300
	Silo, under aeration laterals	228	0	51	354	48	0	0	0	0	0	0	0	36,600	1,290
4	Elevator booth	6	6	9	75	21	24	15	9	3	0	0	3	42	21
	Elevator booth	30	0	21	279	36	0	0	0	0	0	0	0	144	132
	Silo 4	516	66	6	30	15	0	0	0	3	0	0	0	21	2,100
	Silo 5	1,530	330	60	240	90	0	0	0	0	0	0	0	420	6,900
	Conveyor	30	0	120	270	570	0	0	0	0	0	0	0	0	210
5	Shed 10	63	0	15	105	3	0	0	0	0	96	90	0	2,850	3,480
	Shed 10, end wall conveyor	26,400	450	60	1,410	210	0	0	0	0	0	0	30	90	30
6		17,400	480	0	120	0	0	0	0	0	0	0	30	0	270
7	Wheat bins (top), general area	3	0	0	18	36	0	0	0	0	0	0	3	0	18
	Separator, bay 1	10,200	1,470	60	150	30	0	0	0	0	0	0	0	0	2,100

Table 1 (cont). Colony counts from debris/dust samples taken from commercial and on-farm grain stores during 2006 and 2007

Premises	Sample location	Colony counts on DG18 (colony forming units x 10^3 / g debris)												
		Field Fungi		Storage 'moulds'										
		Cladosporium spp.	*Alternaria* spp.	*Penicillium verrucosum*	Other *Penicillium* spp.	*Eurotium* spp.	*Aspergillus versicolor*	*A. flavus*	*A. candida*	*A. niger*	Other *Aspergillus* spp.	Absidia	Aureobasidium	*Wallemia* spp.	Yeasts
8	Rubble reel, 10th floor silo	336	39	0	12	9	0	0	0	0	0	0	0	3	429
	Intake separator, bay 3	3,840	30	90	210	270	0	0	0	0	0	0	30	60	5,010
9	Silo 14, auger	9	0	24	63	3	0	0	3	0	0	0	0	39	5,790
	Silo 14, floor	30	0	3,360	270	120	0	0	0	0	0	0	0	690	7,800
	Elevator boot	0	0	22,200	22,800	600	0	0	0	0	0	2,100	0	0	78,900
10	Elevator spout	1,440	0	330	2,490	60	0	0	0	0	30	0	0	32,400	15,000
11	Conveyor	15	0	0	228	108	0	0	0	0	0	0	0	0	0
12		10	0	0	270	3	0	0	0	0	0	0	0	3	10
13	Site A	4,762	0	893	19,940	1,190	0	0	0	0	0	0	0	0	26,190
	Site A (2)	1,977	0	56	16,102	565	0	0	0	0	0	0	0	0	26,836
	Shed 2; outlet spout	10,784	0	0	53,268	2,288	0	0	327	0	0	0	0	0	6,209
14	Metal wall	63	15	0	3	2	0	0	0	0	0	0	35	0	8
15	Flat-store 1	0	0	0	3,300	120	0	0	0	0	0	0	0	0	0
	Bin-store1	18	3	0	48	30	0	0	0	0	0	0	0	0	228
16	HF around cleaner	6	6	0	21	63	0	TNTC	0	1	0	0	0	0	0
	HF upper walkway	6	0	0	156	94	0	19	0	13	0	0	0	0	13
17	PF wall edge	30	0	0	1,170	750	0	0	0	0	0	0	0	0	43,500
18	Intake over-flow	240	0	30	5,640	90	0	0	0	0	0	0	30	0	180
	Bin 1 conveyor	360	180	0	1,620	0	0	0	0	0	0	0	0	0	180
	Bin 2 converyor	0	0	0	573	21	0	0	0	0	0	0	0	0	0
19	Flat store	48	3	18	87	9	0	0	0	0	0	0	0	0	30
	Equipment store, shed 2	600	0	1,800	6,600	0	0	0	0	0	0	0	0	0	2,100
	Equipment store, shed 1	3	0	3	87	27	0	0	0	0	0	0	0	0	12
	Combine intake	420	240	3	1,230	0	0	0	30	0	0	0	0	0	300

TNTC = too numerous to count

Of the 19 premises tested, 68% of the premises had *P. verrucosum* present in at least one sample. *Penicillium verrucosum* was present in 67% of the samples received. Where *P. verrucosum* was present, levels ranged from 3 to 22,300 cfu x 10^3/g debris, with the range 10–99 cfu x 10^3/g debris the most commonly found (Figure 3).

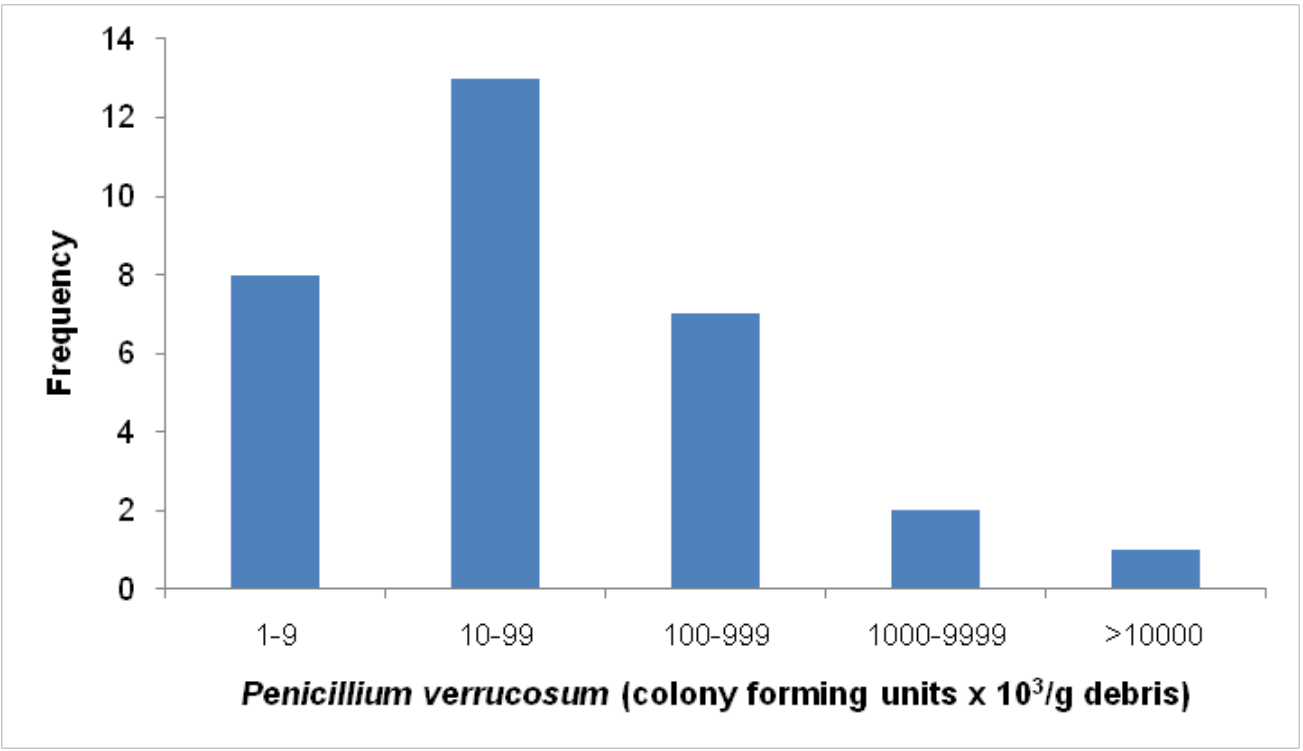


Figure 3. Range of *Penicillium verrucosum* contamination (colony forming units x 10^3/g debris) in debris/dust samples received from grain stores in 2006 and 2007.

Penicillium verrucosum was detected in all five of the broad categories outlined (Figure 4), with the highest levels of *P. verrucosum* found in samples taken from store elevators/conveyors. All samples contained levels above the minimum detectable.

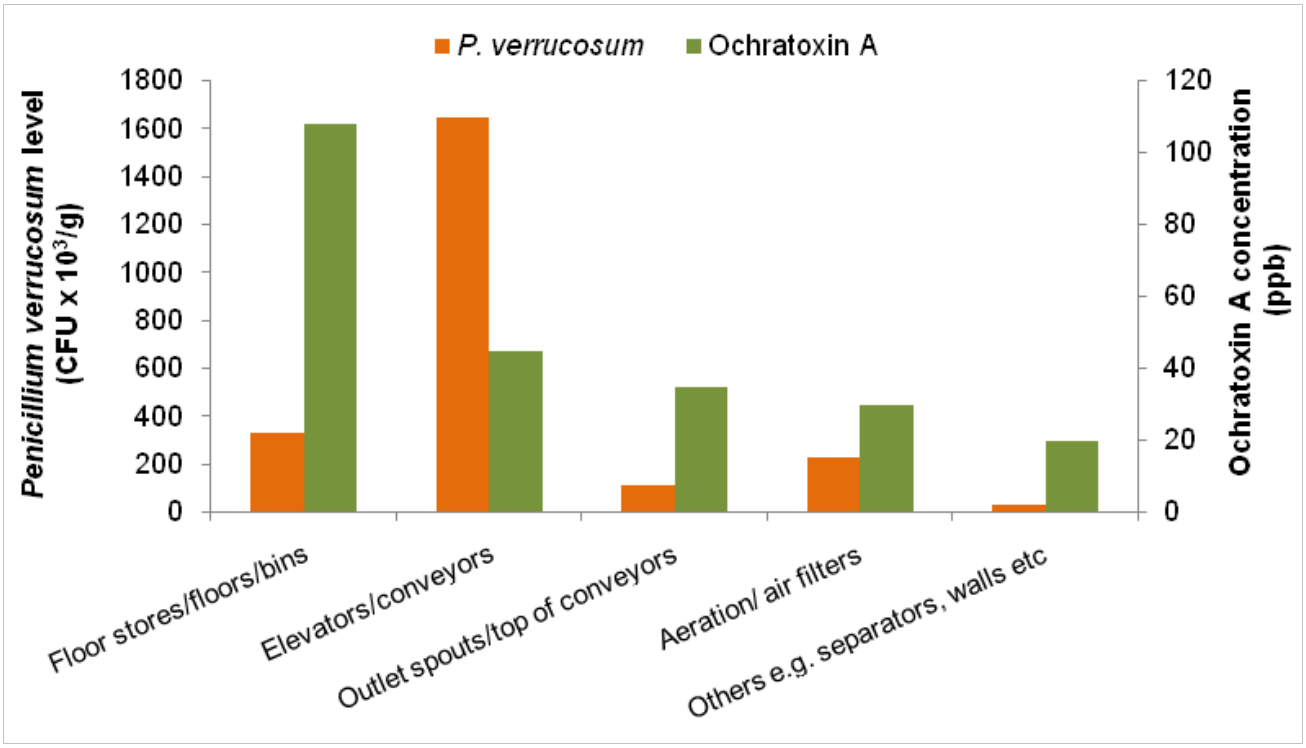


Figure 4. *Penicillium verrucosum* and ochratoxin A levels in dust/debris samples collected from grain stores during 2006 and 2007.

Dust/debris sampling from different locations around a number of grain store premises has indicated that *P. verrucosum* contamination is widespread, with elevator/conveyors being the main areas of contamination. The one sample taken from a combine was positive for the presence of *P. verrucosum*. These results suggest that these areas could be a source of *P. verrucosum* contamination of grain. However, is still not clear what the minimum level of dust/debris contamination is that would subsequently result in levels of ochratoxin A in grain above the legal limit.

1.1.2 Field to store samples

Materials and methods

Samples were collected in both 2007 and 2008. For the field sample approximately 100 whole ears were collected just prior to harvest and sent to Fera for threshing using a Hege single ear thresher. Grain from the same field was then followed into the store and samples taken at various stages in the storage process (i.e. post combine, pre- and post-drying).

On arrival at Fera, grain samples were wetted to give a moisture content of 22% and incubated at 18°C for 10 days. Contamination of the grain sample by *P. verrucosum* was determined using the process described in section 1.1.1. The wetting and incubation stage was used to amplify *P. verrucosum* in a sample to detectable levels.

Results and discussion

In 2007, 24 field samples were taken from the field with follow-up store samples received from 11 of these (1 site provided multiple samples from the store). Unusually, 15 of the 24 field samples, once wetted up and incubated, contained *P. verrucosum*. This would be the first report of *P. verrucosum* in field samples. However, sampling of the Hege single ear thresher indicated that *P. verrucosum* was present within the workings of the machine and the contamination of the field sample could have been introduced at this point.

For the samples arriving in 2008, the threshing machine was thoroughly cleaned before first use. In 2008, 10 field samples were received, with 8 of these also having follow-up storage samples. None of the field samples in 2008 were found to contain *P. verrucosum* (Table 2). Of the samples collected from the store, four of these contained *P. verrucosum*. These were all collected after coming out of the dryer, suggesting that at some point during the drying process or post-drying, but before the sample being taken, contamination of grain was occurring. The moisture content of these grain samples post-drying was at a level where build-up of *P. verrucosum* would not be expected and so ochratoxin A contamination of these samples would also not be expected, provided the grain did not increase in moisture content during storage. Where *P. verrucosum* was found in the post-amplification stage sample, the original pre-amplification stage sample was also analysed for the presence of *P. verrucosum*. In all four instances, *P. verrucosum* was not detected in the pre-amplification sample, suggesting that it was present in these samples at levels below those detectable by the method used here.

Table 2. Colony counts from grain samples taken from field and grain store during 2008

Sample	Collection point	Moisture content (%)		Colony counts (colony forming units x10^3/g grain)									
		Initial	Final	*Penicillium verrucosum*	*Penicillium spp.*	*Wallemia spp.*	*Cladosporium spp.*	*Eurotium spp*	*Yeasts*	*Altenaria spp*	*Fusarium spp*	*Aureobassidium spp*	*Aspergillus candida*
1 (Wheat)	Field	15.2	21.8	0	1,830	0	0	30	0	0	0	0	0
	Post-combine	16.9	21.9	0	498	0	15	0	0	0	0	0	0
	Pre-dryer	18.1	21.7	0	294	210	0	90	0	0	0	0	9
	Post-dryer	15.3	21.7	30	660	0	0	0	0	60	0	0	0
	Post-dryer	14.2	21.8	3	1,110	0	0	0	0	0	0	0	0
2 (Wheat)	Field	23.1	22.9	0	330	0	30	0	0	60	60	0	0
	Store	15.9	21.9	0	1,980	0	0	0	0	0	0	0	0
3 (Wheat)	Field	15.9		0	48	3	3	0	21	6	3	0	0
	Store	17.3	21.3	0	32	0	4	0	1.5	0	1.8	0	0
4 (Wheat)	Field	14.6	21.7	0	507	0	6	0	6	0	0	0	0
	Pre-dryer	18.4	21.9	0	246	39	15	0	0	6	6	0	0
	Post-dryer	13.9	21.9	33	2,070	0	30	0	0	30	0	0	0
5 (Wheat)	Field	19.6		0	3,480	0	0	0	0	0	30	0	0
	Post-combine	24.8	24.3	0	204	0	48	0	48	0	0	0	0
	Post-dryer	13.9	21.8	60	4,080	0	0	60	0	0	0	0	0
6 (Wheat)	Field	17.5		0	243	3	12	0	15	3	3	0	0
	Store	15.6	21.8	0	3,240	0	0	0	0	0	0	0	0
7 (Wheat)	Field	19.6	22.1	0	1,890	0	30	0	0	0	210	0	0
	Store	22.9	22.9	0	1,050	0	30	30	270	0	180	0	0
8 (Barley)	Field	16.1	21.7	0	354	0	3	0	18	6	0	0	0
	Post-combine	16.3	21.9	0	51	6	63	0	60	36	3	6	0
	Post-dryer	15.8	21.9	0	123	0	15	0	27	3	0	3	0
9 (Wheat)	Field	22.7	21.8	0	312	0	33	0	0	3	39	0	0
10 (Wheat)	Field	17.6	21.9	0	2,820	0	0	0	0	0	0	0	0

Lines in red show samples positive for *P. verrucosum*.

3.2.2. Step 1.2 Effectiveness of current hygiene measures in the empty store

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. The studies undertaken for step 1.1 established that *P. verrucosum* can be detected from dust/debris in and around grain stores. The aim of this step was to determine how different hygiene methods affect levels of *P. verrucosum*. This was achieved in two ways, firstly using the controlled conditions of a trial in a flat store in the Food and Environment Research Agency (Fera) experimental grain storage facility, and secondly, through sampling stores pre and post-cleaning.

1.2.1 Fera experimental trial

Materials and methods

***Penicillium verrucosum* inoculum production**

Wheat grain (100g) was adjusted to give a moisture content of 22% and transferred into a 250ml conical flask. The flask was sealed using a non-absorbent cotton wool bung and sterilised by autoclaving at 110°C for 15 min on three consecutive days. The sterile grain were inoculated with 1ml of a *P. verrucosum* conidial suspension containing 10^4 conidia ml^{-1}; the conidia were washed from a 10-day old colony of *P. verrucosum* grown on DG18 at 20°C for 10 days. The inoculated grain was incubated for 7–10 days at 20°C until profuse green sporulation could be seen on the surface of the grain.

Experimental set up

The experiment was established in Fera flat store 1. An area 8.5m x 4m was marked on the store floor and divided into areas 1m x 1m with a 0.5m gap between each area and a fully randomised block design established for five hygiene treatments and a control replicated three times (Figure 5). The treatments consisted of:

- Control (no action)
- Sweep (using dust pan and brush)
- Disinfect (Sorgene 5 @ 1:100, applied at 500 ml/m^2)
- Disinfect (Sorgene 5 @ 1:200, applied at 500 ml/m^2)
- Sweep + Disinfect (Sorgene 5 @ 1:100, applied at 50 ml/m^2)
- Sweep + Disinfect (Sorgene 5 @ 1:200, applied at 50 ml/m^2)

Each treatment and control area was contaminated with 1g of grain heavily infected with *P. verrucosum* 24 hr prior to the pre-treatment sampling. Pre-treatment fungal counts were carried out for each treatment area by wiping a W pattern with a cotton wool swab. Each swab was placed in a sterile 30ml universal and washed thoroughly in 10ml of 0.1% BPW. The BPW was serially diluted to 10^{-4} and 0.1ml plated onto two DG18 agar plates labelled appropriately. Plates were incubated at 25°C for 10 days and colonies were counted with the level of *P. verrucosum* expressed as

colony forming units (cfu) per ml BPW. A post-treatment fungal count was also carried out 24 hrs after treatment.

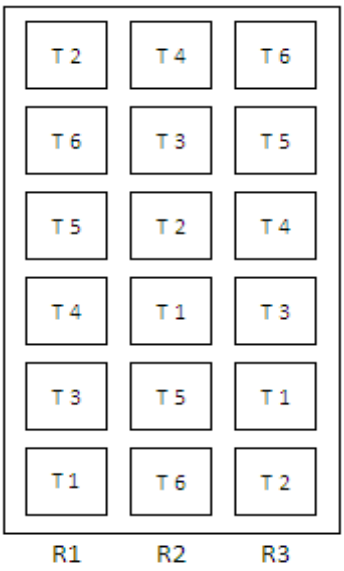


Figure 5. Trial plan for flat store hygiene experiment. T1 = Control, T2 = Sweep, T3 = Sorgene 5 @ 1:100), T4 = Sorgene 5 @ 1:200, T5 = Sweep + Sorgene 5 @ 1:100, T6 = Sweep + Sorgene 5 @ 1:200.

Results and discussion

Consistent levels of *P. verrucosum* were identified across the experimental area (~1x10^7 cfu/ml BPW). Of the hygiene measures tried, the use of Sorgene 5 disinfectant at either dilution rate was the least effective, although there was a dose response seen with the 1:100 dilution giving a greater reduction in *P. verrucosum* levels than 1:200 dilution (Figure 6). Hygiene measures that included sweeping were the most effective, with sweeping alone reducing *P. verrucosum* levels by 98.47%, the addition of the disinfectant reduced levels even further (99.98 and 99.99% for the 1:200 and 1:100 dilution of Sorgene 5 respectively). Again, a disinfectant dose response was seen.

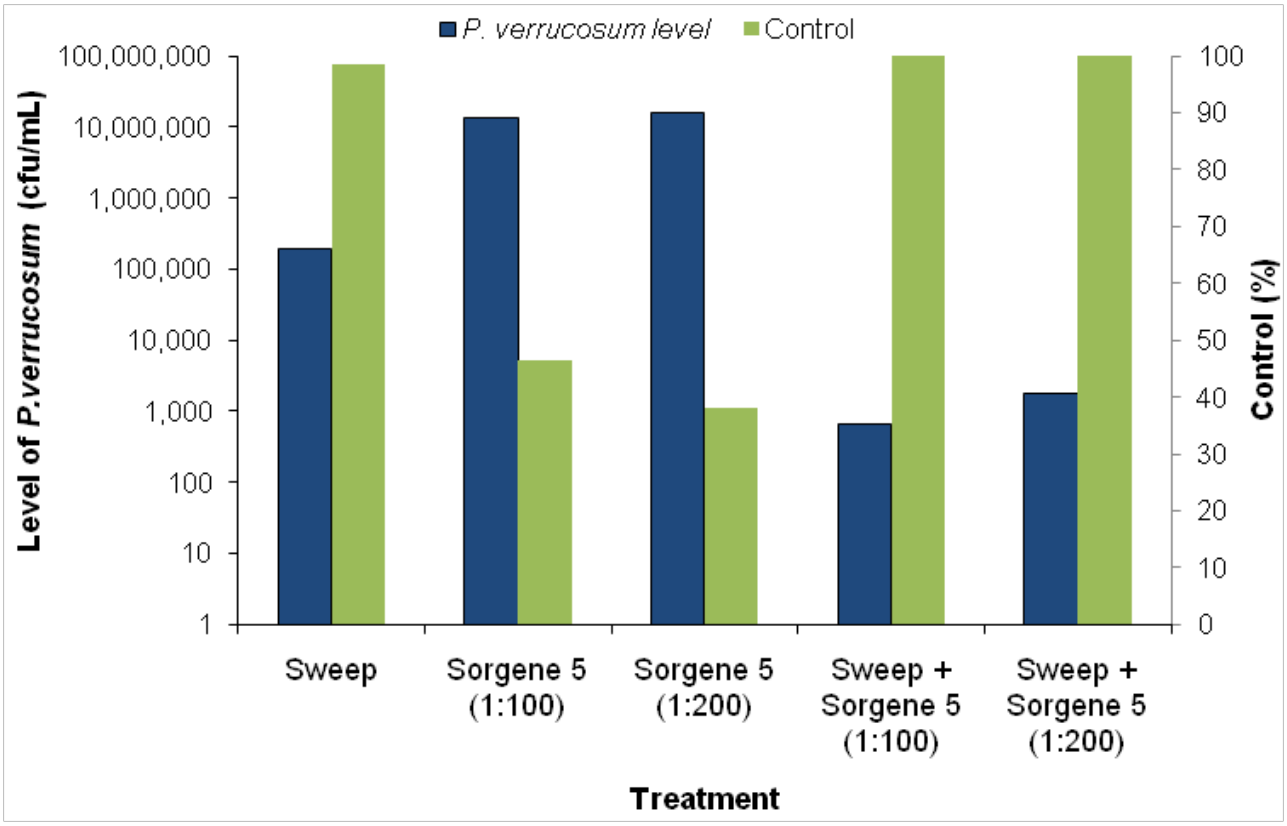


Figure 6. Effect of hygiene measures on *Penicillium verrucosum* levels on in a flat store.

The trial carried out in the Fera flat store has shown 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.

1.2.2 On-farm samples

Materials and methods

Sampling

Cotton wool swabs and pre-labelled 30ml sterile plastic universal tubes were sent to participating premises. Separate sets of swabs/tubes were sent for each area sampled and for the pre- and post-clean sampling. Sampling of the different areas was carried out as follows

1) Floor or bin store/intake pits

The aim was to sample the store in the same five places prior to and after the normal cleaning routine. For the pre-cleaning sample, accumulations of debris/dust were pushed into the universal using the cotton wool swab provided. The sample and swab were then sealed in the universal tube using the screw top. If the accumulations were small the area was wiped with the swab and the swab was placed in the tube and sealed. The tubes were labelled with the sample date and location of the sample.

The post cleaning samples were collected from the same position as the pre-clean sample. For each sample an area approx. 10cm x 10cm was swabbed and the swab sealed into the tube. Again, the date and sampling position were recorded on the tube.

2) Conveyor systems

Areas were sampled where there was a build-up of debris e.g. ends of conveyor runs, the foot of the elevators, the dust collection system or the top surfaces of the conveyor system. For the pre-clean sample, the conveyor was started and run for a short while to dislodge any residues and the debris was collected with a cotton wool swab and plastic tube at an appropriate outlet. Tubes were labelled appropriately. A similar routine was followed when taking the post-cleaning sample

3) Combine harvester

Pre- and post-clean dust or residue samples were collected from hoppers or elevators (if accessible) as described previously.

On arrival at the laboratory, samples were processed as described in section 1.2.1.

Results and discussion

Twenty sites sent samples for testing as part of the hygiene trial. However, only 11 sites sent both pre- and post-cleaning samples (4 sent pre-clean only and 5 sent post-clean only). Sweeping was the main form of cleaning used at all sites where both pre- and post-clean samples were taken. Overall, cleaning gave a reduction in *P. verrucosum* with the count reducing from 8.7×10^4 cfu/ml pre-clean to 1.5×10^4 cfu/ml post-clean. Using the overall count does not tell the whole story, as the *P. verrucosum* count was only reduced on 6 of the 11 sites and was 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 (Table 3).

Conclusion

Overall, the experimental trial and in store sampling both showed that cleaning a store gave benefits in terms of reducing *P. verrucosum* counts and, thus, the level of inoculum present that could potentially contaminate loads of grain arriving at the store. In particular, analysis indicated that the combine harvester was an important source of *P. verrucosum* inoculum.

Table 3. Level of *Penicillium verrucosum* in different areas of the store pre- and post-cleaning.

Area sampled	Number of samples	*P. verrucosum* count (CFUx10^3/mL)	
		Pre-clean	Post-clean
Premises 1			
Floor	5	1.5	0.03
Elevators	2	0.2	0.08
Combine harvester	2	15.0	0
Straw bale dividing wall	1	0	0.2
Premises 2			
Floors/benches	4	0.07	0.04
Intake pit/hopper/bins	8	0.2	0.1
Conveyors/elevators	6	0.02	0.08

3.2.3. Step 1.3 Impact of current storage practices on OTA presence in stored grain

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. The risk associated with extending the safe storage period by aeration of damp grain held in temporary storage was also assessed.

1.3.1 Examining the effects of surface moisture absorption on presence of Penicillium verrucosum.

The aim of this part of the project was to determine whether the increases in surface grain moisture content, that occur during the winter, are sufficient to allow growth of *P. verrucosum*.

Method

Grain samples were obtained from stores identified by industry partners. Once the grain was in store, ten sampling points were identified on the surface of the grain and approximately 100g of the surface layer only (no greater than 5cm depth) was taken from each point. The sampling points were marked so that subsequent samples could be taken from the same spot as the original sample. The ten samples were combined to give a single sample, which was returned to Fera for analysis.

Where possible, the sampling procedure was repeated on another two occasions during the storage period. Ideally, these were in December and January/ February, although the timings were dependant on the length of the storage period. On arrival at Fera, the moisture content and *P. verrucosum* counts of the samples were determined using the methods described previously.

Results and Discussion

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 (Table 4). The moisture content in the surface layer of grain in some of the stores reached levels that would permit growth of *P. verrucosum*. *Penicillium verrucosum* was not found on grain samples at any sampling point in four of the stores (Table 4). Due to the different times at which the samples were taken in each of the stores, interpretation and analysis of the results is difficult. It is likely that where *P. verrucosum* was detected in a sample, but was not detected at the next sampling point. This was due to the sampling methods used, both for collection of the grain sample and for determination of the *P. verrucosum* count.

A greater number of stores and a more rigorous sampling program would be needed to provide robust evidence that 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.

Table 4. Moisture content and presence of *Penicillium verrucosum* in grain samples collected from the surface layer of grain on entering storage and at two further time points whilst in storage.

Store	First sample		Second sample		Third sample	
	M.C.(%)	CFU/g	M.C.(%)	CFU/g	M.C.(%)	CFU/g
1	13.98	0	13.64	120	15.65	0
2	12.82	0	12.71	0	14.36	300
3	15.91	30	16.82	120	15.63	0
4	15.49	0	17.54	0	19.07	0
5	17.16	0	18.06	0	17.46	0
6	15.75	0	15.54	0	13.19	0
7	14.72	0	16.47	0	18.43	0
8	14.77	30	17.26	0	20.24	0
9	16.30	0	17.16	0	17.52	30
10	16.02	0	16.62	300	15.70	2,100

1.3.2 Examining the effect of extension of the safe storage period by aeration of damp grain prior to drying on levels of P. verrucosum and ochratoxin A.

The aim was to investigate the risk associated with extending the safe storage period by aeration of damp grain held in temporary storage prior to drying. This was a laboratory study, which

monitored the levels of *Penicillium 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).

Methods

The moisture content of wheat was adjusted to 18, 20 or 22% m.c., as required. The moisture content was determined using the oven method in compliance with ISO 712 prior to wetting. Grain was then wetted and stored in a room at 10°C for one week. The oven method was used to ensure that the correct moisture content had been achieved. Approximately 10kg of wheat was prepared for each moisture content examined.

Wheat at the appropriate moisture content was inoculated with *P. verrucosum* at two concentration levels (100 and 1000 spores/g grain). In addition, control replicates consisting of untreated wheat were used to determine any background level of *P. verrucosum.* The inoculum was suspended in water and a known volume was applied to the grain. For the control grain, water only was applied. Each replicate consisted of 100g of wheat and three replicate samples were set up for each temperature/moisture content/fungal load/incubation time combination. Additional replicates of the control grain were set up for determination of the moisture content at the two temperatures and each of the four time points.

In the first experiment, grain samples were kept in disposable plastic pots with lids, which had a hole plugged with a non-absorbent cotton wool bung to allow air exchange, at the required temperature (15°C or 30°C) and 70% relative humidity (r.h.). Pots were removed after 1, 2, 4 and 8 days and stored in a freezer at -20°C. The concentration of *P. verrucosum* was determined as described previously. The level of OTA was determined by an ELISA test using a RIDASCREEN®FAST Ochratoxin A kit (R-Biopharm).

To investigate whether airflow affected the growth of *P. verrucosum* grain an additional experiment was conducted. The moisture content of wheat was adjusted to that required (20 or 22%) and inoculated with *P. verrucosum* as described above. The wheat was placed in a 2L aeration vessel and the airflow through the vessel was adjusted to 250ml/min. Wheat was also placed in plastic containers, without an airflow, for comparison. At each sampling point (2, 4 and 8 days), approximately 150g of wheat was removed from the aeration vessel or the plastic container to determine the level of *P. verrucosum* (130g) and the moisture content (20g).

Results

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 (Figure 7). This needs to be considered when interpreting the results.

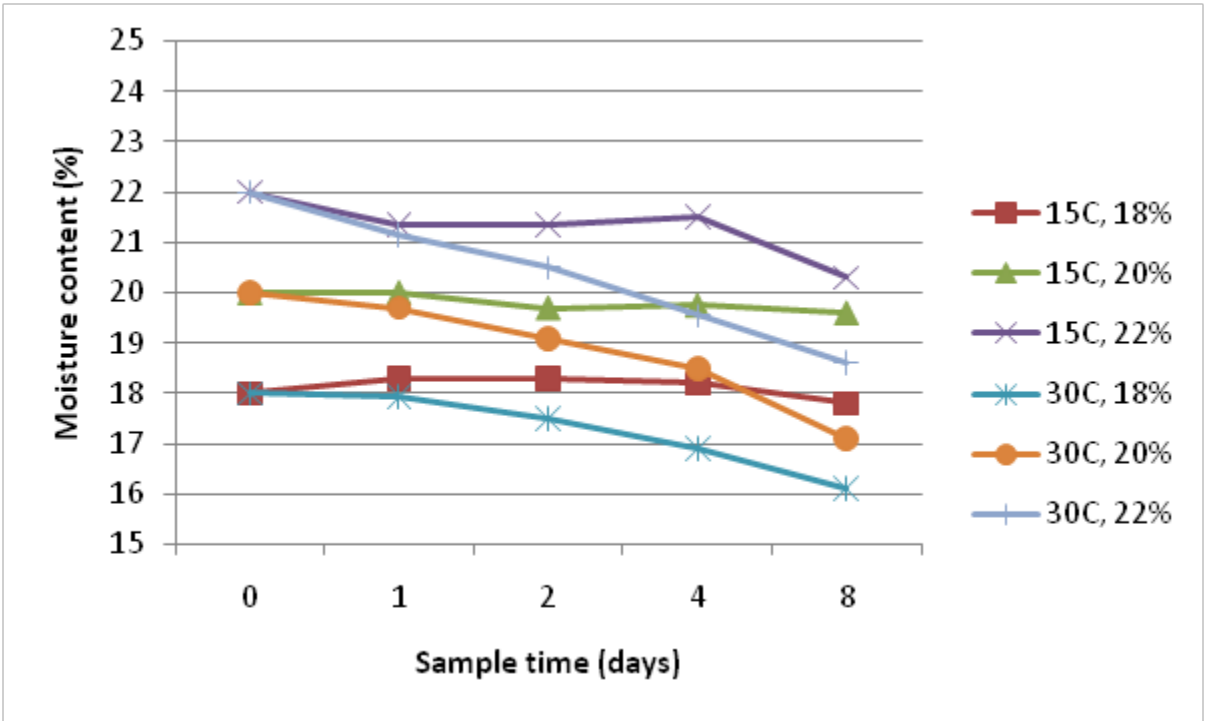


Figure 7. Change in the moisture content of wheat samples held at 15°C or 30°C after 1, 2, 4 and 8 days.

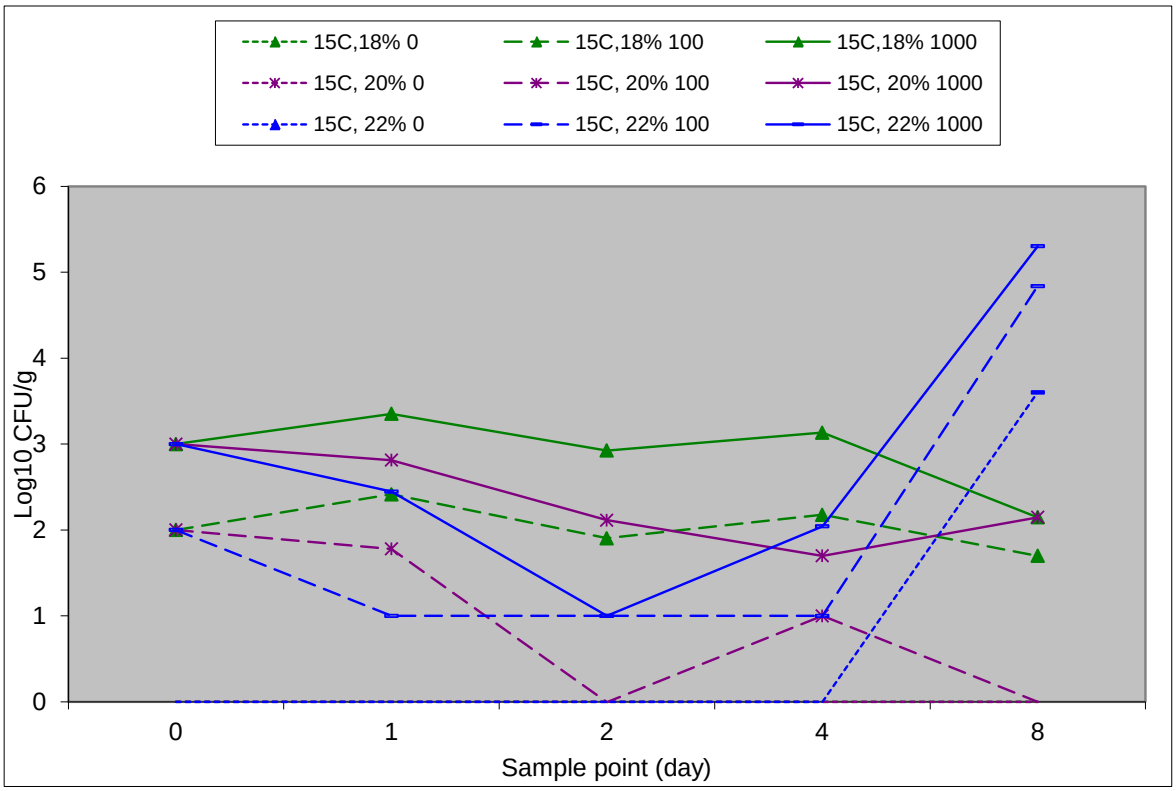
The levels of *P. verrucosum* in the wheat samples taken at 1, 2, 4 and 8 days are shown in Figure 8. *Penicillium verrucosum* was found in the control samples on Day 8 in the 15°C, 22% m.c. treatment and at Day 4 in the 30°C, 18% m.c. treatment. This may have been due to the presence of *P. verrucosum* on the wheat prior to the experiment.

Results showed a wide variation, both within the three replicate plates made for each sample and between the three replicate pots. This may have been due to the sampling method used as only 40 g of grain was used from each pot. The growth phase of *P. verrucosum* at the sampling point may also have affected the ease with which spores could be recovered from the sample and this may have led to no detectable colony forming units at a particular time point, although colony forming units had been detected at the previous sampling point.

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 (Figure 9 b). 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.

(a)



(b)

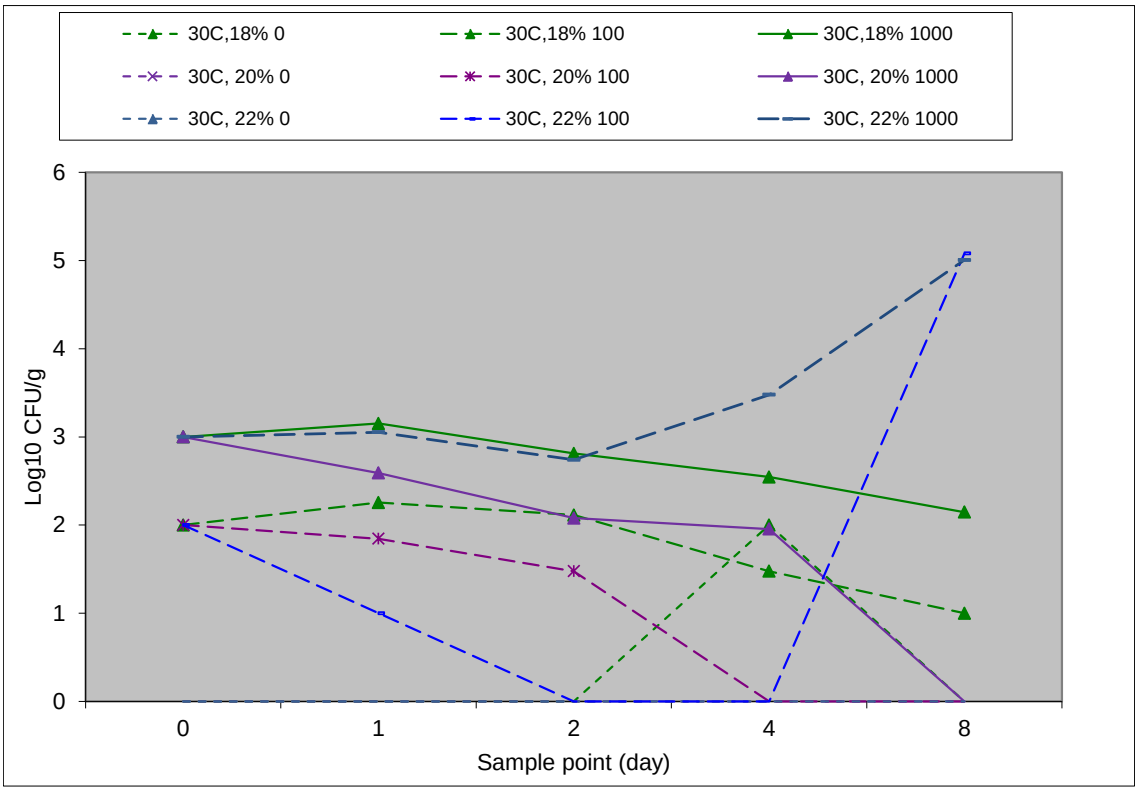
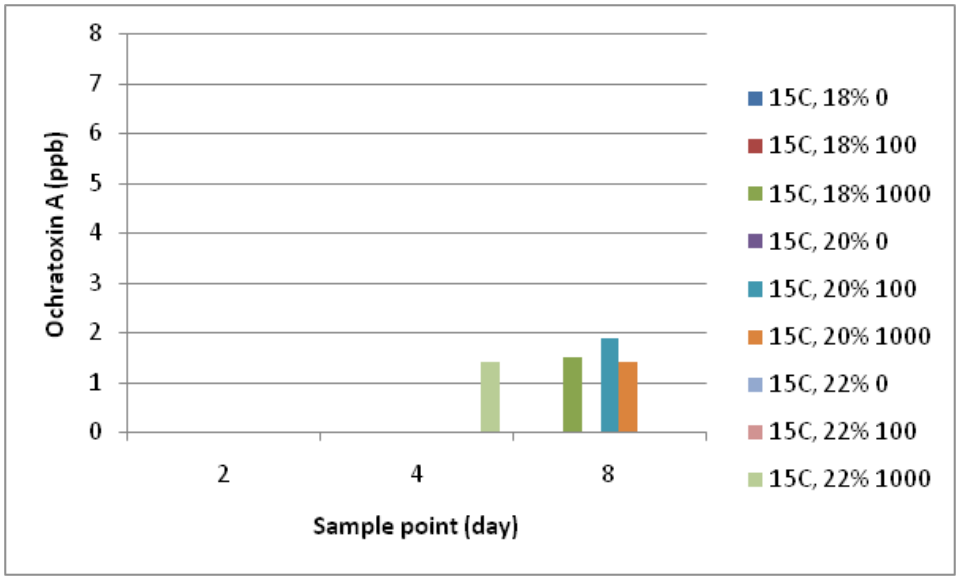


Figure 8. Levels of *P. verrucosum* in wheat samples at three moisture contents at four time points after inoculation with 100 or 1000 spores/g held at 15°C (a) or 30°C (b).

(a)



(b)

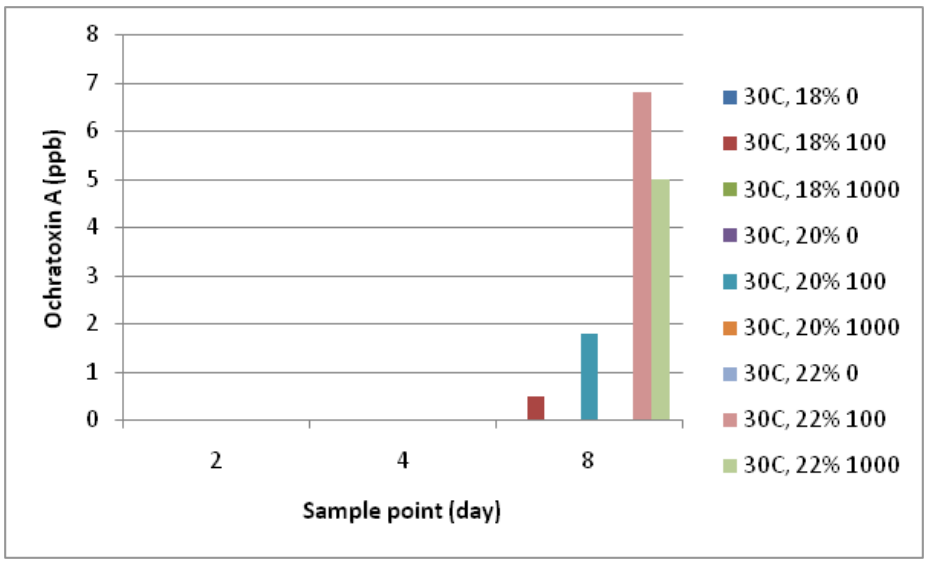


Figure 9. Levels of ochratoxin A in wheat samples at three moisture contents at three time points after inoculation with 100 or 1000 spores/g held at 15°C (a) or 30°C (b).

The results were compared to the model for OTA production produced by Jonsson (Bruce *et al*., 2006), 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% mc, the data from the laboratory test is compatible with the Jonsson model. However, at 15°C, the data show that OTA production occurred at times far shorter than the Jonsson model predicts. For example, if we examine the result at 15°C 22% m.c., which is within the range that Jonsson used to fit his model (10°–25°C, 0.79–0.95 water activity), OTA production occurred in <4 days, whereas the Jonsson model predicts 52 days.

The main difference between the laboratory experiment reported here, the experiments that Jonsson 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.

To examine this further, an additional experiment was undertaken to compare the growth of *P. verrucosum* on wheat at 15°C with and without an airflow. The moisture content of the wheat stored in the plastic containers remained at the required moisture content or increased slightly. The maximum moisture content recorded for non-aerated wheat was 21.4% and 22.8% for wheat at a starting moisture content of 20% and 22%, respectively. The aerated wheat in general lost moisture during the experiment, but the extent of this did differ between the treatments (Table 5) with the minimum moisture content recorded at Day 8.

Table 5. Moisture content of aerated and non-aerated wheat 2, 4 and 8 days after inoculation with 100 spores/g or 1000 spores/g *Penicillium verrucosum*.

20.4% starting moisture content

	Non-aerated			Aerated		
Day	control	100 spores/g	1000 spores/g	control	100 spores/g	1000 spores/g
2	20.9	20.9	20.9	20.9	20.9	20.9
4	21.2	21.3	21.4	20.9	20.5	20.4
8	21.3	21.3	21.3	19.4	20.2	18.8

22.0% starting moisture content

	Non-aerated			Aerated		
Day	control	100 spores/g	1000 spores/g	control	100 spores/g	1000 spores/g
2	22.8	22.8	22.8	21.4	22.0	21.3
4	22.8	22.8	22.8	21.1	22.1	21.6
8	22.8	22.8	22.8	20.9	22.5	22.3

At 20% m.c., *P. verrucosum* was found in only one sample despite the wheat having been inoculated at the start of the experiment (Table 6). At 22% m.c., there were a greater number of CFU/g in non-aerated wheat inoculated with 1000 spores/g compared with aerated wheat, but for wheat inoculated with 100 spores/g the average number of CFU/g found were the same (Table 7).

Table 6. Levels of *P. verrucosum* (CFU/g) in wheat samples at a starting moisture content of 20.4% at three time points after inoculation with 100 or 1000 spores/g held at 15°C.

		***Penicillium verrucosum* (Mean CFU/g)**		
		Day 2	**Day 4**	**Day 8**
Control	Aerated	0	0	0
	Non-aerated	0	0	0
100	Aerated	3,000	0	0
	Non Aerated	0	0	0
1000	Aerated	0	0	0
	Non Aerated	0	0	0

Table 7. Levels of *P. verrucosum* (CFU/g) in wheat samples at a starting moisture content of 22.0% at three time points after inoculation with 100 or 1000 spores/g held at 15°C.

		***Penicillium verrucosum* (Mean CFU/g)**		
		Day 2	**Day 4**	**Day 8**
Control	Aerated	0	0	0
	Non-aerated	0	0	0
100 spores/g	Aerated	0	0	39,000,000
	Non Aerated	2,400,000	0	39,000,000
1000 spores/g	Aerated	0	0	300,000
	Non Aerated	5,400,000	0	1,200,000

Interpretation of the results is difficult due to the high frequency of zero recordings for the presence of *P. verrucosum*. Further research is needed to ascertain why *P. verrucosum* could not be detected in the samples.

3.2.4. Objective 1 Conclusion

- *Penicillium verrucosum* is found frequently in UK grain stores.
- Conveyer systems and combine harvesters may harbour high levels of *P. 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.
- *Penicillium verrucosum* develops rapidly and production of OTA occurs in wheat without an airflow at 15°C. Further research is needed to examine the effect on current predictions of OTA production when there are periods either pre-drying or during bulk drying when grain is unventilated.

3.3. 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. Similarly, the resistance status of the common species has not been established recently. Limited pesticide options could mean that changes in resistance would have a major impact on control strategies. This objective examines the current occurrence and resistance status of storage insect and mite species.

Residual insects invade warm grain after harvest and quickly breed. Mould growth can also occur due to moisture translocation, which occurs as warm air meets cold grain during the natural process of convection. In the presence of storage insects, particularly grain weevils, which can heat up grain by the development of internally-developing stages, mould growth can occur even in previously cooled grain. In this objective, we provide 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, which aids in defining the potential risks of the presence of storage arthropods.

Current best practice is based on an Integrated Pest Management (IPM) system which includes hygiene measures, such as cleaning and pressure washing, to prevent infestation. There is no validation of the effects of these measures and again this information is needed to define risk levels. Alternative hygiene measures, such as the potential of UVC, are explored in this objective. Existing control methodologies may not be realistic for future use due to changes in either legislation or climate. Current models of climate change are used to assess the ability of existing control measures related to cooling to remain effective. These studies reveal vulnerable areas for existing capabilities and form a basis for identifying the requirement for development of alternative control measures in the future.

3.3.1. Step 2.1 Current pest status

2.1.1 Occurrence of stored product insect and mite species in UK stores

Introduction

Information gathering exercises were undertaken in farm stores, commercial stores and animal feed stores throughout the UK between 1985 and 1991 (Prickett, 1992). Since these exercises, there has been no further work to ascertain the current status of arthropod pests although storage practices and control measures have changed considerably. 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.

Methods

The PC trap (Cogan *et al.*, 1991) and PC floor trap (Collins and Chambers, 2003) were used for the detection of insect pests. The traps were used according to current recommendations. PC traps: up to ten pairs of traps were used in each store. For each trap pair, one trap was positioned on the grain surface and the other buried approx. 5–10cm below the surface. Traps were placed 4–5 metres apart. PC floor traps: up to ten traps were used in each store. Traps were placed 4–5 metres apart in corners and at points where the wall and floor meet and under conveyers. Traps were also placed inside empty bins in bin stores. The PC traps and PC Floor traps were checked each week and the contents returned to Fera for analysis. In some cases, participants also provided samples of insects which were found outwith traps.

The BT mite trap (Thind, 2005) was used to monitor stores for mite species. The mite traps were placed in similar positions to, but at least one metre away from, the PC Floor Traps and in areas where high humidity was expected. The traps were left in position for four to six days then returned to Fera for analysis. The number of mites from each of the main genera was recorded and a small number of these mites were identified to species level.

Results

Insects

Samples were received at Fera, from the premises listed in Table 8, between July 2006 and November 2007. The total number of samples received was 240. Of these, 12 were insects found in BT mite traps and 52 did not contain any insects. It was not possible to distinguish between catches of insects from PC Traps and PC Floor Traps because these were not labelled as such when they were returned to Fera. Only one sample received was of unidentifiable origin.

Table 8. Premise types and samples received

Premise type	No. premises	Total no. samples
Grain store (commercial)	5	65
Grain store (farm)	14	94
Maltings and malting grain stores	7	43
Flour and semolina mills and warehouses	7	15
Feed wheat and pet food manufacturers	4	12
Export grain facilities	2	5
Imported wheat and maize	2	3
Soya	1	2

The most common insects found are listed in Tables 9a and 9b for pest and non-pest insect species of stored grain, respectively. In addition, fewer than five individuals of each of the following insects were found. Stored grain pest: *Ptinus tectus*. Non-pests and feeders on decaying material: *Orius* sp. (predatory bug), *Epuraea unicolor* (bark beetle), Hymenoptera, carabid beetles, *Anthicus floralis* (narrow-necked harvest beetle), Parasitoid wasp, *Dermestes ater* larvae (mainly feeds on dried animal material but can feed on dried plant material), sciarid fly (fungus feeder), ant, sphaerocerid fly (dung feeder), *Ectopsocus briggsi* (tree dwelling psocid), Raphidioptera (predatory snake fly), *Glischrochilus hortensis* (sap beetle), *Hofmannophila pseudospretella* (brown house moth – poor hygiene related species), *Anthrenus* sp. (dermestid which mainly feeds on dried animal material), *Anthrenus verbasci* (mainly feeds on dried animal material but can complete its life cycle on stored grain), Woodlouse (Isopoda, Oniscidea), unidentified saprophytic coleopteran, phorid and scatopsid flies (feed on decaying animal and plant material), nitidulid coleopteran (feeds on mouldy plant and animal material), aphids (greenfly), *Anobium punctatum* (woodworm beetle – feeds on the woodwork in the store), leaf beetle (Chrysomelidae), nine unidentified small flies.

Table 9a. Most common stored product insect pest species found

Insect	**Number**
Lepinotus sp. (psocid)	1347
Liposcelis sp. (psocid)	1038
Tribolium confusum (confused flour beetle) – adults + 8 larvae	730
Tribolium castaneum (rust-red flour beetle)	285
Sitophilus granarius (grain weevil)	231
Cryptolestes ferrugineus (rust-red grain beetle)	199
Oryzaephilus surinamensis (saw-toothed grain beetle)	134
Sitophilus oryzae (rice weevil)	79
Rhyzopertha dominica (lesser grain borer)	24
Ptinus fur (white-marked spider beetle)	17
Ahasverus advena (foreign grain beetle) feeds on mould but pest if large numbers	15
Sitophilus zeamais (maize weevil)	8
Ptinus clavipes (brown spider beetle)	6
Ptinus pusillus	5

Table 9b. Most common non-pest species found

Insect	Number
Lachesilla pedicularia (psocid) – lives in straw	580
Limothrips sp. (thrip)	392
Sitona sp. (clover weevil)	80
Collembola (springtails)	44
Cryptophagidae (mould beetles) – presence indicates wet mouldy grain	35
Spider	30
Lathridiidae (plaster beetles) – presence indicates wet mouldy grain	29
Staphylinidae (rove beetles) – predator	18
Ceutorhychus assimilis (seed weevil) – field pest	16
Psychodidae (owl midge) – feeds on wet detritus	12
Drosophila sp. (fruit flies)	9
Sciarid fly (fungus gnat)	5

The most frequently found insects are listed in Tables 10a and 10b for pest and non-pest insect species of stored grain, respectively. 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.

Table 10a. Most frequently found stored product insect pest species

Insect	No. of samples	% samples
Lepinotus sp. (psocid)	49	20.4
Liposcelis sp. (psocid)	19	7.9
Oryzaephilus surinamensis (saw-toothed grain beetle)	15	6.3
Sitophilus granarius (grain weevil)	12	5.0
Tribolium confusum (confused flour beetle) – adults + 8 larvae	13	5.4
Ptinus fur (white-marked spider beetle)	8	3.3
Ahasverus advena (foreign grain beetle) feeds on mould but pest if large numbers	8	3.3
Sitophilus oryzae (rice weevil)	7	2.9
Tribolium castaneum (rust-red flour beetle)	7	2.9
Cryptolestes ferrugineus (rust-red grain beetle)	6	2.5
Ptinus tectus	4	1.7
Sitophilus zeamais (maize weevil)	3	1.3
Ptinus clavipes (brown spider beetle)	3	1.3
Rhyzopertha dominica (lesser grain borer)	3	1.3
Ptinus pusillus	2	0.8

Table 10b. Most frequently found non-pest species

Insect	No. of samples	% samples
Limothrips sp. (thrip)	35	14.6
Spider	25	10.4
Cryptophagidae (mould beetles) – presence indicates wet mouldy grain	21	8.8
Lathridiidae (plaster beetles) – presence indicates wet mouldy grain	19	7.9
Staphylinidae (rove beetles) – predator	17	7.1
Collembola (springtails)	14	5.8
Lachesilla pedicularia (psocid) – lives in straw	9	3.8
Sitona sp. (clover weevil)	8	3.3
Psychodidae (owl midge) – feeds on wet detritus	6	2.5
Ceutorhychus assimilis (seed weevil) – field pest	5	2.1
Sciarid fly (fungus gnat)	4	1.7
Orius sp. (predatory bug)	4	1.7
Carabid beetle (predator)	3	1.3
Epuraea unicolor (bark beetle)	3	1.3
Hymenoptera	3	1.3
Parasitoid wasp	2	0.8
Drosophila sp. (fruit flies)	2	0.8

The most commonly and most frequently found insects in commercial grain stores are listed in Table 11. Since the number of samples per store varied, these are given as average numbers of insects per sample and % of stores in which the insects were found, respectively. Psocids were found most frequently in commercial stores followed by *Sitophilus* species, *Ptinus* species and *O. surinamensis*. *Lepinotus* species psocids were found in the highest numbers in individual stores followed by *O. surinamensis.* Mould feeders (cryptophagids and lathridiids) were found in 40% of the commercial grain stores from which samples were received.

The most commonly and most frequently found insects in farm grain stores are listed in Table 12. Psocids were found most frequently in farm grain stores followed by *O. surinamensis*, *A. advena* and *Ptinus* species. Psocids were found in the highest numbers in individual stores followed by *O. surinamensis.* Mould feeders (cryptophagids and lathridiids) were found in 71% of the farm grain stores from which samples were received.

The most commonly and most frequently found insects in maltings and malting grain stores are listed in Table 13. *Oryzaephilus surinamensis* was found most frequently in maltings and malting grain stores followed by *Liposcelis* species psocids, *Sitophilus* species and *T. castaneum.*

Sitophilus granarius was found in the highest numbers in individual stores followed by *T. castaneum*, *S. oryzae* and *O. surinamensis.* Mould feeders (cryptophagids and lathridiids) were found in 57% of the maltings and malting grain stores from which samples were received. Sciarid and psychodid flies, which prefer a wet environment and the presence of decaying material, were also found in a number of these stores.

The most commonly and most frequently found insects in flour and semolina mills and warehouses are listed in Table 14. *Tribolium* species were found most frequently in flour and semolina mills and warehouses followed by *O. surinamensis* and *Sitophilus* species and *Ptinus* species*. Tribolium confusum* was found in the highest numbers in individual stores followed by *T. castaneum* and *P. clavipes.*

The most commonly and most frequently found insects in pet food mills and feed wheat stores are listed in Table 15. *Tribolium* species were found most frequently in pet food mills and feed wheat stores followed by *O. surinamensis* and *Ptinus* species*. Tribolium confusum* was found in the highest numbers in individual stores followed by *T. castaneum* and *P. clavipes.* Mould feeders (cryptophagids and lathridiids) were found in 50% of the pet food mills and feed wheat stores from which samples were received. Sciarid, psychodid and phorid flies were also found in a number of these stores. Nitidulid beetles were also found in 25% of pet food mills and feed wheat stores suggesting that mouldy material was available to them. *Dermestes. ater* was also found in 25% of stores. These insects feed on proteinaceous material.

Table 11. Most commonly and most frequently found insects on commercial grain stores

Pests				Non-pests			
Most common	Average/sample	Most frequent	% premises	Most common	Average/sample	Most frequent	% premises
Lepinotus sp.	3.28	*Lepinotus* sp.	80	*Limothrips* sp.	1.97	Spider	80
Oryzaephilus surinamensis	0.26	*Liposcelis* sp.	40	Cryptophagidae	0.20	*Limothrips* sp.	60
Ptinus fur	0.06	*Sitophilus granarius*	20	Spider	0.15	Staphylinidae	60
Liposcelis sp.	0.05	*Sitophilus zeamais*	20	Staphylinidae	0.08	Cryptophagidae	40
Sitophilus granarius	0.03	*Sitophilus oryzae*	20	Lathridiidae	0.03	Carabid beetle	20
Sitophilus zeamais	0.02	*Oryzaephilus surinamensis*	20	Psychodidae	0.03	Lathridiidae	20
Sitophilus oryzae	0.02	*Ptinus fur*	20	Carabid beetle	0.02	Sciarid fly	20
Ptinus pusillus	0.02	*Ptinus pusillus*	20	Sciarid fly	0.02	Psychodidae	20

Table 12. Most commonly and most frequently found insects on farm grain stores

Pests				Non-pests			
Most common	Average/sample	Most frequent	% premises	Most common	Average/sample	Most frequent	% premises
Lepinotus sp.	12.06	*Liposcelis* sp.	57	*Limothrips* sp.	2.82	Lathridiidae	50
Liposcelis sp.	6.58	*Lepinotus* sp.	57	*Sitona* sp.	0.86	Cryptophagidae	43
Oryzaephilus surinamensis	0.22	*Oryzaephilus surinamensis*	14	*Ceutorhychus assimilis*	0.17	Staphylinidae	43
Ahasverus advena	0.15	*Ahasverus advena*	14	Lathridiidae	0.16	Collembola	21
Ptinus fur	0.04	*Ptinus fur*	7	Cryptophagidae	0.11	Spider	21
Ptinus clavipes	0.03	*Ptinus clavipes*	7	Collembola	0.11	*Limothrips* sp.	21
				Staphylinidae	0.09	*Orius* sp.	21
				Lachesilla pedicularia	0.04	*Epuraea unicolor*	21
				Orius sp.	0.03	*Lachesilla pedicularia*	21

Table 13. Most commonly and most frequently found insects in maltings and malting grain stores

Pests				Non-pests			
Most common	Average/sample	Most frequent	% premises	Most common	Average/sample	Most frequent	% premises
Sitophilus granarius	5.23	*Oryzaephilus surinamensis*	43	*Drosophila* sp.	0.21	Spider	57
Tribolium castaneum	1.67	*Liposcelis* sp.	29	Lathridiidae	0.09	Lathridiidae	43
Sitophilus oryzae	1.26	*Sitophilus granarius*	29	Spider	0.09	Cryptophagidae	29
Oryzaephilus surinamensis	1.12	*Sitophilus oryzae*	29	Psychodidae	0.07	Staphylinidae	29
Liposcelis sp.	0.30	*Tribolium castaneum*	29	*Anthicus floralis*	0.07	Sciarid fly	14
Lepinotus sp.	0.14	*Lepinotus* sp.	14	Cryptophagidae	0.05	sphaerocerid fly	14
Ptinus tectus	0.09	*Ptinus tectus*	14	Staphylinidae	0.05	Raphidioptera	14
Cryptolestes ferrugineus	0.02	*Cryptolestes ferrugineus*	14	Sciarid fly	0.05	*Glischrochilus hortensis*	14
Ahasverus advena	0.02	*Ahasverus advena*	14	sphaerocerid fly	0.02	*Hofmannophila pseudospretella*	14
Rhyzopertha dominica	0.02	*Rhyzopertha dominica*	14	Raphidioptera	0.02	Psychodidae	14
				Glischrochilus hortensis	0.02	*Anthrenus* sp.	14
				Hofmannophila pseudospretella	0.02	*Drosophila* sp.	14
				Anthrenus sp.	0.02	*Anthicus floralis*	14
				Orius sp.	0.02	*Orius* sp.	14
				Hymenoptera	0.02	Hymenoptera	14

Table 14. Most commonly and most frequently found insects in flour and semolina mills and warehouses

Pests				Non-pests			
Most common	Average/sample	Most frequent	% premises	Most common	Average/sample	Most frequent	% premises
Tribolium confusum	20.20	*Tribolium confusum*	71	*Lachesilla pedicularia*	33.33	*Lachesilla pedicularia*	14
Tribolium castaneum	3.33	*Tribolium castaneum*	29				
Ptinus clavipes	0.20	*Oryzaephilus surinamensis*	14				
Oryzaephilus surinamensis	0.07	*Ptinus fur*	14				
Ptinus fur	0.07	*Ptinus clavipes*	14				

Table 15. Most commonly and most frequently found insects in pet food mills and feed wheat stores

Pests				Non-pests			
Most common	Average/ sample	Most frequent	% premises	Most common	Average/ sample	Most frequent	% premises
Tribolium confusum	8.92	*Lepinotus* sp.	50	*Lachesilla pedicularia*	6.25	Collembola	75
Tribolium castaneum	4.50	*Sitophilus granarius*	50	Collembola	1.67	Spider	75
Liposcelis sp.	0.75	*Ptinus fur*	50	Spider	0.50	*Lachesilla pedicularia*	75
Ptinus fur	0.67	*Cryptolestes ferrugineus*	50	Psychodidae	0.50	Cryptophagidae	50
Cryptolestes ferrugineus	0.58	*Liposcelis* sp.	25	Cryptophagidae	0.25	Staphylinidae	50
Lepinotus sp.	0.42	*Sitophilus zeamais*	25	*Limothrips* sp.	0.17	Lathridiidae	25
Sitophilus granarius	0.33	*Oryzaephilus surinamensis*	25	Staphylinidae	0.17	*Limothrips* sp.	25
Ptinus pusillus	0.33	*Tribolium confusum*	25	*Dermestes ater*	0.17	Psychodidae	25
Sitophilus zeamais	0.08	*Tribolium castaneum*	25	Lathridiidae	0.08	*Dermestes ater*	25
Oryzaephilus surinamensis	0.08	*Ptinus pusillus*	25	Woodlouse	0.08	Woodlouse	25
				Epuraea unicolor	0.08	*Epuraea unicolor*	25
				Sciarid fly	0.08	Sciarid fly	25
				Phorid fly	0.08	Phorid fly	25
				Nitidulid beetle	0.08	Nitidulid beetle	25
				Aphid	0.08	Aphid	25

Mites

Mite traps were received at Fera between July 2006 and July 2007. A total of 198 traps were received from 23 premises/stores. There were 10 farm stores, 5 commercial stores, 5 maltings/malting grain stores, 2 export facilities and one pet food manufacturer represented.

Astigmatic mites were present in 19 of the 23 premises. These belonged to the genera *Acarus, Tyrophagus, Lepidoglyphus and Glycophagus*. Predatory mites were also found at some premises and prostigmatic and mesostigmatic mites were also present. The most frequently occurring mites were *Tyrophagus* spp., followed by *Acarus* spp. and then Glycyphagid mites (Table 16). Some premises had representatives from all three groups. The number of mites found in traps from a single premise ranged from one mite to in excess of 2500.

Table 16. The percentage of stores with astigmatic mites found in BT mite traps

	Acarus spp	*Tyrophagus* spp	Glycyphagid mites
Farm stores*	30	60	30
Commercial stores	40	60	40
Maltings/brewery stores	60	40	40

* In addition, three farm stores had an astigmatic mite species that could not be identified to genera.

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.

Comparison with previous surveys

The methods used in this study for detection of both insects and mites were different to those used in previous exercises; therefore, the results are not directly comparable. There were two main differences: firstly, every site examined was visually inspected during previous exercises, but this was not the case here. Secondly, the insect and mite trapping methods used were different during previous exercises.

The percentage of farm grain stores in which primary beetle pests, i.e. *Oryzaephilus, Cryptolestes* and *Sitophilus* species, were found was 14.3% in the study reported here. This is higher than the percentages of farm stores found to have primary beetle pests in previous studies: 9.7% (Prickett, 1992), 8.5% (Wilson, 1983) and 13% (Anon., 1981). Almost exactly the same percentage of commercial stores were found to have primary beetle pests as were found during the 1988–1989 exercise (Prickett, 1992): 40% of commercial stores in this study had primary beetle pests and 39.5% of commercial stores were found by Prickett (1992) to have primary beetle pests.

Ahasverus advena was found in 14.3% of farm grain stores during this study compared with 6.0% of farm grain stores during the 1987 study. No *Typhaea* were found during the present study.

The presence of another secondary beetle pest, ptinids (spider beetles), was analysed separately by Prickett (1992) due to its high incidence in commercial grain stores. Prickett (1992) found that 49% of commercial grain stores had ptinids compared with less than half this number (20%) found to have ptinids during this study. Fewer farm grain stores than commercial grain stores were again found to have ptinids: 7.1% and 20%, respectively.

The incidence of psocids in farm and commercial grain stores was higher during this study than was found by Prickett (1992): 86% of farm stores and 80% of commercial stores were found to have psocids during this study compared with 51.9% of farm stores and 55% of commercial stores during previous studies.

No primary moth pests were found during this study. In previous studies, primary moths were found at 28.3% of farm stores and 16.6% of commercial stores (Prickett, 1992). This difference may be due to the different trapping methods used for the surveys: previously bait bags and manual inspections of the store were used. These methods would have been more effective for assessing the presence of moths than the more beetle specific trapping methods used for this study.

In previous information gathering exercises for farm and commercial stores, storage mites were found to be widespread with presence detected in 72% of farm stores and 81.3% of commercial stores. This compares with 82.6% of stores found in the current exercise.

In the 1988–1989 study in commercial stores, *Acarus* spp. were found in 59.4% of stores, *Tyrophagus* spp in 25.1% of stores and Glycyphagid mites in 51.9% of stores. The most commonly occurring species was *A. siro* (59% of inspections) followed by *L. destructor* (51.2% of inspections). The most frequently found *Tyrophagus* spp. was *T. longior* (15.9% of inspections) followed by *T. putrescentiae* (12.0%) and *T. palmarum* (1.4% of inspections). It would, therefore, appear that the incidence of *Tyrophagus* spp. in commercial stores has increased, but the small number of samples in the present study and the use of different detection methods in the two studies must be borne in mind.

2.1.2 Resistance status of Oryzaephilus surinamensis and Acarus siro

Introduction

During the last survey of post-harvest insect and mite pests in the UK (1987–9), *O. surinamensis* populations resistant to pirimiphos methyl were found in 27% of farm stores and 82% of commercial stores (Prickett and Muggleton, 1991). Mite populations resistant to pirimiphos methyl were found in 16% of the mite samples collected from farm stores and 64% of the populations collected from commercial stores (Starzewski, 1991). 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*.

Materials & Methods

Insects

Insects were obtained from the traps returned from the stores as described in 2.1.1. Adult *O. surinamensis* were separated and cultured on the standard laboratory diet. Insects were used when they were 1–3 weeks old.

Adult *O. surinamensis* were tested against the established discriminating dose for pirimiphos methyl. The method used for the discriminating dose test was based on FAO method No. 15 (Anon, 1974). The discriminating dose used was 0.12% pirimiphos methyl in total solvent mixture. (0.6% in oil). The laboratory susceptible population was used as a control.

In addition to the filter paper based discriminating dose bioassay, one of the resistant populations (population C) was tested using the recommended field dose of pirimiphos methyl to determine whether the level of resistance could result in a control failure. Pirimiphos methyl was admixed with wheat at a concentration of 4 mg/kg. Fifty grams of treated wheat was placed in 120 ml wide-necked jars and adult *O. surinamensis* were added. The jars were then closed with filter paper lids and left for 7 days, after which time the insects were removed and the number of live and dead adults was determined. The wheat was returned to the jars and after an additional 7 weeks the jars were checked for emergence of progeny.

Mites

The BT Mite Trap was used to collect mites from farm grain stores. Once returned to the laboratory, the trap contents were examined and mites identified. Any adult female mites belonging to the genus *Acarus* were isolated in small cells and allowed to lay eggs. Once eggs had been laid, the adult mites were removed and identified to species. The offspring of any mites found to be *Acarus siro* were combined and bred up in 50ml conical flasks. These populations were then tested

for resistance using the discriminating dose of pirimiphos methyl of 0.11%, equivalent to 15µg/cm^2 (Thind & Muggleton, 1998). In addition, the laboratory strain of *A. siro* (9266/1) was tested as a positive control, as this is considered to be a susceptible population.

The procedure for carrying out the resistance test is described in detail by Thind and Muggleton (1998). Twenty to twenty five adult female mites were placed in each of five replicate cells. The cells were then placed in a desiccator over potassium hydroxide solution to maintain a relative humidity of 80% and were held in an incubator at 20ºC. The mites were exposed to the pesticide treated filter papers for 48 hours, after which time the cells were opened, the number of live and dead mites determined and the mites moved to an untreated recovery cell and fed a little of the standard mite diet of yeast and flour. After a further 24 hours in the recovery cell, the number of live and dead mites was assessed again.

Survivors of the test were bred and re-tested to determine whether the population was resistant or tolerant. If fewer mites were killed in the second test, the population was deemed resistant; if the same or more mites were killed it was deemed tolerant.

In addition to the filter paper based bioassay, one of the resistant populations (population D) was tested using the recommended field dose to see if the level of resistance could result in a control failure. Pirimiphos methyl was admixed with wheat at a concentration of 4 mg/kg. Fifty grams of treated wheat was placed in 120 ml wide-necked jars and a level microspoonful of mite culture added. The jars were then closed with filter paper lids and left for 24 hours, after which time they were opened again and the contents sieved through a 710µm mesh. The number of live mites was determined by placing the seivings in a petri dish over a ‘Solomon’s disc’ (a disc divided into 64 sectors of which 8 are blacked) (Solomon, 1962). The seivings were spread evenly over the petri dish and the numbers of mites in the blacked sections counted. The percentage inhibition of the population was determined by comparing the number of mites present in the treated grain to untreated grain; % inhibition=100-[(Treatment means/Control means)*100].

Results

Insects

Sixteen populations of *O. surinamensis* were collected from different stores and tested with the discriminating dose of pirimiphos methyl. 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 (Table 17). 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.

Table 17. Mean % survival of field collected *Oryzaephilus surinamensis* populations to a discriminating dose of 0.12% pirimiphos methyl in total solvent mixture

Population	% survival at discriminating dose	Resistant or susceptible
A	72	Resistant
B	95	Resistant
C	92	Resistant
D	27	Resistant
E	32	Resistant
F	6	Resistant
G	46	Resistant
H	91	Resistant
I	30	Resistant
J	51	Resistant
K	9	Resistant
L	12	Resistant
M	0	Susceptible
N	17	Resistant
O	12	Resistant
P	12	Resistant

Population C 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.

Mites

Fifteen populations of *Acarus siro* were collected from different stores, and tested with the discriminating dose of pirimiphos methyl. The survivors of thirteen of these populations were bred up and then tested again. The percentage of mites killed for both the initial population and the surviving is given in Table 18.

Table 18. Mean % mortality of field collected *Acarus siro* populations to a discriminating dose of 0.11% pirimiphos methyl and subsequent % mortality of F1 generation bred from survivors.

Population	% mortality Initial Population	% mortality Survivors	Difference in % Kill (+/-)	Resistance or Tolerance
A	85.4	49.3	-36.09	Resistance
B	87.0	55.2	-31.76	Resistance
C	72.1	63.5	-8.59	Resistance
D	44.9	33.8	-11.12	Resistance
E	73.5	43.2	-30.37	Resistance
F	75.7	51.9	-23.81	Resistance
G	33.6	30.2	-3.37	Resistance
H	52.9	37.1	-15.78	Resistance
I	50.0	18.94	-31.06	Resistance
J	98.4	n/a	n/a	Unconfirmed
K	76.2	45.0	-31.2	Resistance
L	93.9	96.0	2.1	Tolerance
M	98.4	n/a	n/a	Unconfirmed
N	94.5	97.7	3.2	Tolerance
O	36.5	40.9	4.4	Tolerance

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.

Population D 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 field population D 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 (Table 19).

Table 19. % inhibition of a laboratory susceptible and a field collected resistant *Acarus siro* population on wheat treated with 4mg/kg pirimiphos methyl.

Population	Treatment/Dose	Mean number of mites	% inhibition
Laboratory strain 9266/1	Control	2555.2	99.97%
	Pirimiphos methyl (4 mg/kg)	0.8	
Field Population D	Control	1265.6	39.32%
	Pirimiphos methyl (4 mg/kg)	768.0	

Conclusion

From this limited study, it would appear that the incidence of resistance to pirimiphos methyl in *O. surinamensis* populations has increased, with only one of the populations tested being susceptible to the discriminating dose. However, this does not necessarily mean that this will result in a control failure for this species. As shown in this study, one of the populations showing high survival to the discriminating dose failed to survive and breed on wheat treated with the recommended concentration of pirimiphos methyl, and thus, even though the population is resistant, with correct application, a control failure should not occur.

In the current study, all mite populations tested survived the discriminating dose, with 66.7% of the populations confirmed as resistant. Comparison with previous studies is more difficult for this species as different test methods were used. The present study used a discriminating dose of 0.11% pirimiphos methyl, whereas the previous study used wheat treated with 8 mg/kg pirimiphos methyl to determine resistance (Starzewski, 1991). Previously, based on exposure to 8 mg/kg treated wheat, 71% of the *A. siro* populations tested from commercial stores were classed as resistant to pirimiphos method (Starzewski. 1991).

A resistant mite population was able to survive the recommended concentration for wheat treated with pirimiphos methyl (4 mg/kg). Therefore, with the widespread presence of resistant populations in stores, control failures when using this insecticide are possible. However, it should be noted that for mite pests, careful control of the physical conditions in the store, particularly the grain moisture content, will be effective in preventing the development of mite populations.

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 to 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.

3.3.2. Step 2.2 Population growth under current storage practices

Detailed knowledge of the biological limits of insect and mite pests over at least the range of temperatures and moisture contents likely to be experienced in stored grain is essential to predict the potential risk of infestation if the recommended store management conditions cannot be attained.

A review of the literature for two of the most frequently occurring insect pests of UK grain stores, *Oryzaephilus surinamensis* and *Sitophilus granarius* was conducted in order to identify deficiencies

in the published data. The review concluded that the published demographic data for both *S. granarius* and *O. surinamensis* is incomplete and often inconsistent. In particular, data for the duration of the oviposition period and the rate of egg deposition is often lacking under controlled conditions. Existing population models for these species are largely based on the estimates given by Howe (1965). In turn, these estimates are based on the earlier work reported by Eastham & McCully (1943) and Eastham & Segrove (1947) for *S.granarius*. Estimates for *O.surinamensis* are based on data collected by Howe (1965), but on artificial or composite substrates that optimise population growth. They are of little direct relevance to the stored grain environment. Even the more recent studies have employed deliberately damaged or kibbled grain (Jacob & Fleming, 1989, 1990; Beckett and Evans, 1994) and are of limited practical value. Furthermore, the significance of acclimation to conditions at the lower temperature margins has often been disregarded. It was concluded that, despite being common pests, there is a paucity of detailed, relevant information for both *S. granarius* and *O. surinamensis* even on wheat. Information for barley is even more limited, being restricted to a comparison of grain weevil development at an optimal temperature. Similarly for storage mites, although studies on population growth have been published, these were conducted on artificial diets and data for population growth on the cereals and oilseeds stored is lacking.

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.

Population growth of Oryzaephilus surinamensis and Sitophilus granarius

Materials & Methods

Sitophilus granarius (Witham) and *O. surinamensis* (Hartley) were used. These were recently collected field strains. Insects were initially bred at 25°C, 70% r.h. to increase numbers, but were subsequently maintained at 20°C, 70% r.h. on either wheat or barley for at least a further generation prior to experimental use. *Sitophilus granarius* was kept on whole grains, but *O. surinamensis* was bred on coarsely kibbled grain.

Wheat (Hereward) and barley (Riviera) were used. These were tested and determined as pesticide free prior to use. To ensure homogeneity of the grain, debris and dockage was removed prior to use using a Rationel Kornservice, SLN 3 sample grain cleaner using screens recommended by the

manufacturer and in compliance with EU guidelines. Cleaned grain was placed in a domestic freezer (~ -18°C) for 3 weeks to destroy any possible insect and mite contamination prior to use.

Cereals were tempered in constant environment rooms at the required conditions prior to use. Where the specific conditions required were not available, grain was held in desiccators over potassium hydroxide solutions of appropriate specific gravity to achieve the required relative humidity. The moisture content of the cereals was determined using the standard oven method in compliance with ISO 712.

Insects were allowed to develop in cultures at 20°C, 70% r.h. until progeny started to emerge, at which point all adults were removed. All progeny that emerged subsequently were removed at regular intervals (2–3 days), and held for a further period to ensure mating. The adults were then sexed and arranged in to 10 cohorts composed of 20 insects, of uniform sex ratio, and acclimated to the appropriate experimental conditions. If the difference in temperature at which the insects were to be held was greater than 5°C for a lower target temperature, an interim temperature step was used. Acclimation to higher temperatures does not appear to be as critical. It is not known whether acclimation to different humidities is beneficial, but transfer to a lower humidity on food results in a gradual change in equilibrium similar to acclimation.

The experimental insects were placed in 120ml glass jars, containing 50g of prepared grain and sealed with filter paper and bolting nylon (20µm aperture), held in place by a perforated screw-top lid. It has previously been observed that approximately 5%, by weight, of feed grain sampled from a bulk of wheat may be damaged. To simulate this, a similar proportion of coarsely kibbled, tempered grains (dockage) was added to experimental samples.

The jars were held at the appropriate conditions for two or three weeks, depending on the temperature, after which time, all adults were removed from the jars. The number and sex of any dead adults was recorded and the remaining insects were transferred to a new jar. This continued until all of the parental female insects had died.

Jars were returned to the experimental conditions and examined weekly until the emergence of the F_1 generation was observed. Upon emergence, the progeny were removed from the jar and the number of individuals was noted. This continued on a weekly basis until no further progeny emerged.

Population growth of Acarus siro and Tyrophagus putrescentiae

Materials & Methods

Field strains of *Acarus siro* and *Tyrophagus putrescentiae* were used. Adult female mites of each genus were isolated in small cells and allowed to lay eggs. The adults were then removed and identified to species. Offspring of those adults identified to be the correct species were then combined and bred up on the standard mite diet of yeast and flour. Once established on this diet the populations were gradually introduced onto the commodities for the test (90% whole grain and 10% kibbled).

Prior to setting up the experiments, the wheat, barley and oilseed rape (Expert) were conditioned to the required relative humidity by placing the grains in a thin layer inside a large, sealed nylon mesh bag. The bags were then placed in controlled environment rooms maintaining the required relative humidity, and left for a minimum of 10 days. Once conditioned, the grains were thoroughly mixed and the moisture content was checked. In addition, the mite populations were conditioned to the required temperatures for one week.

Sample tubes were created by bonding nylon mesh to 10 cm lengths of PVCu plumbing waste pipe. Approximately one hundred mites (larvae, nymphs and adults) were placed in each tube with 50g of wheat, barley or oilseed rape (90% whole, 10% kibbled). The tubes were then covered with nylon mesh held firmly in place with elastic bands and tightly sealed with electrical tape to prevent any escapes. The tubes were numbered and placed in large circular plastic boxes over solutions of potassium hydroxide to maintain the required humidity. The plastic boxes were then placed in either controlled environment rooms or in incubators maintaining the required temperature. Thirty replicates were set up for each species on each commodity and for each combination of temperature and RH. This allowed six replicates to be removed every 21 days at 1, 22, 43, 64 and 85 days. A random number generator was used to determine which tubes to remove at each sampling point.

On removal from the plastic boxes, the tubes were placed in labelled, sealable polythene bags and frozen for a minimum of 24 hours prior to analysis. Analysis was carried out by placing the contents of each tube into a beaker and rinsing the nylon mesh lid and the tube itself with water. Industrial methylated spirits was then added to make a 50% aqueous alcohol solution. The beakers were then placed in an ultrasonic bath for 10 minutes to aid removal of the mites from the kernels. The samples were analysed using the modified flotation method described by Thind (2000). The filter papers were then examined under a binocular microscope and the number of mites counted. For *A. siro* on wheat, the mites were differentiated by life stage (larvae, nymphs and adults). For all other tests only a total mite count was done. Where large numbers of mites were present in the sample, it was necessary to dilute the samples. This was done by suspending the mites in a known

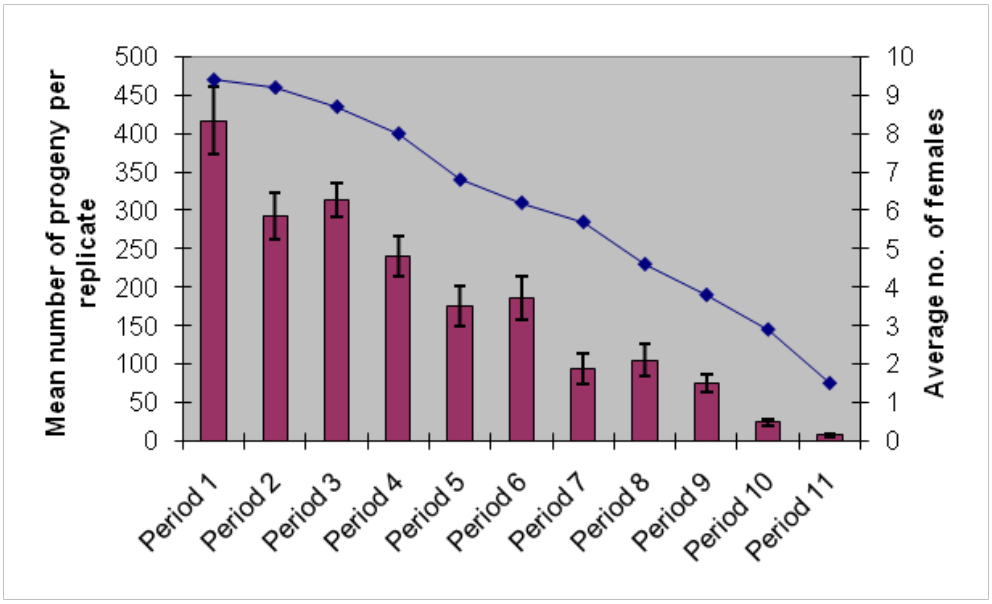
volume of glycerol and alcohol (46:54 v/v) in a measuring cylinder. The cylinder was sealed with Parafilm and inverted 20 times, distributing the mites evenly within the liquid. A known volume aliquot was then taken and placed on a filter paper and counted as before. The number of mites in the original sample was then estimated by multiplying the number in the aliquot by the dilution factor.

Results

Insects

Sitophilus granarius did not develop at 12.5°C. *Oryzaephilus surinamensis* did not develop at 17.5°C. At all other temperatures tested (15°, 17.5°, 20°, 22.5°, 25°, 27.5° and 30°C for *S. granarius* and 20°, 22.5° and 25°C for *O. surinamensis*) progeny were produced. As expected, progeny developed more rapidly at the higher temperatures. However, at the higher temperatures, female insects died more quickly. Productivity was generally greater at 70% r.h. than at 60% r.h. Typical results for wheat and barley for *S. granarius* are shown in Figure 10 for conditions of 25°C, 70% r.h.

A. Wheat



B. Barley

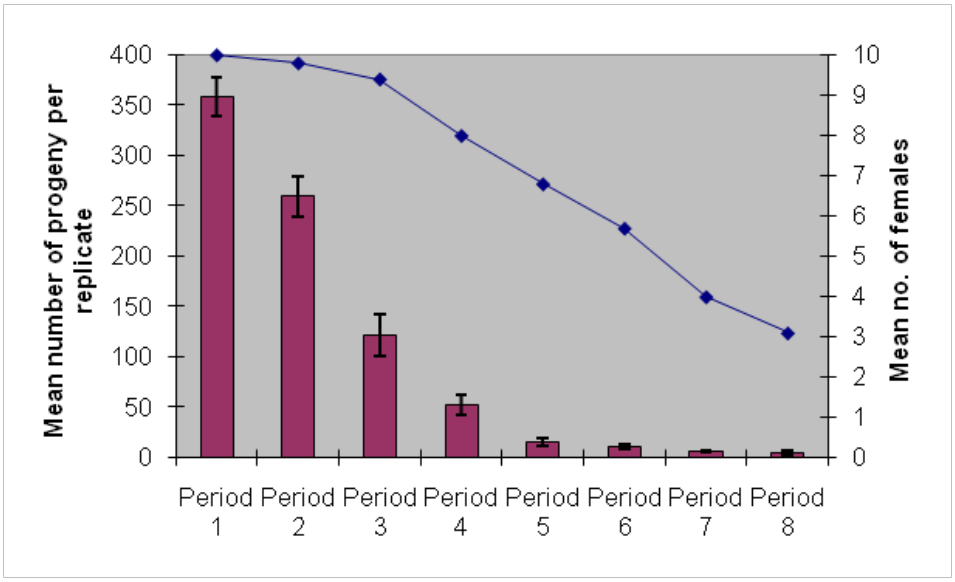


Figure 10. Example of data collected for population study for *S. granarius* on wheat and barley at 25°C, 70% r.h.

Mites

In general, the optimal temperature for development for both species was between 20ºC and 25ºC with a relative humidity in the range of 75–85% (Table 20). It was noted that at the lower humidity the moisture content supports the mites, but with little population growth. As the humidity increased, the population also increased, regardless of the type of commodity. Population growth was also directly related to temperature; as the temperature increased, the population growth was greater. An example of typical growth curves is shown in Figure 11 for *A. siro* on wheat. Uptake of water vapour by astigmatic mites cannot occur below 71% and so longevity is indirectly proportional to the saturation deficit of the air (Boczek, 1991). Longevity depends upon temperature, relative humidity, food and reproductive activity. Population explosions are associated with a congruence of favourable environmental factors (especially temperature, humidity and fungal growth).

All data for insects and mites was provided to Drs Holt and Knight at Imperial College for production of the growth models.

Table 20. Optimal and least favourable conditions for mite population growth based on increase/decrease in total mite numbers from Day 1 to Day 85.

Species	Commodity	Optimum conditions	Least favourable conditions
Acarus siro	Wheat	20ºC & 85% RH	10ºC & 65%RH
	Barley	15ºC & 85% RH	10ºC & 65%RH
	Oilseed rape	25ºC & 85% RH	20ºC & 65%RH
Tyrophagus putrescentiae	Wheat	20ºC & 85% RH	10ºC & 65%RH
	Barley	25ºC & 75% RH	15ºC & 65%RH
	Oilseed rape	25ºC & 85% RH	15ºC & 75%RH

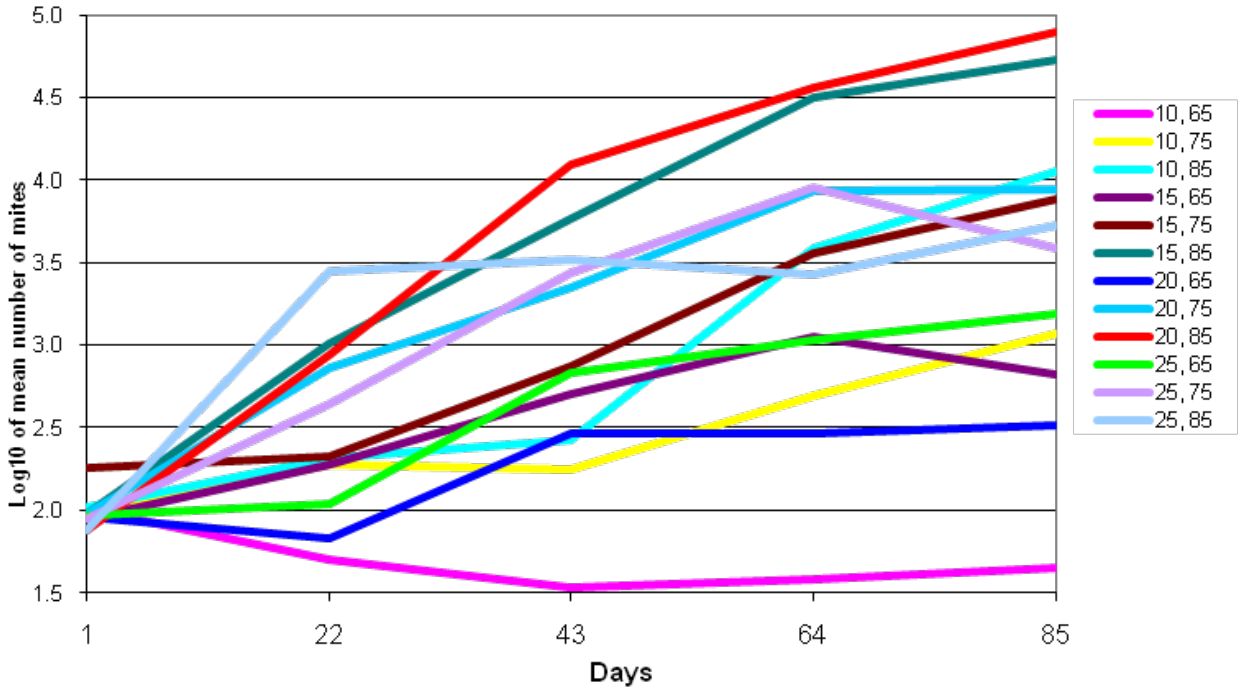


Figure 11. Example of population curves produced for *Acarus siro* bred at different temperatures and relative humidities on wheat.

Fitting models to the experimentally-derived insect and mite population growth rates over ranges of temperature and humidity

Calculating daily rates of population increase

Cohort life table data were collected at two relative humidities (60% and 70%) and three temperatures (20°C, 22.5°C and 25°C) for the two insect species, and three relative humidities (65%, 75% and 85%) and four temperatures (10°C, 15°C, 20°C and 25°C) for the two mite species. The life table data were used to calculate intrinsic rates of increase (r_m) for each of 10 replicate cohorts subjected to each temperature / humidity regime. Calculated estimates of r_m were

converted to finite rates of increase λ, which were expressed as a daily rate for purposes of model fitting. This is the average fold of increase of the population per day, so for example, a value of 1.1 means that if there are 100 insects on a particular day then we predict 110 on the following day.

Although a relatively small set of combinations of temperature and humidity were available, other knowledge could usefully be taken into account. For example, Beckett and Evans (1994) found that the rate of increase of *Oryzaephilus* peaked at 32.5°C (at both 50% and 70% r.h.) before declining steeply at 35°C. The data suggested a near-linear response to increase in temperature, but the slope differed for the two humidities. There was therefore an interaction between temperature and humidity with population growth rate showing an increased response to temperature when humidity was higher.

For the two beetle species, the data consisted of records of adult progeny emerged from a cohort of 20 original adults. To obtain life table statistics from this we proceeded as follows. We have the daily progeny emergence per week for the cohort. Each week, however, adults die, so progeny emergence automatically takes this into account so is directly equivalent to $l_x m_x$ where l_x is the number surviving to week x and m_x is the fecundity in week x. Progeny emergence is recorded at the end of the week so a better estimate of x is to take the midpoint of the week concerned, making x = week number - 0.5 weeks.

The adults were transferred to new containers every two weeks so it was necessary to sum the emerged progeny from the succession of containers across each value of x.
Taking then the 'per adult value of $l_x m_x$, the total number of adults emerged per original adult is

$$R_0 = \sum_x l_x m_x \qquad \text{(Eq1)}$$

and for *O. surinamensis* at 25°C, 70% r.h. for example, R_0 was approximately 8.9 adults per adult.
The intrinsic rate of increase can be estimated from the equation

$$r = \ln R_0 / T_c \qquad \text{(Eq2)}$$

where T_c is the cohort generation time which in turn is given by

$$T_c = \sum_x x l_x m_x \Big/ \sum_x l_x m_x = \sum_x x l_x m_x \Big/ R_0 \qquad \text{(Eq3)}$$

This equation has a clear biological interpretation but an exact value of r can be calculated from the equality

$$\sum_x \exp(-rx) l_x m_x = 1 \qquad \text{(Eq4)}$$

As we have the necessary values of x, l_x and m_x this equation can be solved numerically for r. For *O. surinamensis* at 25°C, 70% r.h., the r (per week) was approximately 0.22.

Having obtained a valued of *r* from Eq4, we can estimate T_c from Eq2, the value for *O. surinamensis* at 25°C, 70% r.h. was approximately 9.8 weeks. This is the average time between adult emergence in an individual and the adult emergence of its own offspring.
The multiplication rate of the population is $\lambda = \exp(r)$ with corresponding value of 1.25 per week.
This relates directly back to R_0 by the relationship

$$\lambda^{T_c} = R_0 \qquad \text{(Eq5)}$$

i.e. if the population grows as a rate of 1.25 times per week for a period of one generation (9.8 weeks) then this equates to growth of 8.9 times in one generation, the value of R_0.
When instead the generation time was calculated from Eq 3, the value was approximately 11.4 weeks and the estimate of *r* then calculated using Eq 2 was about 13% less than that obtained from Eq 4, a fairly significant discrepancy suggesting that use of Eq 4 is worthwhile. Eq 3 does not take into account the fact that some offspring may themselves produce offspring during the reproductive life of their parent.

Cubic spline interpolation

A series of models were fitted by cubic spline interpolation to the finite rates of increase *λ* obtained above but expressed daily rather than weekly:

- *Tyrophagus putrescentiae* on wheat, barley and oilseed rape (OSR)
- *Acarus siro* on wheat, barley and OSR
- *Oryzaephilus* on wheat and barley
- *Sitophilus granarius* on wheat and barley

The mathematical modelling software, Mathcad (Mathcad 2000i Professional, © 1986–2001 MathSoft Engineering & Education, Inc.) was used to develop and fit the models. Cubic spline interpolation allows a curve to be passed through a set of points in such a way that the first and second derivatives of the curve are continuous across each point. This curve is assembled by taking three adjacent points and construction of a cubic polynomial passing through those points. These cubic polynomials are then strung together to form the completed curve.

The curves were fitted using built-in Mathcad functions and three alternatives are provided for the cubic spline interpolation. These differ according to the assumptions made about the spline curve at the end-points. ‘Cspline’ is cubic at the endpoints, ‘pspline’ is parabolic at the endpoints and ‘lspline’ is linear at the endpoints. It was found that the assumption of linearity at the end-points gave interpolations with least complex shapes and ‘lspline’ was used throughout.

In two-dimensional cubic spline interpolation, the surface passes through all data points exactly and the surface is smooth because the gradient is continuous across each point. It cannot be

represented by a simple equation, so model predictions are presented in the form of a surface plot which can be referenced as a 'look-up' table of the desired precision. Table 21 shows an example of the table obtained by interpolation with values shown at 1°C and 1% r.h. intervals

Table 21. Example of a table of interpolated values of the daily rates of increase of *Acarus siro* on wheat obtained by fitting the spline. The values highlighted in yellow are the actual data points.

RH %	Temp C															
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	0.979	0.994	1.008	1.019	1.028	1.032	1.031	1.026	1.019	1.013	1.009	1.008	1.011	1.015	1.022	1.029
66	0.982	0.997	1.01	1.022	1.03	1.034	1.034	1.03	1.024	1.018	1.014	1.014	1.016	1.021	1.027	1.034
67	0.986	1	1.013	1.024	1.032	1.036	1.036	1.033	1.029	1.024	1.021	1.02	1.023	1.027	1.032	1.038
68	0.99	1.003	1.015	1.025	1.033	1.038	1.039	1.037	1.033	1.03	1.027	1.027	1.029	1.033	1.037	1.042
69	0.995	1.006	1.017	1.027	1.034	1.039	1.041	1.04	1.038	1.036	1.034	1.034	1.036	1.039	1.042	1.046
70	1	1.01	1.02	1.028	1.035	1.04	1.043	1.043	1.043	1.042	1.041	1.042	1.043	1.045	1.047	1.05
71	1.005	1.014	1.022	1.03	1.036	1.041	1.045	1.046	1.048	1.048	1.048	1.049	1.05	1.051	1.052	1.053
72	1.01	1.017	1.024	1.031	1.037	1.042	1.046	1.05	1.052	1.054	1.055	1.056	1.056	1.057	1.057	1.057
73	1.015	1.021	1.027	1.033	1.038	1.044	1.049	1.053	1.057	1.06	1.062	1.063	1.063	1.062	1.061	1.06
74	1.019	1.024	1.03	1.035	1.04	1.045	1.051	1.056	1.061	1.065	1.068	1.069	1.069	1.067	1.065	1.063
75	1.024	1.028	1.032	1.037	1.042	1.048	1.054	1.06	1.066	1.07	1.073	1.074	1.074	1.072	1.069	1.066
76	1.027	1.031	1.035	1.04	1.045	1.05	1.057	1.064	1.07	1.075	1.078	1.079	1.078	1.076	1.072	1.068
77	1.03	1.034	1.038	1.043	1.048	1.054	1.06	1.067	1.074	1.079	1.082	1.083	1.082	1.079	1.075	1.071
78	1.033	1.037	1.042	1.046	1.052	1.058	1.064	1.071	1.078	1.083	1.086	1.086	1.085	1.082	1.078	1.073
79	1.035	1.04	1.045	1.05	1.056	1.062	1.069	1.075	1.081	1.086	1.089	1.089	1.087	1.084	1.08	1.075
80	1.037	1.043	1.049	1.055	1.061	1.067	1.073	1.079	1.085	1.089	1.091	1.091	1.089	1.086	1.082	1.077
81	1.038	1.045	1.052	1.059	1.066	1.072	1.078	1.084	1.088	1.092	1.093	1.093	1.091	1.088	1.084	1.079
82	1.04	1.048	1.056	1.064	1.071	1.077	1.083	1.088	1.092	1.094	1.095	1.094	1.092	1.089	1.085	1.081
83	1.041	1.05	1.06	1.068	1.076	1.083	1.088	1.092	1.095	1.096	1.097	1.095	1.093	1.09	1.087	1.083
84	1.042	1.053	1.063	1.073	1.082	1.089	1.094	1.097	1.098	1.099	1.098	1.096	1.094	1.091	1.088	1.085
85	1.043	1.055	1.067	1.078	1.087	1.094	1.099	1.101	1.102	1.101	1.099	1.097	1.095	1.092	1.089	1.087

Initial models based on quadratic polynomials

At the start of the work, an alternative to the fitted spline was used, which took the form of a quadratic polynomial. This polynomial model was used in a series of simulations exploring different cooling and drying scenarios in the grain store (Section 2.3.3). The simulations were carried out for *O. surinamensis* on wheat and in this case, the difference between the polynomial and the spline models was sufficiently small so that, for practical purposes, there was expected to be very little impact on the result of the simulations. For the other species / grain combinations examined in the project, the errors associated with a quadratic polynomial were much larger, which is why the alternative approach with cubic splines was used.

Results

The population growth rates and the fitted splines

A series of figures (Figure 12) show the daily population growth rates for each species – grain combination together with the 95% confidence intervals for the growth rate for each set of conditions under which an experiment was carried out. The lines are the fitted splines. These lines can be thought of as three (sometimes two) slices through the fitted two-dimensional surface corresponding to the specific relative humidity values at which experiments were carried out.

In general, the confidence intervals were quite narrow, indicating that the replicates of each experiment gave quite consistent results. It would perhaps be expected that the curves would be

monotonic or uni-modal and this was usually the case. In one or two cases, the curves did not rise (or fall) entirely consistently in response to temperature and relative humidity changes and this may have been due to some uncontrolled factor in the experiments. After project discussions it was decided to fit models that were faithful to the actual data rather than to force more simplified curves based on expectations of what the curves 'should' look like. As discussed below, the apparent anomalies are not sufficiently large to affect the practical conclusions for grain drying and cooling which can be drawn from the results.

Comparison of models developed in this project with earlier ones

There have been a range of models developed over the years covering a range of insect species and mites (for example, Berreen, 1974; Flinn *et al.*, 2004, Hagstrum and Flinn, 1990; Hagstrum and Thorne,1989; Kawamoto *et al.,* 1991*;* Sanchez-Ramos *et al.*, 2007). However, whilst many of these have been developed over a range of temperatures they are often based on data for a single relative humidity. The other overriding problem has been the substrate used to rear the mites or insects. In many cases, whole grain is not used; instead broken grain or a rearing diet consisting of a mixture of the germ, flakes of grain and yeast in the case of mites is supplied. Therefore, it is unlikely that the rates of development observed under these conditions will reflect what happens in grain stores. The models derived from these experiments, whilst having some biological interest are incomplete in trying to define the population changes expected under dynamic temperature and relative humidity conditions in farm and commercial stores. A further complication is that the models themselves are designed to predict different things. For example the models of Hagstrum and Flinn (1990) and Hagstrum and Thorne (1989) are designed to give the population of insects in a bulk of grain after a particular period of time for a given volume of grain. They also incorporate immigration into the model making direct comparison impossible. It is therefore, extremely difficult to make any direct comparisons between any of the models that have been developed.

Models of *Acarus siro*

There appear to be very few models of *Acarus siro* and none for *Tyrophagous putrescentiae* with which to compare the current model. Kawamoto *et al.* (1991) produced a very similar type of model based on a bivariate linear interpolation of the data taken from Cunnington (1984) and Solomon (1969). Table 22 shows a comparison between the daily rates of increase for the two models. (Figures for Kawamoto *et al.* (1991) are estimated from Figure 1 in the paper).

Table 22. Comparison of daily rates of increase from the model produced in this project and that produced by Kawamoto *et al.* (1991)

RH% /Temp °C	10		15		20		25	
	LINK model	Kawamoto *et al.* (1991)	LINK model	Kawamoto *et al.* (1991)	LINK model	Kawamoto *et al.* (1991)	LINK model	Kawamoto *et al.* (1991)
65	0.979	1.00	1.032	0.98	1.009	0.99	1.029	0.81
70	1.000	1.02	1.04	1.04	1.041	1.02	1.05	1.01
75	1.024	1.05	1.048	1.10	1.073	1.10	1.066	1.09
80	1.037	1.06	1.067	1.10	1.091	1.14	1.077	1.20
85	1.043	1.08	1.094	1.11	1.099	1.14	1.087	1.17

There are clear differences between the two models which stem from the data that are used to build and test the model. There are considerable gaps in the data used by Kawamoto *et al.* (1991) which would probably lead to greater uncertainty and inaccuracy of the results obtained. The proposed model probably provides the best estimate of *A. siro* growth rates under a range of conditions on wheat, barley and oilseed rape.

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.

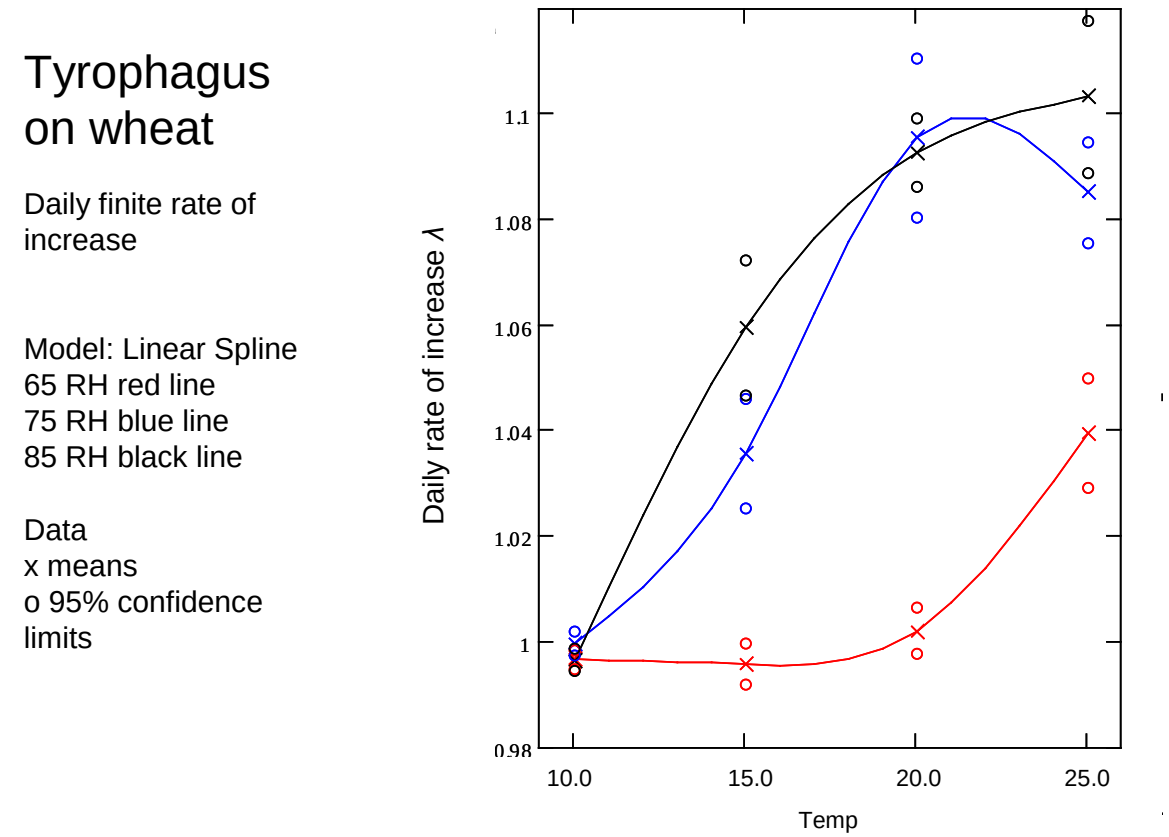
Results expressed as tables of population doubling times

For practical purposes, it may be more useful to express the results not in terms of population growth rates but as doubling times: that is the time it takes for the population to double in size. This is more readily understood than a growth rate, so for example, if the population doubles in less than a month, this may be considered a serious situation.

Figure 13 shows each set of results as a table of doubling times in weeks. Thus, by selecting the column and row values of temperature and humidity, the number of weeks it takes for the population to double can be read at where the column and row intersect. To make the tables easier to read at a glance, they are colour coded so that periods of 4 weeks or less are shaded red, 12 weeks or more yellow and intermediate periods orange. The colour coding is a guide only and should not be interpreted as red to mean unacceptable, and yellow acceptable, because levels of infestation also depend on starting number and the period for which it is intended to store the grain influences the acceptability of the conditions.

The use of the earlier quadratic polynomial for grain storage simulations

A comparison between the cubic spline and the quadratic polynomial models is shown for the case of *O. surinamensis* on wheat (Figure 14). For *O. surinamensis,* a four-term quadratic fitted the data reasonably well, but the polynomial can be seen to overestimate slightly at 22.5°C and 70% r.h. and underestimate slightly at 20°C at both humidities (Figure 14). This type of model was used initially because other information implied a curve in the response surface such that growth ceased at around 17°C. Ideally, growth should peak at around 35°C, but a more complex model would be needed to capture both the near linear response between 20°C and 25°C as well as a sharp decline from 32.5°C upwards. The model can only be regarded as reasonably accurate up to 32.5°C. The equation of the polynomial was, daily rate of increase $\lambda = ax^2 + bxy + cy^2 + f$ where x is the temperature (°C), and y the relative humidity (%), and a, b, c and f are parameters. In the case of *O. surinamensis* on wheat (Figure 14), a = -1.05 x10^{-4}, b = 1.2 x 10^{-4}, c = -0.17 x 10^{-4} and f = 0.969.

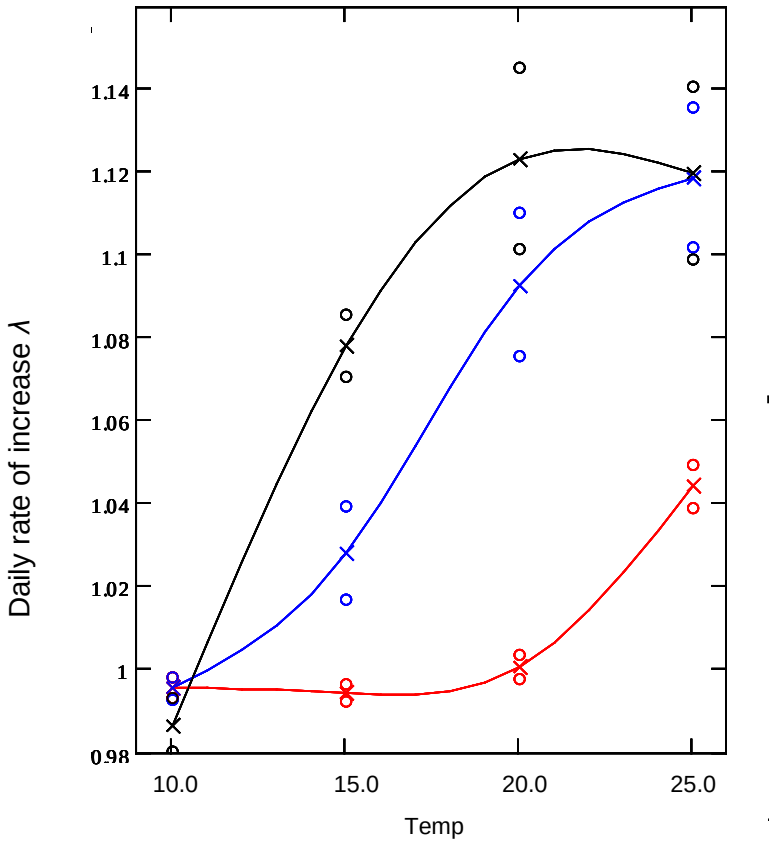


Tyrophagus on barley

Daily finite rate of increase

Model: Linear Spline
65 RH red line
75 RH blue line
85 RH black line

Data
x means
o 95% confidence limits

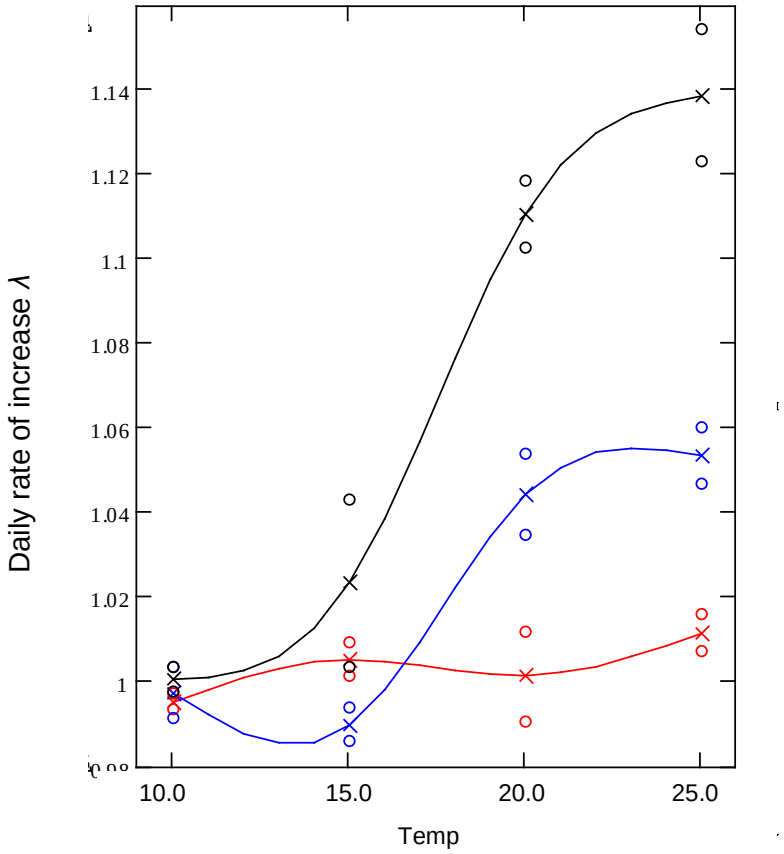


Tyrophagus on OSR

Daily finite rate of increase

Model: Linear Spline
65 RH red line
75 RH blue line
85 RH black line

Data
x means
o 95% confidence limits

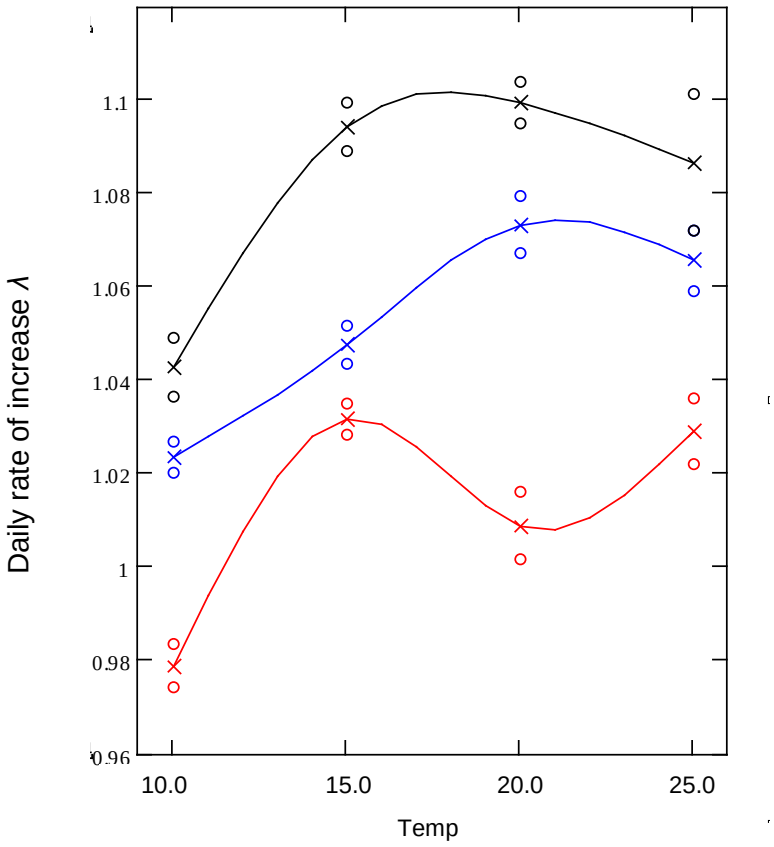


Acarus on wheat

Daily finite rate of increase

Model: Linear Spline
65 RH red line
75 RH blue line
85 RH black line

Data
x means
o 95% confidence limits

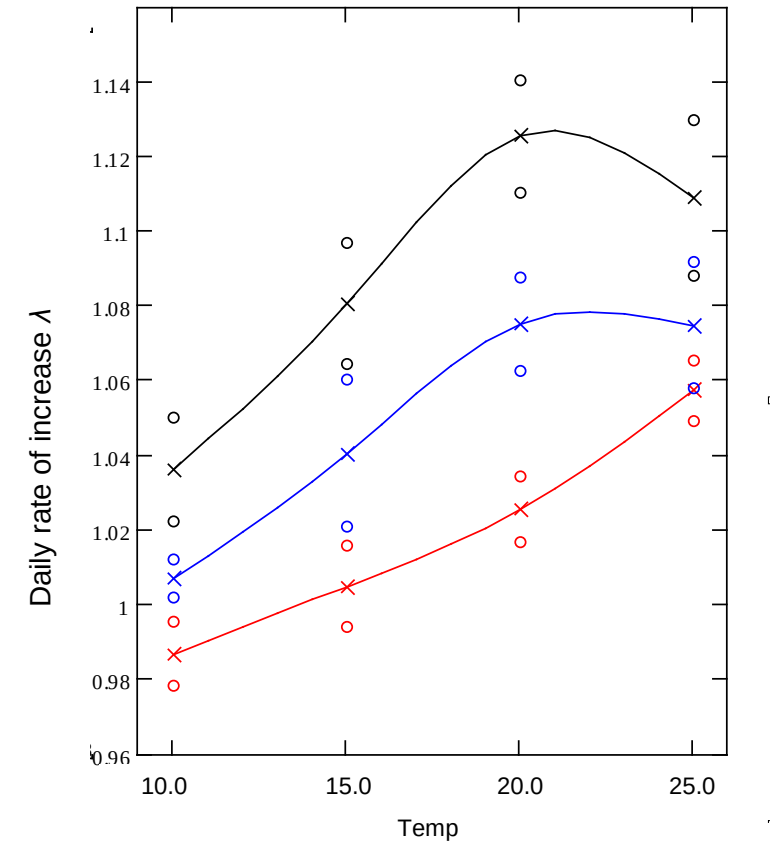


Acarus on barley

Daily finite rate of increase

Model: Linear Spline
65 RH red line
75 RH blue line
85 RH black line

Data
x means
o 95% confidence limits

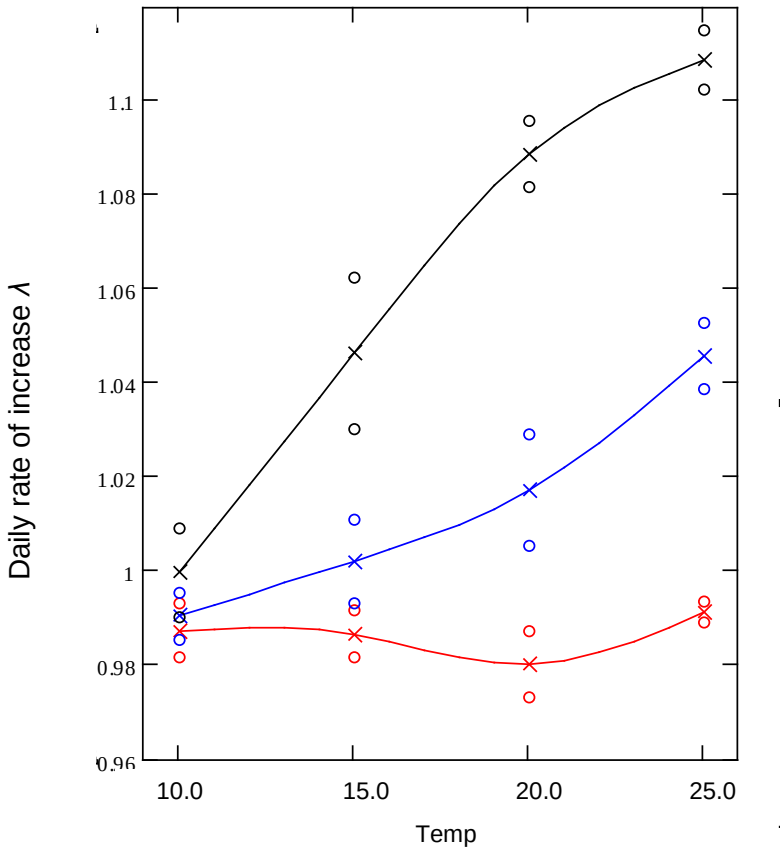


Acarus on OSR

Daily finite rate of increase

Model: Linear Spline
65 RH red line
75 RH blue line
85 RH black line

Data
x means
o 95% confidence limits

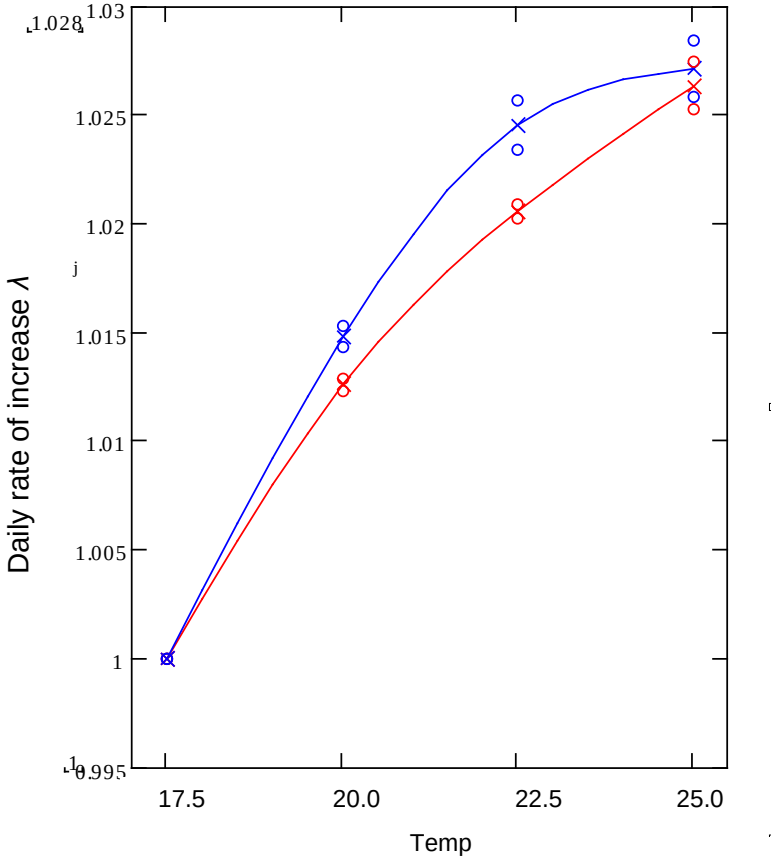


Oryzaephilus on barley

Daily finite rate of increase

Model: Linear Spline
60 RH red line
70 RH blue line

Data
x means
o 95% confidence limits

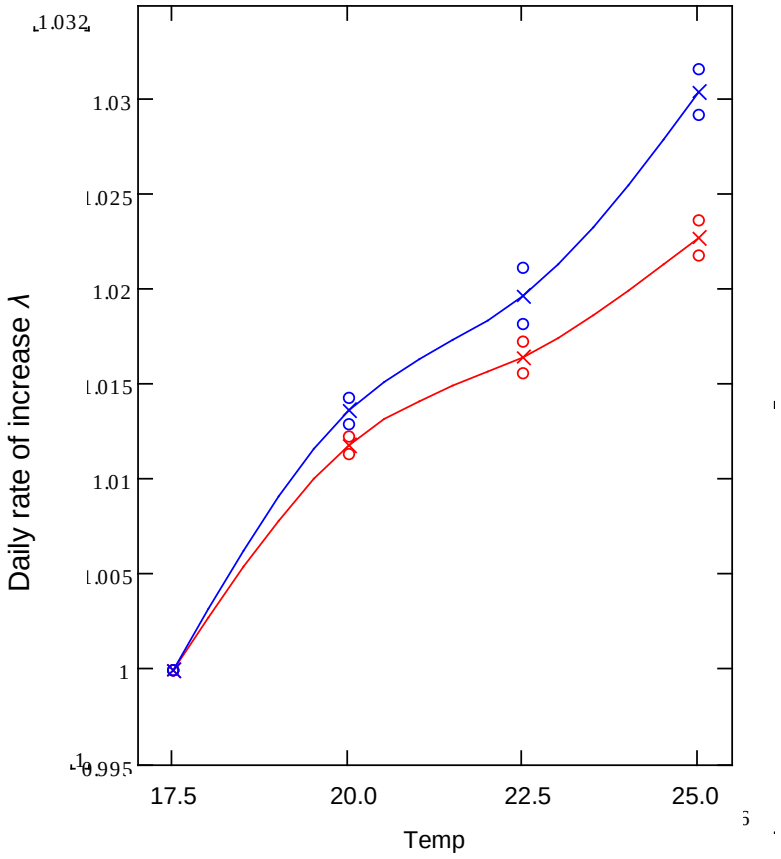


Oryzaephilus on wheat

Daily finite rate of increase

Model: Linear Spline
60 RH red line
70 RH blue line

Data
x means
o 95% confidence limits

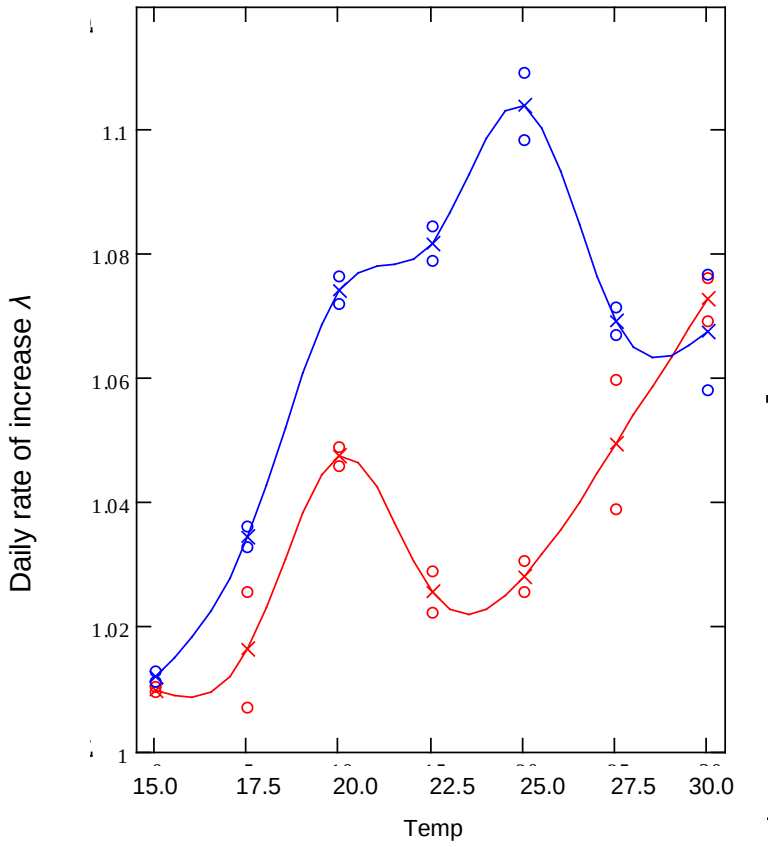


Sitophilus on barley

Daily finite rate of increase

Model: Linear Spline
60 RH red line
70 RH blue line

Data
x means
o 95% confidence limits



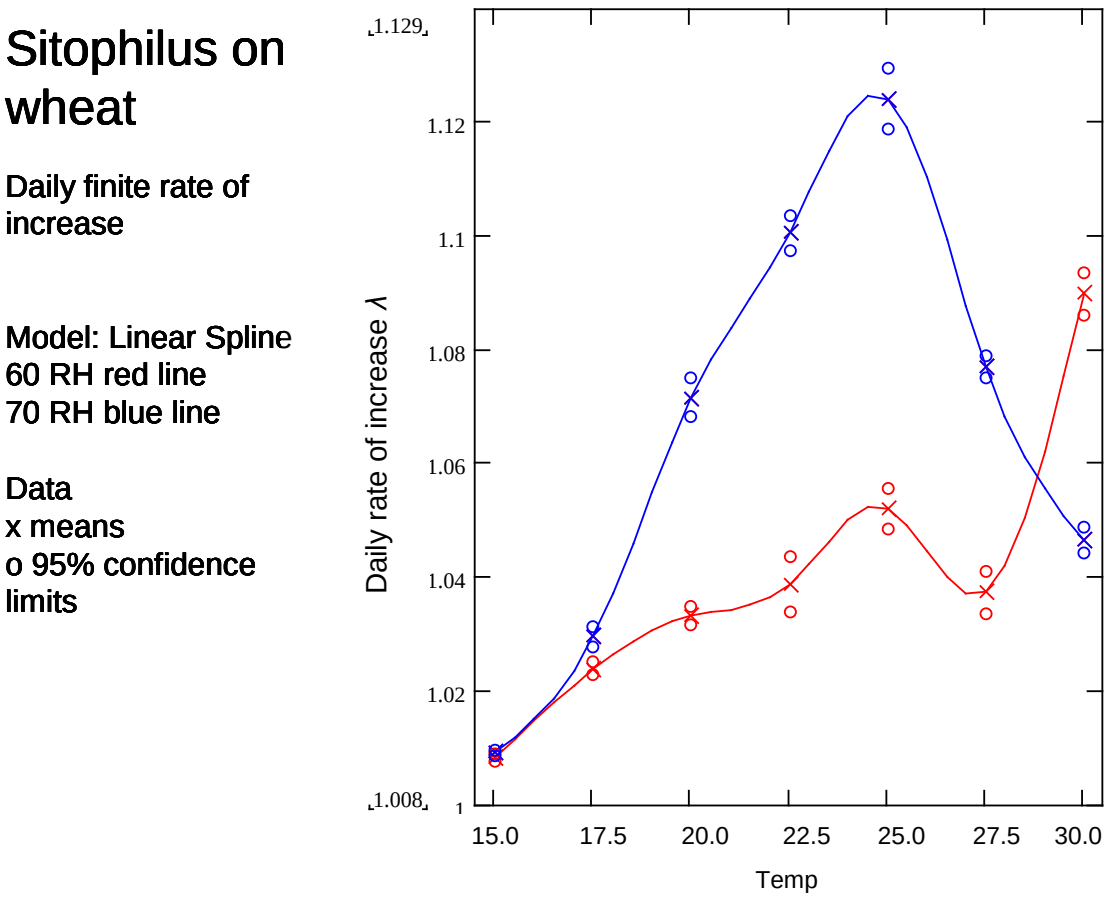


Figure 12. Daily rates of population increase with confidence limits under different temperature / relative humidity combinations obtained for each insect/mite on each host grain. The lines show an interpolated line fitted to the data using a linear spline.

Tyrophagus putrescentiae on wheat

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	50	14	7	5	3	3
66	∞	∞	∞	∞	∞	∞	∞	∞	50	20	11	7	5	4	3	2
67	∞	∞	∞	∞	∞	50	25	17	10	7	6	4	3	3	2	2
68	∞	∞	∞	99	33	20	11	8	6	4	4	3	3	2	2	2
69	∞	∞	99	33	17	11	7	5	4	3	3	2	2	2	2	2
70	∞	∞	50	20	11	7	5	4	3	2	2	2	2	2	2	2
71	∞	99	25	14	8	6	4	3	2	2	2	2	2	1	1	1
72	∞	50	20	10	7	4	3	2	2	2	1	1	1	1	1	1
73	∞	33	14	8	5	4	3	2	2	1	1	1	1	1	1	1
74	∞	25	11	7	5	3	2	2	1	1	1	1	1	1	1	1
75	∞	20	10	6	4	3	2	2	1	1	1	1	1	1	1	1
76	∞	20	8	5	4	3	2	2	1	1	1	1	1	1	1	1
77	∞	17	8	5	3	2	2	1	1	1	1	1	1	1	1	1
78	∞	14	7	4	3	2	2	1	1	1	1	1	1	1	1	1
79	∞	14	6	4	3	2	2	1	1	1	1	1	1	1	1	1
80	∞	12	6	4	3	2	2	1	1	1	1	1	1	1	1	1
81	∞	12	5	3	2	2	2	1	1	1	1	1	1	1	1	1
82	∞	11	5	3	2	2	2	1	1	1	1	1	1	1	1	1
83	∞	11	5	3	2	2	2	1	1	1	1	1	1	1	1	1
84	∞	10	4	3	2	2	2	1	1	1	1	1	1	1	1	1
85	∞	10	4	3	2	2	1	1	1	1	1	1	1	1	1	1

Tyrophagus putrescentiae on barley

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	99	14	7	4	3	2
66	∞	∞	∞	∞	∞	∞	∞	∞	∞	33	12	7	5	3	2	2
67	∞	∞	∞	∞	∞	∞	99	33	17	10	6	4	3	3	2	2
68	∞	∞	∞	∞	∞	50	20	12	8	5	4	3	2	2	2	1
69	∞	∞	∞	∞	50	20	11	7	5	4	3	2	2	2	1	1
70	∞	∞	∞	99	25	12	7	5	3	3	2	2	2	1	1	1
71	∞	∞	∞	33	17	8	5	4	3	2	2	2	1	1	1	1
72	∞	∞	99	25	11	7	4	3	2	2	2	1	1	1	1	1
73	∞	∞	50	17	8	5	3	2	2	2	1	1	1	1	1	1
74	∞	∞	33	12	7	4	3	2	2	1	1	1	1	1	1	1
75	∞	∞	20	9	6	4	3	2	2	1	1	1	1	1	1	1
76	∞	∞	17	8	5	3	2	2	1	1	1	1	1	1	1	1
77	∞	99	12	6	4	3	2	2	1	1	1	1	1	1	1	1
78	∞	99	10	5	3	2	2	1	1	1	1	1	1	1	1	1
79	∞	50	8	4	3	2	2	1	1	1	1	1	1	1	1	1
80	∞	33	7	4	3	2	2	1	1	1	1	1	1	1	1	1
81	∞	25	6	3	2	2	1	1	1	1	1	1	1	1	1	1
82	∞	25	5	3	2	2	1	1	1	1	1	1	1	1	1	1
83	∞	20	5	3	2	2	1	1	1	1	1	1	1	1	1	1
84	∞	17	4	2	2	1	1	1	1	1	1	1	1	1	1	1
85	∞	14	4	2	2	1	1	1	1	1	1	1	1	1	1	1

Tyrophagus putrescentiae on oilseed rape

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	99	33	20	20	20	25	33	50	99	50	25	17	11	8
66	∞	∞	∞	50	33	25	25	25	25	25	25	20	14	11	8	7
67	∞	∞	∞	∞	50	33	33	25	20	17	14	11	9	8	7	6
68	∞	∞	∞	∞	∞	99	50	25	17	11	9	8	7	6	5	5
69	∞	∞	∞	∞	∞	∞	99	25	12	9	7	6	5	5	4	4
70	∞	∞	∞	∞	∞	∞	∞	25	11	7	5	5	4	4	4	4
71	∞	∞	∞	∞	∞	∞	∞	20	9	6	4	4	3	3	3	3
72	∞	∞	∞	∞	∞	∞	∞	20	8	5	4	3	3	3	3	3
73	∞	∞	∞	∞	∞	∞	∞	17	6	4	3	3	2	2	2	2
74	∞	∞	∞	∞	∞	∞	∞	14	5	3	3	2	2	2	2	2
75	∞	∞	∞	∞	∞	∞	∞	10	5	3	2	2	2	2	2	2
76	∞	∞	∞	∞	∞	∞	∞	8	4	3	2	2	2	2	2	2
77	∞	∞	∞	∞	∞	∞	50	6	3	2	2	2	2	1	1	2
78	∞	∞	∞	∞	∞	∞	20	5	3	2	2	1	1	1	1	1
79	∞	∞	∞	∞	∞	∞	11	4	3	2	1	1	1	1	1	1
80	∞	∞	∞	∞	∞	99	8	3	2	2	1	1	1	1	1	1
81	∞	∞	∞	∞	∞	20	6	3	2	1	1	1	1	1	1	1
82	∞	∞	∞	∞	99	11	4	3	2	1	1	1	1	1	1	1
83	∞	∞	∞	∞	20	7	4	2	2	1	1	1	1	1	1	1
84	∞	∞	∞	33	11	5	3	2	1	1	1	1	1	1	1	1
85	99	99	33	17	8	4	3	2	1	1	1	1	1	1	1	1

Acarus siro on wheat

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	12	5	4	3	3	4	5	8	11	12	9	7	5	3
66	∞	∞	10	5	3	3	3	3	4	6	7	7	6	5	4	3
67	∞	∞	8	4	3	3	3	3	3	4	5	5	4	4	3	3
68	∞	33	7	4	3	3	3	3	3	3	4	4	3	3	3	2
69	∞	17	6	4	3	3	2	3	3	3	3	3	3	3	2	2
70	∞	10	5	4	3	3	2	2	2	2	2	2	2	2	2	2
71	20	7	5	3	3	2	2	2	2	2	2	2	2	2	2	2
72	10	6	4	3	3	2	2	2	2	2	2	2	2	2	2	2
73	7	5	4	3	3	2	2	2	2	2	2	2	2	2	2	2
74	5	4	3	3	3	2	2	2	2	2	2	1	1	2	2	2
75	4	4	3	3	2	2	2	2	2	1	1	1	1	1	1	2
76	4	3	3	3	2	2	2	2	1	1	1	1	1	1	1	2
77	3	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1
78	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1
79	3	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1
80	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
81	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
82	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
83	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
84	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
85	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1

Acarus siro on barley

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	∞	∞	99	20	12	8	6	5	4	3	3	2	2	2
66	∞	∞	∞	∞	25	12	8	6	5	4	3	3	2	2	2	2
67	∞	∞	∞	50	17	9	7	5	4	3	3	3	2	2	2	2
68	∞	∞	∞	25	11	7	5	4	3	3	3	2	2	2	2	2
69	∞	∞	50	14	8	6	4	4	3	3	2	2	2	2	2	2
70	∞	∞	20	10	7	5	4	3	3	2	2	2	2	2	2	1
71	∞	50	14	8	5	4	3	3	2	2	2	2	2	2	2	1
72	∞	20	10	6	5	3	3	2	2	2	2	2	2	2	1	1
73	50	14	8	5	4	3	3	2	2	2	2	2	1	1	1	1
74	25	10	6	4	3	3	2	2	2	2	1	1	1	1	1	1
75	14	8	5	4	3	3	2	2	2	1	1	1	1	1	1	1
76	10	6	4	3	3	2	2	2	1	1	1	1	1	1	1	1
77	8	5	4	3	3	2	2	2	1	1	1	1	1	1	1	1
78	7	5	3	3	2	2	2	1	1	1	1	1	1	1	1	1
79	6	4	3	3	2	2	2	1	1	1	1	1	1	1	1	1
80	5	4	3	2	2	2	1	1	1	1	1	1	1	1	1	1
81	4	3	3	2	2	2	1	1	1	1	1	1	1	1	1	1
82	4	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1
83	3	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1
84	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
85	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1

Acarus siro on oilseed rape

Temperature °C

Relative humidity %

	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
65	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
66	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
67	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
68	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	25
69	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	25	11
70	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	25	11	7
71	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	20	11	7	5
72	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	50	20	10	7	5	4
73	∞	∞	∞	∞	∞	∞	∞	∞	99	33	17	10	7	5	4	3
74	∞	∞	∞	∞	∞	∞	99	33	20	12	8	6	5	4	3	3
75	∞	∞	∞	∞	∞	50	20	14	10	8	6	5	4	3	3	2
76	∞	∞	∞	∞	33	20	12	8	7	5	4	4	3	3	2	2
77	∞	∞	∞	50	20	11	8	6	5	4	3	3	3	2	2	2
78	∞	∞	99	20	11	8	6	5	4	3	3	2	2	2	2	2
79	∞	∞	33	14	8	6	5	4	3	3	2	2	2	2	2	1
80	∞	∞	20	9	6	5	4	3	3	2	2	2	2	2	1	1
81	∞	99	12	7	5	4	3	3	2	2	2	2	1	1	1	1
82	∞	33	10	6	4	3	3	2	2	2	2	1	1	1	1	1
83	∞	20	8	5	4	3	2	2	2	2	1	1	1	1	1	1
84	∞	14	7	4	3	2	2	2	2	1	1	1	1	1	1	1
85	∞	11	6	4	3	2	2	2	1	1	1	1	1	1	1	1

Oryzaephilus surinamensis on wheat

Temperature °C

Relative humidity %

	18		19		20		21		22		23		24		25	
60	∞	33	20	12	10	8	8	7	7	6	6	6	5	5	5	4
	∞	33	17	12	10	8	8	7	7	6	6	6	5	5	5	4
61	∞	33	17	12	10	8	7	7	7	6	6	6	5	5	5	4
	∞	33	17	12	9	8	7	7	6	6	6	6	5	5	4	4
62	∞	33	17	12	9	8	7	7	6	6	6	5	5	5	4	4
	∞	33	17	12	9	8	7	7	6	6	6	5	5	5	4	4
63	∞	33	17	11	9	8	7	7	6	6	6	5	5	5	4	4
	∞	33	17	11	9	8	7	6	6	6	6	5	5	5	4	4
64	∞	33	17	11	9	8	7	6	6	6	6	5	5	4	4	4
	∞	33	17	11	9	8	7	6	6	6	6	5	5	4	4	4
65	∞	33	17	11	9	8	7	6	6	6	5	5	5	4	4	4
	∞	33	17	11	9	8	7	6	6	6	5	5	5	4	4	4
66	∞	33	17	11	9	8	7	6	6	6	5	5	5	4	4	4
	∞	33	17	11	9	8	7	6	6	6	5	5	5	4	4	4
67	∞	33	17	11	9	8	7	6	6	6	5	5	5	4	4	4
	∞	33	17	11	9	7	7	6	6	6	5	5	5	4	4	3
68	∞	33	17	11	8	7	7	6	6	6	5	5	4	4	4	3
	∞	33	17	11	8	7	7	6	6	6	5	5	4	4	4	3
69	∞	33	17	11	8	7	7	6	6	6	5	5	4	4	4	3
	∞	33	17	11	8	7	7	6	6	6	5	5	4	4	4	3
70	∞	33	17	11	8	7	7	6	6	6	5	5	4	4	4	3

Oryzaephilus surinamensis on barley

Temperature °C

Relative humidity %

	18	19	20	21	22	23	24	25
60	∞ 33	20 12	10 8	7 6	6 5	5 5	4 4	4 4
	∞ 33	20 12	10 8	7 6	6 5	5 5	4 4	4 4
61	∞ 33	20 12	9 8	7 6	6 5	5 5	4 4	4 4
	∞ 33	17 12	9 8	7 6	6 5	5 5	4 4	4 4
62	∞ 33	17 12	9 8	7 6	5 5	5 5	4 4	4 4
	∞ 33	17 12	9 8	7 6	5 5	5 4	4 4	4 4
63	∞ 33	17 12	9 8	7 6	5 5	5 4	4 4	4 4
	∞ 33	17 12	9 8	7 6	5 5	5 4	4 4	4 4
64	∞ 33	17 12	9 8	6 6	5 5	5 4	4 4	4 4
	∞ 33	17 11	9 7	6 6	5 5	5 4	4 4	4 4
65	∞ 33	17 11	9 7	6 6	5 5	5 4	4 4	4 4
	∞ 33	17 11	9 7	6 6	5 5	4 4	4 4	4 4
66	∞ 33	17 11	9 7	6 6	5 5	4 4	4 4	4 4
	∞ 33	17 11	8 7	6 6	5 5	4 4	4 4	4 4
67	∞ 33	17 11	8 7	6 5	5 5	5 4	4 4	4 4
	∞ 33	17 11	8 7	6 5	5 5	5 4	4 4	4 4
68	∞ 33	17 11	8 7	6 5	5 5	5 4	4 4	4 4
	∞ 33	17 11	8 7	6 5	5 4	4 4	4 4	4 4
69	∞ 33	17 11	8 7	6 5	5 4	4 4	4 4	4 4
	∞ 33	17 11	8 7	6 5	5 4	4 4	4 4	4 4
70	∞ 33	17 11	8 7	6 5	5 4	4 4	4 4	4 4

Sitophilus granarius on wheat

Temperature °C

Relative humidity %

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
60	12 8	7 6	5 4	4 3	3 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	2 3	3 3	2 2	2 1 1
	12 8	7 6	5 4	4 3	3 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	2 3	3 2	2 2	2 1 1
61	12 8	7 6	5 4	4 3	3 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	2 3	3 2	2 2	2 1 1
	11 8	7 6	5 4	4 3	3 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	2 3	2 2	2 2	1 1
61	11 8	7 5	5 4	4 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1
	11 8	7 5	5 4	4 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1
62	11 8	7 5	5 4	3 3	3 3	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1
	11 8	7 5	5 4	3 3	3 3	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1
63	11 8	6 5	5 4	3 3	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1
	11 8	6 5	5 4	3 3	3 3	2 2	2 2	2 2	2 2	2 2	1 1	2 2	2 2	2 2	2 2	1 1
63	11 8	6 5	4 4	3 3	3 2	2 2	2 2	2 2	2 2	2 1	1 1	2 2	2 2	2 2	2 2	1 1
	11 8	6 5	4 4	3 3	3 2	2 2	2 2	2 2	2 1	1 1	1 1	2 2	2 2	2 2	2 2	1 1
64	11 8	6 5	4 4	3 3	3 2	2 2	2 2	2 2	2 1	1 1	1 1	2 2	2 2	2 2	2 2	1 1
	11 8	6 5	4 4	3 3	3 2	2 2	2 2	2 2	2 1	1 1	1 1	2 2	2 2	2 2	2 2	1 1
65	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	2 1	1 1	1 1	1 2	2 2	2 2	2 2	1 1
	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	2 1	1 1	1 1	1 2	2 2	2 2	2 2	1 1
65	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	2 1	1 1	1 1	1 2	2 2	2 2	2 2	2 1
	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	2 1	1 1	1 1	1 1	2 2	2 2	2 2	2 1
66	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	2 1	1 1	1 1	1 1	2 2	2 2	2 2	2 1
	11 8	6 5	4 4	3 3	2 2	2 2	2 2	2 2	1 1	1 1	1 1	1 1	2 2	2 2	2 2	2 1
67	11 8	7 5	4 4	3 3	2 2	2 2	2 2	2 1	1 1	1 1	1 1	1 1	2 2	2 2	2 2	2 2

Sitophilus granarius on barley

Temperature °C

Relative humidity %

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
60	10 11	11 10	8 6	4 3	3 2	2 2	2 2	3 3	4 4	5 4	4 4	3 3	3 2	2 2	2 2	2 1
	10 11	11 10	8 6	4 3	3 2	2 2	2 2	3 3	4 4	4 4	4 4	3 3	3 2	2 2	2 2	1 1
61	10 11	11 10	8 6	4 3	3 2	2 2	2 2	3 3	4 4	4 4	4 3	3 3	2 2	2 2	2 2	1 1
	10 10	10 9	8 6	4 3	2 2	2 2	2 2	3 3	3 4	4 4	4 3	3 3	3 2	2 2	2 2	1 1
61	10 10	10 9	7 6	4 3	2 2	2 2	2 2	2 3	3 4	4 4	3 3	3 3	2 2	2 2	2 2	1 1
	10 10	10 9	7 5	4 3	2 2	2 2	2 2	2 3	3 3	3 3	3 3	3 3	2 2	2 2	2 2	1 1
62	9 10	10 8	7 5	4 3	2 2	2 2	2 2	2 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	1 1
	9 10	9 8	7 5	4 3	2 2	2 2	2 2	2 3	3 3	3 3	3 3	3 2	2 2	2 2	2 2	1 1
63	9 9	9 8	7 5	4 3	2 2	2 2	2 2	2 2	3 3	3 3	3 2	2 2	2 2	2 2	2 2	1 1
	9 9	9 8	6 5	4 3	2 2	2 2	2 2	2 3	3 3	3 2	2 2	2 2	2 2	2 2	2 1	1 1
63	9 9	8 8	6 5	3 3	2 2	2 2	2 2	2 3	3 3	2 2	2 2	2 2	2 2	2 2	2 1	1 1
	9 9	8 7	6 5	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
64	9 9	8 7	6 4	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
	9 8	8 7	6 4	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
65	9 8	8 7	6 4	3 3	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
	9 8	8 7	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
65	9 8	8 6	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
	9 8	7 6	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
66	9 8	7 6	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 1	1 1
	9 8	7 6	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1	2 2	2 2	2 1	1 1
67	9 8	7 6	5 4	3 2	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1	2 2	2 2	2 1	1 1

Figure 13. The preceding series of tables give the doubling times (weeks) of the mite and insect populations over a range of temperatures and relative humidities. The tables are colour coded to distinguish three ranges of values: four weeks or less (red), more than four weeks and up to 12 weeks (orange) more than 12 weeks (yellow). The values were obtained by interpolation of measured population growth rates from a series of experiments carried out at different temperatures and relative humidities.

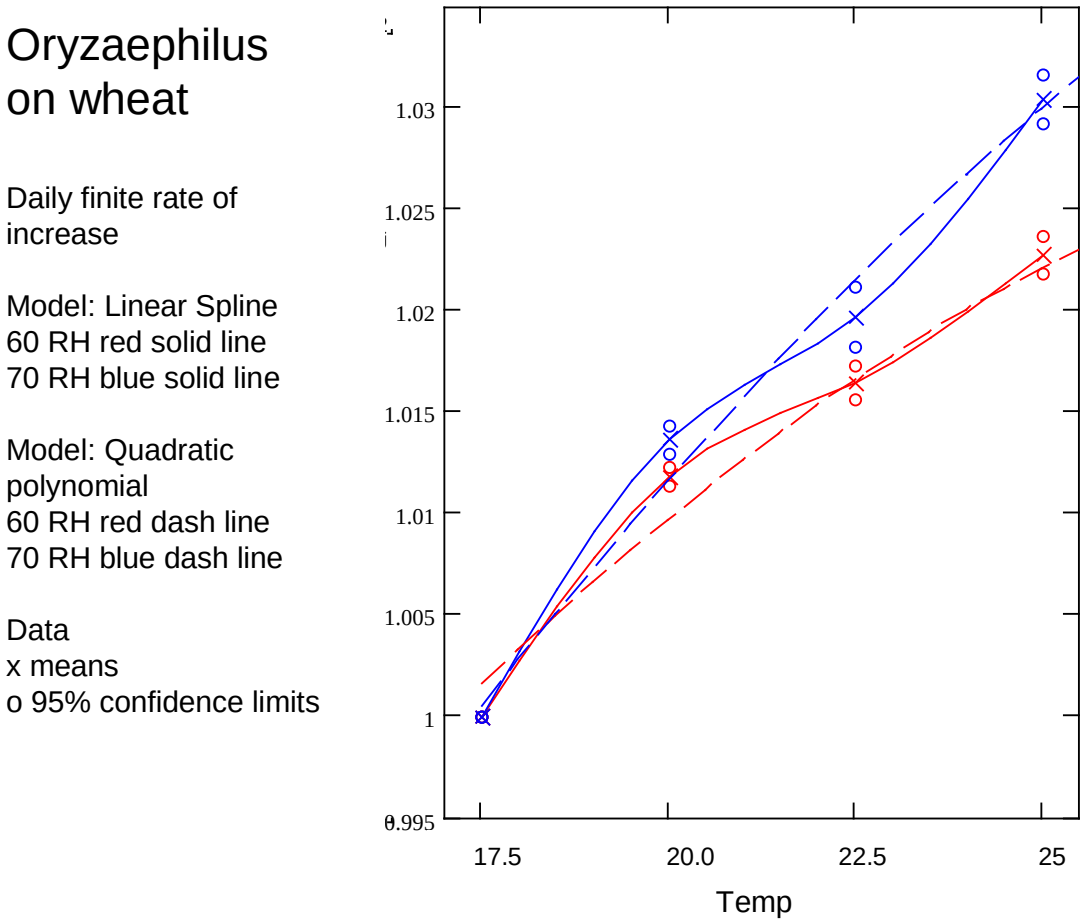


Figure 14. Comparison between a fitted spline, a fitted quadratic polynomial and the experimentally–derived population growth rates for *Oryzaephilus* on wheat

Conclusions

The pest population growth rates or population doubling times (Fig. 13) derived here are presented as a series of tables encompassing the ranges of temperature and relative humidity examined experimentally. A resolution of half or one C and half or one % RH point was used for the tables. For purposes of pest population prediction in the grain store, the predictive model can therefore take the values from the tables according to the environmental conditions pertaining to each time step in the simulation. It was the preferred approach to use such 'look-up tables' because a reasonably low-order polynomial model could not be obtained which accurately reflected the patterns on the data. As a result there is no single equation to represent growth rate as a function of temperature and humidity but instead the interpolation algorithm provided results from which the 'look-up' tables were compiled.

The use of interpolation means that the tables represent a completely faithful representation of the experimental results. There was some unexplained variation in some of the results in which growth rates appeared to exhibit some bimodality in relation to increasing temperature (Fig. 12). This was the case for *Acarus siro* on wheat in which at low humidity (65% RH) growth rate was higher at both 15°C and 25°C than it was at 20°C. A similar unexpected pattern also occurred with *Sitophilus granarius* on barley. It was decided better to reflect the actual results rather than assume a monotonic change without a rational basis to do so. As can be seen from the doubling-time tables (Fig. 13), these potentially anomalous results do not occur in regions of the table which would usually alter storage decision-making.

For both *Oryzaephilus surinamensis* and *Sitophilus granarius* on wheat and barley, it was clear that there was relative insensitivity to RH whereas, as expected from earlier work, with *Tyrophagus putrescentiae* and *Acarus siro* both RH and temperature were important predictors of population growth rate. Some of the doubling time graphs may not encompass the full range of time periods which can be of relevance to grain storage. In particular, the tables for on wheat and barley did not include doubling times longer than 12 weeks because the conditions which would have resulted in slower growth rates were not included in the experiments.

The results presented nonetheless provide a uniquely comprehensive set of organism population growth rates which in most cases encompass the time-frame, temperature and humidity which is of practical relevance for grain storage management. The rates were derived from experiments carried out under conditions which as far as possible resembled the grain-store environment and were consistent across the pest species and crop commodities concerned. They provide a valuable resource and good basis by which pest dynamics in grain storage can be predicted.

3.3.3. Step 2.3 Impact of current control methods

2.3.1 The effectiveness of current hygiene measures

Introduction

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.

Materials and Methods

Farmers and storekeepers were supplied with the PC floor trap (Collins and Chambers, 2003) for detection of insect pests and the BT mite trap (Thind, 2005) for detection of insect pests. The traps

were used as described in 2.1.1. Traps were positioned prior to store cleaning and returned to Fera for analysis. The cleaning method used was also described. Traps were re-positioned after the store had been cleaned and were then returned to Fera for analysis. If large numbers of mites were present these were recorded as > 500 or >1000. Fourteen stores took part in the exercise to examine the effectiveness of hygiene measures for insect pests and sixteen stores in the exercise for storage mites, but these were not necessarily the same stores.

Results

The most commonly used cleaning methods were sweeping or sweeping and vacuum, but a range of different methods were used (Table 23). Two stores did not have any insects detected either pre- or post-cleaning. Primary insect pests were found in four of the stores pre-cleaning. The other stores had secondary pest species, for example *Ahasverus advena,* psocids and cryptophagid spp*.* Post-cleaning, primary insect pests were found in three of the four stores that had primary insect pests pre-cleaning. Only one of these three stores had used a pesticide treatment. In ten of the stores, secondary insect pests were detected post-cleaning.

Stored product mites were present in 15 of the 16 stores pre-cleaning. Post-cleaning storage mites were found in 12 of the 16 stores (Table 23). This demonstrates that storage mites are extremely difficult to eradicate, even when insecticide treatments are used. This may be as a result of the presence of insecticide resistant populations as highlighted in 2.1.1.

Table 23. The presence of stored products mites in stores pre- and post-cleaning using different hygiene methods.

Store	Mites Pre-clean	Mites Post-clean	Hygiene method used
1	12 *Tyrophagus spp.*	3 *Tyrophagus* spp. All dead	Swept
2	21 *Tyrophagus* spp.	33 *Tyrophagus* spp. & Glycyphagid mites	Swept
3	111 Glycyphagid mites	2 *Acarus* & *Tyrophagus* spp.	Swept
4	32 *Tyrophagus* spp.	None	Swept & vacuum
5	> 887 *Acarus* & *Tyrophagus* spp.	> 759 *Acarus, Tyrophagus* spp. & Glycyphagid mites	Swept & insecticide
6	None	None	Swept & insecticide
7	51 *Acarus* & *Tyrophagus* spp.	None	Swept & vacuum
8	> 2500 *Tyrophagus* spp.	58 *Tyrophagus* spp. (30 dead)	Swept
9	59 *Tyrophagus* spp. & Glycyphagid mites	> 2000 *Acarus* & *Tyrophagus* spp.	Sprayed (disinfectant?)
10	3 *Acarus* & *Tyrophagus* spp.	1 *Tyrophagus* spp.	Swept, vacuum, disinfectant & insecticide
11	> 650 *Acarus* & *Tyrophagus* spp.	35 *Acarus* & *Tyrophagus* spp. (33 dead)	Swept, vacuum, disinfectant & insecticide
12	12 *Acarus*, *Tyrophagus* spp. & Glycyphagid mites	None	Swept, vacuum & insecticide
13	>3386 *Acarus* & *Tyrophagus* spp.	1 *Tyrophagus* spp.	Compressed air, swept & insecticide
14	>7624 *Acarus*, *Tyrophagus* spp. & Glycyphagid mites	14 *Acarus* spp. & Glycyphagid mites	Compressed air, swept & insecticide
15	162 *Tyrophagus* spp. & Glycyphagid mites	215 *Tyrophagus* spp.	Swept & vacuum
16	287 *Tyrophagus* spp.	12 *Tyrophagus* spp.	Swept & vacuum

Conclusion

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 cannot be ascertained from this study due to the small sample sizes. 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.

2.3.2 The effect of ultraviolet C radiation on stored product pests

Introduction

An integrated pest management strategy is critical for the safe storage of post-harvest commodities. The use of effective hygiene measures and chemical protectants are integral parts of this strategy. However, the number of pesticides currently approved for the protection of stored commodities from insect and mite pests is very limited and there may be further restrictions as a result of the EU Thematic Strategy for Pesticides. Efficacy may also be affected by the development of pesticide resistant pest populations. Alternative non-chemical control measures are sought which can be incorporated into this pest management strategy.

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. UVC is short wavelength (100 – 280nm) radiation and is primarily used for the disinfection of air, surfaces and liquids from microbial contaminants. The UV destroys the DNA of bacteria and other microbial contaminants, thereby preventing further replication and growth. The use of UVC radiation as a method of pest control, however, has not been extensively investigated due to the perceived risks to human health and the lack of penetration through substrates (Bruce and Lum, 1978). The limited penetration of UVC through substrates, therefore, precludes its use as a treatment on bulk commodities. It may, however, offer potential as a surface hygiene treatment in empty stores.

The efficacy of UVC has been demonstrated against house dust mites and stored product pests (Calderon and Navarro, 1971; Bruce, 1975; Bruce and Lum, 1978; Calderon *et al.,* 1985; Needham *et al.*, 2006; Ghanem and Shamma, 2007; Faruki *et al.,* 2007) although sensitivity varies with species and lifestage (Beard, 1972). It is, however, difficult to make direct comparisons between studies as the level of UV dose achieved is not always stated and UV intensities vary with light sources.

Photoreactivation is an important consideration with UV treatments. Eggs of *Plodia interpunctella* (aged 8 to 48 hours old) placed in lighted areas after exposure to UVC radiation required a longer

exposure period than those placed in the dark to produce an equivalent lethal effect (Bruce and Lum, 1978). For example, the LT_{95} for 8 hour old eggs was 15 minutes when placed in the light after treatment compared to 3.5 minutes when put in the dark. Similarly with *T. putrescentiae*, mites placed in the dark had higher mortalities than those placed in the light following equivalent exposure to UVC (Bruce and Lum, 1978).

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.

Materials and Methods

Beetles

Preliminary experiments used recently collected field strains of each species. However, the number of *O. surinamensis* eggs collected over a 24-hour period was very low. This strain is known to survive the discriminating dose of pirimiphos-methyl. There may therefore be a fitness cost associated with pesticide resistance which may have affected the numbers of eggs produced. It was, therefore, decided to use laboratory susceptible strains in the experiments.

The beetles used were laboratory organophosphate (OP) susceptible strains of *Oryzaephilus surinamensis* and *Tribolium castaneum*. Both were reared in constant conditions of $25 \pm 2^{o}C$ and $70 \pm 5\%$ r.h., without exposure to pesticides.

Mites

Laboratory OP susceptible strains of *Acarus siro* and *Tyrophagus putrescentiae* were used. The strains were reared in constant conditions of $15°C \pm 2^{o}C$ and $90\% \pm 5\%$ r.h., without exposure to pesticides.

Fungus

Spores of the storage fungus *Penicillium verrucosum* were used in the experiments.

UVC light source

A UVP CX-2000 crosslinker was used to generate the UVC at a wavelength of 254 nm. The crosslinker was calibrated using a UVP radiometer with UVX-25 sensor and found to deliver a light irradiance of approximately 9 mW/cm^2. The samples to be treated were placed in a pull-out drawer tray that was pushed back under the light source. During treatment the samples were approximately 9 cm from the light source.

Effect of UVC on beetle eggs

At least two hundred adults of each insect species were placed in separate glass crystallising dishes (20 cm diameter) containing a thin layer of sieved (125 μm) flour. The sides of the dishes used to contain *O. surinamensis* were previously coated with a layer of 'fluon' to prevent the adults escaping. The dishes were covered with nylon mesh held in place with an elastic band and placed in a controlled environment (CE) room set at 25 ± 2°C and 70 ± 5% r.h. for 24 hours. The adults were then removed and the eggs collected by passing the flour through a sieve (180 μm).

Twenty eggs of each species were counted out and put into separate glass petri dishes (48 mm in diameter, 18 mm high). Six replicates were prepared for each UVC dose, including two sets of controls. One set of controls were left untreated. In the other set, the petri dishes were covered with a glass lid, wrapped in foil and then placed under the UVC light source and exposed to the highest energy level used in the tests. This was to assess whether any ozone was generated from the light source, which may have affected mortality.

The dishes containing the eggs to be treated with UVC, were placed singly under the light source (without lids) and exposed to five different energy levels (10,000; 20,000; 40,000; 60,000 and 120,000 μj/cm^2). After exposure, the eggs were transferred into glass bioassay jars (120 ml) half-filled with the appropriate laboratory diet for the species. The jars were put into a CE room set at 30 ± 2°C and 60 ± 5% r.h. with a 15hr light : 9hr dark light regime and incubated until adult emergence.

To assess the potential effect of photoreactivation, experiments were set up as described above but after exposure to UVC, the bioassay jars were incubated in darkness, until adult emergence.

To assess the potential effect of substrate, experiments were set up as described above but the eggs were covered with flour (1 g) after being placed in the petri dishes, but before exposure to the UVC. This aimed to assess the effect of direct and indirect exposure to UVC on egg hatch and insect development.

Effect of UVC on mite eggs

A mixed stage mite culture was sieved (150 μm) to remove any existing larvae and eggs. The nymphs and adults were returned to the culture flask and placed in controlled conditions of 25°C and 70% r.h. for *T. putrescentiae* and 25°C and 80% r.h. for *A. siro*, for 72 hours.

Twenty eggs of each species were then counted out from the mite culture and put into separate mite cells using a single haired brush. The mite cells consisted of a chamber made of a piece of black paper (42.5 cm in diameter). The paper was moulded to create a depression (33 mm in

diameter, 2 mm deep) and secured, using 'Pritt stick' glue, to a glass square (75 x 75 x 4 mm), in the centre of which a hole (35 mm in diameter) had been drilled. The chamber was then covered with a thin glass square (75 x 75 x 2 mm) and secured with bulldog clips. Six replicates for each dose were prepared, including the two sets of controls. One set of controls were left untreated. In the other set, the mite cells were wrapped in foil and then placed under the UVC light source and exposed to the highest energy level used in the tests.

The mite cells containing the eggs to be treated with UVC, were placed singly and uncovered under the light source and exposed to five different energy levels (2000, 4000, 8000, 16000 and 20000 µj/cm^2 for *T. putrescentiae* and 500, 1000, 1500, 2000 and 4000 µj/cm^2 for *A. siro*). After exposure, some mite food (wheatgerm and yeast) was added to the cells, the glass lids were put on and the cells were placed in the appropriate controlled conditions and incubated until adult emergence.

The effect of photoreactivation and substrate was also investigated as described with the beetles except that the eggs were covered with approximately 0.03 g of food.

The effective doses (ED) required to produce 50% and 95% mortality of each pest species were calculated from the dose-response data using the probit analysis program PROBIT (version 7). The linear relationship between the logarithm of the dose and the probit of the percentage response was estimated using the maximum likelihood method (Finney, 1971).

Effect of UVC on egg laying

Pupae of *T. castaneum* were sexed, separated into males and females, put into separate jars containing laboratory diet and incubated until adult emergence. Newly emerged adults of *O. surinamensis* were sexed soon after eclosion, separated into males and females, put into separate jars containing laboratory diet and held until needed for testing. Nymphs of *T. putrescentiae* and *A. siro* were put singly into mite cells containing food, incubated until adult emergence and then sexed.

Single virgin pairs of each species were left for fixed periods of time (6 days for the beetles and 24 hours for the mites) to allow mating, but before egg laying commenced. The males and females were then separated and the female insects and mites were put singly into petri dishes and mite cells respectively. Ten replicates were prepared for each pest species and each treatment (including untreated controls). The females were then exposed to the UVC for periods of time found to be sub-lethal to the adults in preliminary experiments (2 hours for the beetles and 12 seconds for the mites). Food was then added to the petri dishes and mite cells, which were then incubated in the appropriate controlled conditions until the development of the F_1 generation. The

female parent insects were removed after 2 weeks. The numbers of progeny were compared to those produced by the untreated females in the controls.

Effect of UVC on *P. verrucosum*

Preliminary tests used *P. verrucosum* infected wheat grains that had been cut in half and exposed on both sides to UVC doses ranging from 5,000 to 80,000 µj/cm^2. However, limited effect was detected and it was thought that this may have been due to the spores not being in direct contact with the UVC and being shielded by the grain kernel. Therefore, a known number of spores, spread thinly on agar plates were treated so that a more uniform treatment and precise assessment could be undertaken. Other workers have also found that fungal spores are most susceptible to UVC when spread in a monolayer onto an agar surface (Begum *et al.,* 2009).

Four grains heavily infected by *P. verrucosum* were added to 20 ml of sterile distilled water (SDW). The grains were vortex mixed to release the spores into the SDW and the spore levels counted using a haemocytometer. The spore suspension was diluted to give a final concentration of 1x10^4 spores per ml of water.

100 µl of the diluted spore suspension was inoculated onto DG18 plates giving approximately 1000 spores/plate. The 100 µl droplet was carefully spread around the centre of the plate. Care was taken to avoid the plate edges to ensure that no spores were shielded from the UV treatment. Five replicates were prepared for each treatment including two sets of controls (one set untreated and the other set wrapped in foil and treated with the highest dose of UVC). The plates were placed singly and uncovered under the light source and exposed to five different energy levels (5,000, 10,000, 15,000, 20,000 and 25,000 µj/cm^2).

Following UV exposure, the plates were incubated for 24 hours at room temperature (~20°C). From each plate, 100 spores were assessed microscopically and the % spore germination calculated. A spore was classed as germinated if the germ tube was longer than the length of the spore. Where possible, germination counts were repeated after 2 days. This accounted for any delay in germination time caused by the UVC treatment. Colony growth was assessed on each plate after 7 days to ensure the plates had not been contaminated during any stage of the process.

Results and Discussion

Effect of UVC on egg hatch

Oryzaephilus surinamensis

Egg hatch in the control samples ranged from 73% to 81% (Table 24). There was no significant difference in the egg hatch recorded for the two different sets of controls indicating that when shielded from the light source, egg hatch was unaffected.

The mean percentage egg hatch for eggs incubated in the light ranged from 32.5% to 2.5%, when exposed to UVC doses ranging from 10,000 to 120,000 µj/cm^2 with 35% to 3.3% hatch for eggs incubated in the dark (Table 24). There was no significant difference in egg hatch between those incubated in the light and dark. When treated with food, egg hatch ranged from 68% to 54% (Table 24).

The ED_{50} and ED_{95} values for eggs exposed to UVC and incubated in light conditions were 8,456 µj/cm^2 and 96,549 µj/cm^2 respectively compared to 6,726 µj/cm^2 and 91,515 µj/cm^2 when incubated in the dark (Table 25). No probit line could be fitted to the data for the eggs exposed with food because the slope was insignificant, indicating that there was no dose response effect.

Tribolium castaneum

Egg hatch in the control samples ranged from 77% to 92% (Table 24) with no significant difference in the egg hatch recorded for the two different sets of controls.

The mean percentage egg hatch for eggs incubated in the light ranged from 28% to 0.8%, when exposed to UVC doses ranging from 10,000 to 120,000 µj/cm^2 with 23% to 0.8% hatch for eggs incubated in the dark (Table 24). There was no significant difference in egg hatch between those incubated in the light and dark. When treated with food, egg hatch ranged from 44% to 66% (Table 24).

The ED_{50} and ED_{95} values for eggs exposed to UVC and incubated in light conditions were 5,678 µj/cm^2 and 59,069 µj/cm^2, respectively compared to 4,502 µj/cm^2 and 32,009 µj/cm^2 when incubated in the dark (Table 25). No probit line could be fitted to the data for the eggs exposed with food because the slope was insignificant, indicating that there was no dose response effect.

Other workers have reported that the lethal times to reduce egg hatch by 95% in *T. castaneum*, ranged from 181.8 seconds for 24-hour old eggs to 79.4 seconds for 96-hour old eggs (Calderon et al., 1985). These are equivalent to energy levels of 109,080 µj/cm^2 and 47,6400 µj/cm^2 (given that the intensity was stated as being 600 µW/cm^2). These are higher than those calculated in this experiment and may have been due to different methodologies and light sources used.

Tyrophagus putrescentiae

There was no significant difference in egg hatch between the two sets of controls in each experiment. There was, however, a difference in the control egg hatch between the different experiments with 82–92% egg hatch in the tests incubated in the light, 55–61% egg hatch in the

test incubated in the dark and 69–73% egg hatch in the tests with food (Table 24). The reason for this difference is unknown; however, the probit analysis corrected for any control responses.

The mean percentage egg hatch for eggs incubated in the light ranged from 51% to 4%, when exposed to UVC doses ranging from 2,000 to 20,000 µj/cm^2 with 18% to 2% hatch for eggs incubated in the dark (Table 24). There was a significant difference in egg hatch between those incubated in the light and dark suggesting a photoreactivation effect. This effect has also been demonstrated in *T. putrescentiae* by other workers (Bruce and Lum, 1978). When treated with food, egg hatch ranged from 37% to 62% (Table 24).

The ED_{50} and ED_{95} values for eggs exposed to UVC and incubated in light conditions were 2,358 µj/cm^2 and 22,014 µj/cm^2 respectively compared to 669 µj/cm^2 and 14,290 µj/cm^2 when incubated in the dark (Table 25). Respective ED_{50} and ED_{95} values of 22,716 µj/cm^2 and 805,255 µj/cm^2 were calculated for the treatments with food, however there was a lot of variation between replicates and there was a significant difference between the experimental data and the fitted line.

Acarus siro

There was no significant difference in egg hatch between the two sets of controls in each experiment. The mean percentage egg hatch ranged from 44% to 2.5% and 46% to 8% when incubated in the light and dark, respectively, following exposure to UVC doses ranging from 500 to 4000 µj/cm^2 (Table 24). There was no significant difference in egg hatch between those incubated in the light and dark. When treated with food, egg hatch ranged from 74% to 82% (Table 24).

The ED_{50} and ED_{95} values for eggs exposed to UVC and incubated in lighted conditions were 751 µj/cm^2 and 3802 µj/cm^2, respectively, compared to 760 µj/cm^2 and 10,371 µj/cm^2 when incubated in the dark (Table 25). No probit line could be fitted to the data for the eggs exposed with food because the slope was insignificant, indicating that there was no dose response effect.

Table 24. Mean % egg hatch of beetle and mite species exposed to UVC (n=6)

Species	Dose (µj/cm^2)	Mean % egg hatch		
		In Light	In Dark	With food
O. surinamensis				
	Control 1 (untreated)	74.2	80.8	72.5
	Control 2 (exposed to top dose but wrapped in foil)	76	79.2	76.7
	10,000	32.5	35	67.5
	20,000	21.7	15.8	58.3
	40,000	12.5	12.5	63.3
	60,000	5.8	5.8	54.2
	120,000	2.5	3.3	60
T. castaneum	Control 1 (untreated)	81.7	91.7	80
	Control 2 (exposed to top dose but wrapped in foil)	80	90	76.7
	10,000	28.3	22.5	60
	20,000	14.2	11.7	44.2
	40,000	8.3	1.7	60
	60,000	4.2	0.8	53.3
	120,000	0.8	0.8	65.8
T. putrescentiae	Control 1 (untreated)	91.7	60.8	72.5
	Control 2 (exposed to top dose but wrapped in foil)	81.7	55	69.2
	2000	50.8	18.3	61.7
	4,000	29.2	8.3	56.7
	8,000	20	5.8	52.5
	16,000	7.5	2.5	40.8
	20,000	4.2	2.5	36.7
A. siro	Control 1 (untreated)	73.3	75	85
	Control 2 (exposed to top dose but wrapped in foil)	63.3	71.7	80.8
	500	44.2	45.8	81.7
	1,000	34.2	27.5	78.3
	1,500	16.7	25.8	79.2
	2,000	11.7	26.7	74.2
	4,000	2.5	8.3	79.2

Table 25. UVC doses required to produce a 50% and 95% reduction in egg hatch (derived from probit analysis) (n=6)

Species	Test conditions	ED_{50} (µj/cm^2)	95% fiducial limits for ED_{50}	ED_{95} (µj/cm^2)	95% fiducial limits for ED_{95}	Slope ± s.e.	Chi-squared	DF	P-value
O. surinamensis	In light	8456	5041, 11671	96549	68750, 167571	1.56 ± 0.22	26.04	23	0.3
	In dark	6726	3668, 9690	91515	64933, 160642	1.45 ± 0.21	24.58	22	0.32
T. castaneum	In light	5678	3032, 8240	59069	44322, 93034	1.62 ± 0.24	28.76	18	0.05
	In dark	4502	2308, 6568	32009	25486, 44,986	1.93 ± 0.3	10.72	12	0.39
T. putrescentiae	In light	2358	1747, 2938	22014	16541, 33254	1.7 ± 0.18	18.58	18	0.41
	In dark	669	152, 1299	14290	9444, 32208	1.24 ± 0.26	11.62	18	0.87
	With food	22716	13191, 175380	805255	128460, 1.70201 x10^{10}	1.06 ± 0.36	55.2	28	0.0016*
A. siro	In light	751	595, 889	3802	2969, 5555	2.34 ± 0.28	18.98	23	0.7
	In dark	760.3	519, 968	10371	6170, 27538	1.45 ± 0.24	25.94	28	0.58

Effect of UVC on egg laying

Although lower numbers of progeny were recorded with the UVC-treated females of *O. surinamensis*, *T. castaneum* and *T. putrescentiae*, these did not differ significantly from the numbers of progeny produced by untreated females (Table 26). There was, however, a lot of variation between individual replicates which may have been due to several factors e.g. no mating had occurred, variation in egg laying between individual females, death of female during the experiment. The insects also had a habit of running around the edge of the petri dish, which may have shielded them from the effects of the UVC.

Table 26. Mean numbers of progeny (± s.e.) produced following UVC treatment of mated females (n=10, *n=9)

Species	Treatment time	Mean nos of progeny ± s.e.
O. surinamensis	Control	106.4 ± 21.3
	2 hours	83.5 ± 6.4
T. castaneum	Control	92.8 ± 20.9
	2 hours	43.1 ± 22.7
T. putrescentiae	Control	20.9 ± 7 *
	12 seconds	7.4 ± 2
A. siro	Control	21.9 ± 6 *
	12 seconds	1.7 ± 1 *

There was, however, a significant reduction in the numbers of *A. siro* progeny produced by UVC-treated females compared to untreated females. However, in five of the nine replicates the females had died during the incubation period before any eggs had been laid. It is known from the experiments on egg hatch, that *A. siro* was the most sensitive species to UVC and it may have been that a 12 second exposure was too long to produce a sub-lethal effect, even though the females were observed to be active and moving freely immediately after treatment.

Effect of UVC on *P. verrucosum*

There was 100% spore germination in both sets of control replicates. In the UVC treatments, there was 75.4%, 62% and 19% spore germination at 5,000, 10,000 and 15,000 µj/cm^2 respectively, with 0% spore germination at 20,000 µj/cm^2 (Table 27). At 25,000 µj/cm^2, there were no spores present on the plates at all and it was concluded that the UVC had completely destroyed the spores at this dose.

Table 27. Mean % spore germination of *P. verrucosum* exposed to UVC (n=5)

Dose (µj/cm^2)	Mean % spore germination
Control 1 (untreated)	100
Control 2 (exposed to top dose but wrapped in foil)	100
5,000	75.4
10,000	62
15,000	19
20,000	0
25,000	0

The calculated ED_{50} and ED_{95} values were 8,853 and 21,393 µj/cm^2 ,respectively; however, there was a significant difference in the goodness of fit between the doses and the fitted line which may have been due to the limited number of data points, because the top two doses produced 0% germination (Table 28).

Table 28. UVC doses required to produce a 50% and 95% reduction in spore germination of *P. verrucosum* (derived from probit analysis)

ED_{50} (µj/cm^2)	95% fiducial limits for ED_{50}	ED_{95} (µj/cm^2)	95% fiducial limits for ED_{95}	Slope ± s.e.	Chi-squared	DF	P-value
8853	7465, 10149	21393	17833, 28241	4.29 ± 0.53	196.27	15	0.0001*

As with arthropods, the effect of UVC on fungal spores is also known to vary among genera, with spores that are thin walled and have a lighter pigmentation being most sensitive (Begum *et al*., 2009).

Conclusions

These experiments have demonstrated that UVC is effective at reducing egg hatch and spore germination in storage pests. However, the doses required to elicit the responses varied greatly with species. The calculated ED_{95} values for *O. surinamensis*, *T. castaneum*, *T. putrescentiae*, *P. verrucosum* and *A. siro* were 96,549; 59,069; 22,014; 21,393 and 3,802 µj/cm^2, respectively, when incubated in the light.

Although lower ED_{50} and ED_{95} values were recorded for *O. surinamensis* and *T. castaneum* incubated in the dark compared to the light, the difference was only significant for *T. putrescentiae*, suggesting a photoreactivation effect. 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 cracks and crevices. Therefore in order for UVC treatments to be fully effective, the pests must be in direct contact with the UVC for the required

duration or higher doses may need to be applied. Anything that is likely to shield the pest target from the exposure will affect the efficacy of the treatment.

No effect was demonstrated on progeny development with *O. surinamensis*, *T. castaneum* and *T. putrescentiae*, with no difference in the numbers of progeny produced by untreated females and females treated with a sub-lethal dose of UVC. However, there was a large degree of variation in the number of progeny produced by individual females. It was however observed throughout the experiment that development following UVC treatment was slower compared to development in the untreated controls. Lower numbers of *A. siro* progeny were recorded with UVC treated females compared to untreated females, however, over half of the females had died during the incubation period before any eggs had been laid.

Aspects not investigated in this study were the effects of UVC on pheromone degradation, which may offer an additional control technique by disrupting the ability of males to detect females (Bruce and Lum, 1978). However, there may also be implications where pheromone baited monitoring devices are utilised.

A practical application of UVC to a grain surface has been demonstrated by Hidaka and Kubota (2006). The recirculating grain sterilisation equipment consisted of two UV lights, a grain tank and a conveyor system. A thin layer of grain, inoculated with micro-organisms, was sterilised as it passed through the light sources whilst moving along the conveyor system. The time required to obtain a 90% sterilisation rate was 6.3 hours for bacteria (*Bacillus* and *Pseudomonas* spp.) and 5.6 hours for mould (*Aspergillus* and *Pencillium* spp.) using 97 W/m^2. They also found that grain quality was not affected by the UV irradiation used in their experiments. Although this appears to be a practical application of UVC to a grain surface, it is difficult to envisage how the entire grain surface would be treated effectively, as our preliminary experiments showed that fungal spores appeared shielded from the direct effects of the UVC by the grain kernel.

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. Results from this and previous research projects (Defra project AR0604) have found that the conveyor system may be a source of localised pest infestations, despite rigorous treatment and disinfestation of the fabric of the building prior to storage. However, cleaning prior to UVC treatment is an important consideration as UVC has limited penetrative ability and the presence of food, dust and debris will affect efficacy. The costs and safety implications of using UVC should also be considered.

2.3.3 Cooling strategies: current and future implications

Introduction

The simulation program 'Storedry', the fundamentals of which are described by Sharp (1984), includes a mathematical model of the equilibrium type due to Morey *et al.* (1979), originally developed for simulating drying of a deep bed of grain with air at near ambient temperature. This Morey model has been shown to be suitable for calculating the cooling of a deep bed in the HGCA-sponsored project 'BulkCool' (Bruce, 2008), in which calculated temperatures were compared with data for cooling of deep beds of wheat from a doctoral thesis (Epperly, 1989) and from an HGCA-sponsored experiment on oilseed rape at Fera (unpublished data). In the present project, the emphasis has been on implementing modifications to 'Storedry' to allow it to run simulations of cooling such that various strategies and options for controlling insect pests could be explored. To this end, a model developed in section 2.2.2 by Drs Holt and Knight of Imperial College 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.

The simulations produced a very large data set, only a few key values from which are presented here.

Phase 1. Risk of insects in grain

Simulations

Hourly weather data sets for Odiham, Hants, and Wittering, Cambs, from 1998 – 2002 represented respectively a Southern England location where cooling was considered likely to be most difficult to achieve reliably, and a cooler region further north. Data for Waddington, Lincs, for 1951–1970 was also available.

Bed depth was expected to have a significant influence on the success of cooling, and therefore, the influence of depth, from 2 – 6 m, on cooling success was explored. A differential thermostat is considered to be a good method of cooling fan control and is readily available commercially so a 'diffstat' control policy was implemented and the effectiveness of using diffstat settings of 3°C and 5°C throughout the cooling process was examined. For the implementation of this strategy, the

temperature to be compared with the temperature of the ambient air was that from a single sensor at a depth of 0.5 m. If the temperature at that location was more than the differential setting above ambient, the fan was turned on. As the Storedry program uses time steps of 1 h, this meant cooling for 1 h was calculated and then the fan control decision was taken again using the next historical weather data point.

In fact, this strategy caused a problem in the idealised circumstances of the simulation. The grain near the inlet air became warm as it absorbed a small amount of moisture, so its rate of respiration and consequent heat production rose. In one particular year, 1953, when the grain was left with no ventilation for many days because of mild weather late in the year, as might well happen, this single grain layer overheated. In practice, it would lose heat by convection but that was not active in the simulation. The problem could not be resolved, so as an expedient, a one hour period of ventilation was added to the strategy every night at 4 a.m. to ensure this layer remained cool.

Other initial conditions were:- temperature of bed of wheat, 25°C, start date for cooling, 20 August each year, airflow 0.0032 m^3/s.t dry matter (10 m^3/h.t wet matter), concentration of insects (*O. surinamensis*) everywhere in the bed, 1 insect/kg. It was not necessary to use other initial concentrations because in the simulation, the concentration of insects was low enough that it did not influence their growth rate, *e.g.* by generating significant heat or moisture to change conditions in the grain bed, or by competing for resources. This means that results for insect concentration were proportional to initial concentration. So, if initial concentration were 5 insects/kg, results would simply be 5 times greater than if starting with 1 insect/kg.

It should be noted that no movement of the pests was modelled. Insects and mites were assumed to be evenly distributed at the start of the simulation. During cooling, their concentration became dependent on depth in the bed because temperature and relative humidity (r.h.) conditions differed between layers of the grain bed so the pest growth rate was affected, but the pests originating in a grain layer were assumed to remain within that layer.

The model of multiplication of insect numbers was in the form of a multiplier. This was daily rate of increase of the insect on the substrate, expressed in terms of r.h. and temperature. In the model, relative humidity and temperature of the air within the grain layer were used to calculate the daily rate of increase, altered to an hourly rate to suit the model's time steps. The temperature at which the growth rate was 1.0 was in effect the target temperature for cooling. Once this value was achieved, the insect concentration would not increase so the peak concentration had been reached (provided the temperature of the grain layer did not rise again above the critical value).

Although the model predicted negative growth rates at lower than critical temperatures, predictions in this region were by extrapolation from the positive growth rate region and so were not reliable. As the critical temperature for *O. surinamensis* was around 17°C and the target temperature for cooling was 5°C, there was likely to be a reduction in live insect concentration owing to effects of cold. To calculate whether and by how much live insect concentration reduced as temperatures dropped, experimental data (at 10°C and 5°C, 50% and 70% r.h.) were provided (Fleming, unpublished). A probit model was fitted to these data to describe cold mortality. In the software, a transition from the growth model to the cold mortality model was made for each layer once the temperature of that layer reached the critical temperature by the growth model.

Insect concentration is likely to rise from its initial value to reach a plateau once reproduction stops. As cold mortality takes effect, the concentration of live insects would fall, perhaps to below initial concentration. Low peak concentration is desirable so that the insects, whether dead or alive, are not likely to show up in a grain sample. Low final concentration is desirable so that, if any warming of the bed occurred, the insect concentration would take time to increase to a problem level. It was considered that a reasonable value for initial concentration was between 1 insect per 2 kg and 1 per 5kg.

There was also consideration of what concentration of insects would be acceptable once cooling to a target temperature was achieved. The number of insects in a grain sample was required to be zero. So the acceptable concentration would depend on how likely it would be that sampling would capture one or more. It was thought that a concentration of 1 insect per 5kg would be most unlikely to be detected by sampling whereas 5 insect per kg would almost certainly be detected.

A growth model for the mite species *Acarus siro* became available later than the one for *O. surinamensis.* Its critical temperature was around 10°C so the control of mite numbers by cooling would be more challenging. This mite growth model was used for some simulations. Unfortunately, there was no data available for cold mortality of *Acarus siro* so when the temperature in the grain bed was cooled to below the critical temperature, the mite concentration was simply maintained constant, i.e. it was assumed that the *Acarus siro* mites were not killed by cold.

Results and Discussion

Initial simulations showed that, surprisingly, cooling of the grain bed below the breeding threshold temperature was achieved sufficiently quickly that insect concentrations did not reach even 10% above the initial value (the insect growth and cold mortality models were checked and were found to be working correctly.). For this reason, using only the final insect concentration to judge the success of cooling strategies would not discriminate well between cooling strategies that cooled more or less quickly to the critical temperature. Instead, whilst insect concentrations were still

calculated for completeness, it was decided to compare simulation results with the practical advice in the HGCA Grain Storage Guide 2nd edition (HGCA, 2003) (GSG) which sets out temperature targets for cooling grain as follows:- 15°C within 2–3 weeks, 12°C within 4 months and 5°C by the end of December. For initial investigations, the last of these, 5°C by end of December, was implemented as the target. As will be shown, this target is the most demanding one so if it is met, the others are almost certainly met too. The temperature targets in the GSG are likely to be grounded in practical results from stores, in which cooling of the grain is not always even. For example, in zones of the store where airflow is less or where there is warmth from an adjacent structure, cooling would be slower and insects would have longer to multiply.

Key results from the first set of simulations are given in Table 29. The simulations used two locations, Waddington and Odiham representing wheat growing regions in north and south England, two settings of diffstat, 3°C and 5°C, and three values of bed depth, 2m, 4m and 6m. Initial insect concentration was assumed to be 1 insect per kg. Twenty years historical weather data from Waddington (1951–1970) and four years from Odiham (1998–2001) were used. The target for cooling was that the maximum temperature anywhere in the bed should be ≤ 5°C by 31 December, as required by the HGCA Grain Storage Guide.

Target met, % of years. Of the 20 years in each row of the table for Waddington or 4 years for Odiham, this is the % for which the temperature target was met, i.e. the warmest location in the grain bed reached 5°C by end December. A value of 80% indicates that in 4 out of the 20 years the target was not reached before the end of the run, which was at end December in this case.

The remaining columns are for results from years when the target temperature was met and the run terminated. Runs in which the target was not met are not included in the calculation of the minimum, average and maximum values of the parameter over the 20 years, hence the row of ‘not applicable’ where the target was not met in any of the years.

Elapsed time. Time from start of cooling, on 20 August, to when the cooling target was reached.

Fan time. Cumulative time for which fan was run until cooling target was met.

Insect average concentration in bed at end of cooling. Based on the models of insect growth and cold mortality, the average number of live insects per kg in the bed as a whole when cooling target was achieved. The min and max are the results for two of the years out of the twenty years of weather data used for each row in the table. The min is for the year in which cooling was most effective by preventing the pests from breeding and causing high cold mortality. Max was for the year in which cooling was least effective but nonetheless achieved the target.

Insect peak concentration in bed during cooling. This is the result that shows the concentration of pests, live plus dead, that could in principle be found in the grain bed by a sampling test from one particular location. This peak occurs before cold mortality starts to reduce the concentration of live insects, but allows for the fact that the dead bodies would still be present The worst case location in the bed for the worst of the 20 years is selected and the insect concentration at that layer is tabulated as max. Min is from the best case location in the best year.

Table 29. Key results from simulations of successful cooling a bed of wheat at two locations, using two settings of differential thermostat, to control the pest species *O. surinamensis*.

Location	Diffstat setting, C	Bed depth, m	Target met, % of years	Elapsed time, h		Fan time, h			Insect avg concn in bed at end of cooling, /kg		Insect peak concn in bed during cooling, /kg	
				min	max	min	avg	max	min	max	min	max
Waddington	3	6	80	2184	3064	566	722	879	0.22	0.97	1	1.03
		4	85	2185	3064	559	715	873	0.20	0.97	1	1.03
		2	85	2171	3068	506	672	797	0.22	0.96	1	1.03
	5	6	55	2246	3198	395	466	558	0.24	0.93	1	1
		4	60	2246	3179	389	462	537	0.28	0.91	1	1
		2	40	2317	3083	364	419	485	0.20	0.91	1	1
Odiham	3	6	100	2269	3137	552	643	733	0.82	0.99	1	1.04
		4	100	2282	3137	543	642	724	0.81	0.98	1	1.04
		2	100	2603	3142	542	614	688	0.79	0.87	1	1.04
	5	6	50	2991	3179	468	475	481	0.64	0.79	1	1
		4	50	2987	3179	468	472	475	0.62	0.78	1	1
		2	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

The cooling target was that the maximum temperature anywhere in the bed should be equal to or less than 5°C by 31 December of that year. As seen in column headed ‘Target met, % of years’, at Waddington the success rate varied between 40 and 85% of the 20 years explored, the 3°C diffstat setting being much more successful than the 5°C. At Odiham, the 3°C diffstat setting was also very successful, with the cooling target being met in all four years. The 5°C diffstat setting worked poorly. It was clear from this investigation that the diffstat setting was a very influential parameter. The diffstat setting that was less successful in both locations resulted in the fan being on for less time, the inference being that the less successful setting did not allow sufficient fan running time to cool the bed to the target.

It is clear that bed depth did not have much influence on cooling success. Fan running time did not change between 4 m and 6 m, but was reduced at 2 m though this is not really deep enough for cooling in commercial practice. But recall that the airflow was kept constant *per tonne*, which means the 6 m bed was ventilated with three times the volumetric airflow of the 2 m bed. At the airflow used, recommended in the Guide, a cooling front would pass through the bed in a relatively short time, *e.g.* 72 h, so even a short period or series of periods of cool weather would suffice. The resistance of a deeper bed would result in the incoming air being heated more but the difference in temperature rise between the 2 m and the 6 m bed was very small.

The columns of Table 29 showing insect average concentration demonstrate that the initial concentration of 1 per kg was reduced by the end of cooling to a value less than 1/kg even in the 'worst' layer in the bed. For the best layer, probably but not necessarily the layer nearest the air inlet, the concentration had fallen well below 1/kg, showing that cold had killed off a significant proportion of the insects. At Waddington the minimum insect concentration had been reduced to around 0.2/kg, whereas at Odiham it was still around 0.6–0.8/kg. This is a result of colder weather at Waddington. The maxima do not reflect this difference because in all cases they will have been for the layers that had just reached 5°C when cooling was completed.

The final two columns record the maximum insect concentration, wherever and whenever it occurred in the bed, and thus they record a peak. The modest values, up to 1.04, show that insects only managed a very small increase in numbers before breeding was stopped by the fall in temperature. For the *O. surinamensis* species, breeding stops at about 17°C according to the model of Holt and Knight, so the low peak shows that the maximum temperature everywhere in the bed of wheat was reduced below 17°C sufficiently quickly to control insect activity.

The elapsed time columns show little variation, with a minimum of around 2200 hours (3 months) needed for success. The maximum is less significant because it simply approaches the time until end December, 3198 h, required by the worst successful case.

Fan running time was influenced by location and diffstat setting. Location determined how much cool weather was available and diffstat setting how much of it the fan was able to use. It appears odd that at Waddington the average fan running time was longer than at Odiham, a more southern location. Comparing weather at the two locations, it was 0.97°C colder at Waddington than at Odiham over the period from harvest to end of the year. However, the air conditions selected by the diffstat when set at 3^{o}C were within 0.1°C at the two locations. The difference in cooling fan times may be due to the inefficiency of the enforced 1h running at 4a.m. used to control self-heating.

Of course, including only results from the years in which cooling to the target was successful does not give the whole picture because it does not show what happened in those years when the grain bed did not cool to the target. When the simulation reached the end of the calendar year, i.e. the target temperature had not been reached, a measure of the closest approach was needed. This was the lowest temperature during the whole cooling process of the warmest layer anywhere in the bed. Figure 15 shows results for all 20 years simulated, whether successful or not, for Waddington, diffstat settings of 3°C and 5°C and a 6 m depth of bed. Values of the maximum grain temperature anywhere in the bed during the run are presented in ascending order for all 20 years. Successful runs were terminated once this maximum temperature had fallen below 5°C so that these runs form a horizontal line just below 5°C, after which the temperatures for the unsuccessful runs form a rising line above 5°C. The worst result is at the right hand side of the graph. This way of displaying the results gives a good picture of not only how many of the years were successful but how far the failed years were from success. The lowest value of maximum bed temperature during cooling, as shown, did not necessarily occur at the end of the cooling time, i.e. 31 December, because the bed may have warmed from this minimum as the diffstat continued to operate. However, the minimum is a useful measure of the performance in failed years because it shows how close to the target the strategy approached.

In Figure 15, diffstat settings of 3°C and 5°C are compared at Waddington for a 6 m deep bed. Four of the 20 years did not meet the target with the 3°C diffstat setting, though only three were significantly above the 5°C line. With the 5°C setting, nine years were above 5°C, seven significantly so. So it is clear that the 3°C setting is the better in terms of pass/fail and also in closest approach to target. Table 29 shows that at 3°C the diffstat ran the fan for an average of 722 h whereas at the 5°C setting only 466 h were run. This drop in fan running time reduced success from 80% to 55%, and clearly resulted in insufficient exposure of the bed to cool air. In Figure 16, in which the same comparison is made for the four years at Odiham, the superiority of the 3°C diffstat setting is again clear.

Although the target temperature was not met in some of these runs, the temperature was none the less reduced such that activity of the insect species being modelled, *O. surinamensis*, was well controlled.

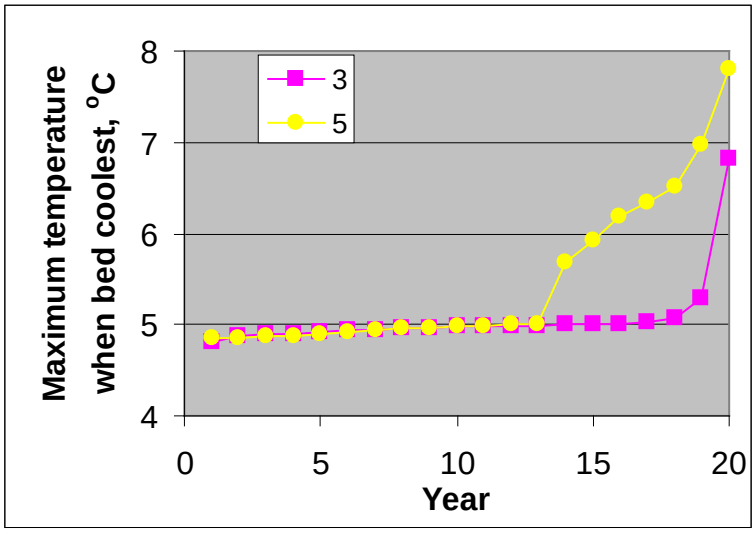


Figure 15. Waddington, cooling a 6m bed to a target of warmest layer less than 5 °C with a diffstat at 3 and 5 °C. The maximum bed temperatures at end of cooling for each of the 20 years were sorted to show the pattern.

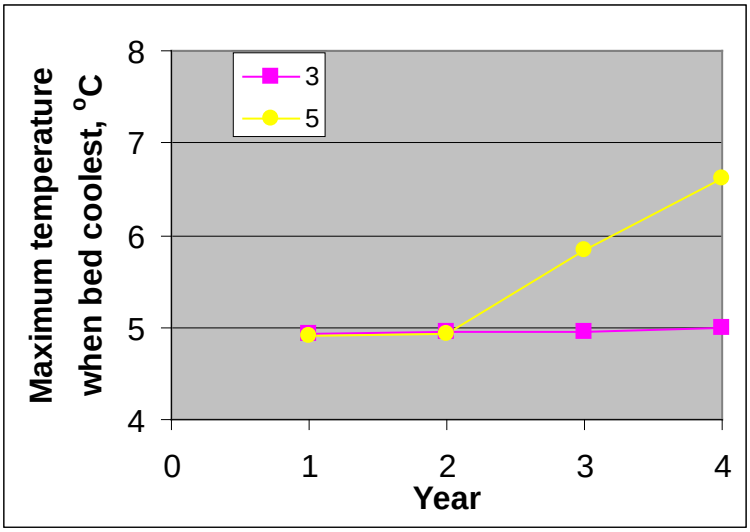


Figure 16. Odiham, cooling a 6 m bed to a target of warmest layer less than 5 °C with a diffstat at 3**°C** and 5°C. The maximum bed temperatures at end of cooling for each of the 4 years were sorted to show the pattern.

A simulation is necessarily a simplification of the real world because it cannot include all influences. Storedry is a one-dimensional model and as such cannot represent influences on cooling of grain from its proximity to the edge of the store, for example, of having grain piled against a warmed wall. If, however, something influences all the store, not just the edges, it should be considered for inclusion in the simulation. The structure of the store is an example in that air from the atmosphere may have to traverse under-floor ducts which will not be at the same temperature as the incoming air. They will tend to warm the air if cold air is selected, and thus reduce the cooling effect on the grain bed. The size of this 'thermal mass' between the fan and the grain bed will depend on the particulars of the store but may in some cases compromise the

cooling, particularly towards the final target when the air is cold but heat from the ground may be conducted to the base of the floor ducts, and hence warm the air. Such a thermal mass was not included in Storedry , being beyond the scope of this study. The effect of this thermal mass could be large enough to influence the results, and could be included in the simulation. Its inclusion should be considered in order to improve the model further.

It was also noted that absorption of moisture by the layer of grain closest to the air inlet was significant, sufficient in many cases to cause the model of Ochratoxin A risk within Storedry to indicate a problem. This absorption from the cool and sometimes humid inlet air had been noted previously in validating studies of both Storedry and BulkDry, developed in a project by HGCA. The degree to which absorption occurs may be less in practice than predicted by simulation but, because even a thin layer of re-wetted grain could harbour fungi and mycotoxins, this is an aspect of cooling that needs clarifying.

Conclusions from Phase 1

1. Simulation of cooling at Odiham and Waddington locations showed that the setting of the differential thermostat was the most important element in determining whether cooling was successful and the number of fan running hours needed. A 3°C diffstat setting was significantly more successful than a setting of 5°C.

2. In those years where cooling achieved the Grain Storage Guide target bed temperature of 5°C by end December, the growth of the pest species *O. surinamensis* was very well controlled. Even when the final stage of cooling was comparatively slow, the critical temperature for this pest species of about 17°C was reached quickly. As a result the growth of this pest in the bed was controlled and, in all cases, simulated the final concentration of live insects which was below the initial value, owing to cold mortality effects.

3. Adsorption of moisture by grain near the air inlet may be problematic.

Phase 2. Effect of cooling strategies on risk of insects

Simulations

From results of Phase 1, it was clear that the strategy for operating the cooling fan was an important factor in the success of cooling, so a range of strategies was explored in Phase 2. Other factors were varied one at a time to examine their effect on success in reaching target temperature.

Table 30 summarises the simulation runs carried out for the Odiham location. Each line shows a set of initial conditions and a strategy that was run for all four year's weather data for that location. Electricity for all the simulations in this report was priced at 20 p/kWh on-peak rate and 5 p/kWh off-peak (0:00 to 7:59).

Table 30. Simulation runs carried out to investigate effect of changing variables. Runs are all at Odiham for wheat of initial m.c. = 14.5% with initial insect or mite concentration = 1/kg. Target maximum temperature = 5 ^{o}C. Years simulated 1998–2001. Colour shows which variable was changed for that run.

Run	Cooling strategy	Diffstat: Temp vs plenum	Time-clock	Start date	Bed depth, m	Airflow, m^3/s/tdm	Initial grain temp, ^{o}C	Comments
1	Diffstat at 4°C	at 0.5 m +1h	on 0:24	20-Aug	4	0.0032	25	Temperature sensor at 0.5m from top of bed. Fan on for 1h at 4a.m.
2	Diffstat at 4°C	at 1.5 m +1h	on 0:24	20-Aug	4	0.0032	25	Temperature sensor at 1.5m from top of bed. Fan on for 1h at 4a.m.
3	Diffstat at 4°C	avg + 1h	on 0:24	20-Aug	4	0.0032	25	Temperature averaged through bed. Fan on for 1h at 4a.m.
4	Diffstat at 4°C	max + 1h	on 0:24	20-Aug	4	0.0032	25	Temperature at warmest location in bed. Fan on for 1h at 4a.m
5	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0032	25	Temperature averaged throughout bed.
6	Diffstat at 4°C	max	on 0:24	20-Aug	4	0.0032	25	Temperature at warmest location in bed.
7	Diffstat at 4°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Temperature sensor at 0.5m from top of bed.
8	Diffstat at 4°C	at 1.5 m	on 0:24	20-Aug	4	0.0032	25	Temperature sensor at 1.5m from top of bed.
9	Diffstat at 6°C	avg	on 0:24	20-Aug	4	0.0032	25	
10	Diffstat at 2°C	avg	on 0:24	20-Aug	4	0.0032	25	
11	Diffstat at 3°C	avg	on 0:24	20-Aug	4	0.0032	25	
12	Diffstat at 5°C	avg	on 0:24	20-Aug	4	0.0032	25	
13	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0032	25	As File 5 but with full output
14	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0048	25	Airflow increased by 50%
15	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0024	25	Airflow reduced by 25%
16	Diffstat at 4°C	avg	on 0:8	20-Aug	4	0.0032	25	Fan only allowed off peak
17	Diffstat at 4°C then 6°C at <9°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Diffstat setting increased when maximum temperature in bed falls below 9^{o}C

18	Diffstat at 4°C then 5°C at <9°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Ditto
19	Diffstat at 4°C then 7°C at <9°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Ditto
20	1st 72h fan on then Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0032	25	Continuous ventilation for 3d then diffstat used
21	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0032	25	*Acarus siro* mites, not *Oryzaephilus surinamensis* insects
22	Diffstat at 4°C	avg	on 0:24	20-Aug	6	0.0032	25	Deeper bed
23	Diffstat at 4°C	avg	on 0:24	20-Aug	2	0.0032	25	Shallower bed
24	Diffstat at 4°C	avg	on 0:24	20-Jul	4	0.0032	25	Cooling starts earlier
25	Diffstat at 4°C	avg	on 0:24	20-Sep	4	0.0032	25	Cooling starts later
26	Diffstat at 4°C	avg	on 0:24	20-Aug	4	0.0032	35	Higher initial temperature
27	Diffstat at 15°C	avg	on 0:24	20-Aug	4	0.0032	25	High setting, testing insect model
28	Diffstat at 4°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	As File 7 but with full output
29	4°C until T at 0.5m=7°C, then 2°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Full output
30	Diffstat at 4°C then 6°C at <7°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Diffstat setting increased
31	Diffstat at 4°C then 6°C at <11°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Ditto
32	Diffstat at 4°C then 6°C at <8°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Ditto
33	Diffstat at 4°C then 6°C at <10°C	at 0.5 m	on 0:24	20-Aug	4	0.0032	25	Ditto

Results and Discussion

Table 31 summarises the results of the simulation runs outlined in Table 30. Columns in Table 31 are as explained for Table 29 with the addition of the following.

Closest approach when target missed. This is the minimum value of the warmest temperature in any of the grain beds simulated during the 20 years, and indicates how far the cooling strategy was away from success. It shows the temperature that could have been achieved in that worst year if ventilation had been stopped at that point. But since it was not (necessarily) at the end of December, the cooling strategy would have continued to operate and the bed may have ended the year warmer than this value. Where there is 100% success in the previous column, this value is 5^{o}C because all beds were cooled to this value.

Total electricity. Cost of electricity to power the fan, the only energy using device in the cooling system, for on- and off-peak usage combined.

Table 31. Results for simulation runs at Odiham, Hants, outlined in Table 29. The Run numbers in each Table correspond.

Run	% years when target reached	Closest approach when target missed, °C	Elapsed time, h		Fan run time, h			Total electricity, p/tonne		Average *O. surinamensis* concentration in bed at end of cooling, /kg		Max peak *O. surinamensis* concentration in bed during cooling, /kg	
			min	max	min	avg	max	min	max	min	max	min	max
1	50	5.7	2867	3137	544	547	550	2.8	2.9	0.73	0.82	1.00	1.00
2	50	5.7	2919	2983	426	453	479	2.2	2.4	0.58	0.90	1.00	1.00
3	75	5.1	2939	3180	429	466	502	2.1	2.5	0.58	0.86	1.00	1.00
4	100		2269	3127	635	755	836	3.6	4.9	0.83	0.98	1.00	1.07
5	100		2799	3150	234	255	279	1.2	1.5	0.45	0.83	1.00	1.14
6	100		2273	3106	383	416	470	2.2	2.8	0.75	0.94	1.00	1.07
7	100		2551	3128	318	358	399	1.9	2.3	0.71	0.81	1.00	1.08
8	100		2583	3130	253	286	311	1.4	1.7	0.67	0.82	1.00	1.10
9	0	8.1	3198	3198									
10	100		2484	3092	446	513	589	2.5	3.4	0.75	0.84	1.00	1.09
11	100		2580	3110	305	329	357	1.7	1.9	0.69	0.82	1.00	1.11
12	25	6.3	3198	3198	224	224	224	1.1	1.1	0.43	0.43	1.00	1.00
13													
14	100		2583	3129	163	181	198	1.6	2.1	0.53	0.78	1.00	1.08
15	50	5.3	2933	3156	338	346	353	1.1	1.2	0.61	0.78	1.00	1.00
16	0	7.1	3198	3198									
17	100		2801	3136	264	329	362	1.6	2.0	0.38	0.87	1.00	1.08
18	100		2580	3125	274	335	370	1.7	2.0	0.72	0.84	1.00	1.08
19	50	6.9	2992	3177	356	358	360	1.9	2.0	0.52	0.75	1.00	1.00
20	100		2799	3149	263	292	319	1.5	1.8	0.44	0.82	1.00	1.07
21	100		2799	3150	234	255	279	1.2	1.5	1.18	1.18	1.00	2.49

22	75	5.0	2799	3151	237	259	275	2.1	2.7	0.67	0.84	1.00	1.00
23	100		2799	3148	235	256	282	0.7	0.9	0.44	0.83	1.00	1.13
24	100		3543	3894	270	290	313	1.3	1.6	0.44	0.80	1.00	1.17
25	100		2055	2405	224	257	278	1.3	1.6	0.48	0.85	1.00	1.13
26	100		2798	3147	255	279	307	1.4	1.7	0.49	0.87	1.00	1.15
27	0	24	3198	3198						11.6	13.4	48.7	56.9
28													
29	100		2241	3127	353	396	514	1.9	3.1	0.76	0.98	1.00	1.08
30	50	5.1	2799	3140	360	368	376	2.0	2.1	0.73	0.73	1.00	1.00
31	75	5.03	2801	3150	260	315	352	1.6	1.9	0.62	0.85	1.00	1.00
32	100		2800	3146	272	334	369	1.7	2.1	0.40	0.87	1.00	1.08
33	100		2801	3149	262	321	359	1.6	1.9	0.42	0.86	1.00	1.08

For those runs where not all the years, out of 4, reached the cooling target, the lowest value of the maximum bed temperature is shown in column 3. For those runs were no years were successful, no data is given (except for Run 27, see comment immediately below).

Effect of inadequate cooling. To test the model when the cooling was completely inadequate, a diffstat setting of 15°C was used to operate the fan (Run 27), which gave the expected result of cooling the bed only to an average temperature of about 15°C above ambient. Only 42 and 52 fan hours were run, the cooling target of 5°C was of course missed and by the end of December the concentration of insects was at the least 11.6/kg and at the worst layer was 57/kg. This important test of the simulation confirmed the expected result that, with minimal cooling, the insects would multiply quickly to an unacceptable concentration.

Depth of grain bed temperature sensor. If only one sensor is available for measuring grain bed temperature, which location is most suitable? Runs 1 and 2 compared sensor depths of 0.5m and 1.5 m in a bed 4 m deep. A differential temperature of 4°C was used, with an additional hour of fan operation from 4–5 a.m. Table 3 shows that for both runs, in only 50% (2 out of 4) years was the target maximum temperature of 5°C achieved. The highest temperature in the years when target was missed was 5.7°C, so the target was not missed by a large margin. Positioning the sensor at 1.5 m, run 2, resulted in lower fan running times (an average of 453 h against 547 h). The fan was the only device using energy so energy costs were proportional to fan running time. Insect concentration was controlled in all years with either sensor position.

Grain bed temperature signal. With several sensors, it is possible to use the average temperature in the bed (run 3) or the maximum temperature (run 4) or values approximating to these derived from a finite number of sensors. Comparing runs 3 and 4 with runs 1 and 2, the target maximum temperature was reached 75% and 100% of years in runs 3 and 4 so these arrangements for grain temperature signal were better. Where the average was used, the one year that missed target only did so by 0.1°C but the fan running times were much shorter (a maximum of 466 h *vs* 755 h) so the use of the average bed temperature was adopted for a number of later runs.

Effect of additional 1h ventilation. The additional hour, introduced to avoid problems with a particular year of the Waddington data set, was not necessary for the Odiham set, so the conditions of runs 1–4 were repeated in runs 5–8 without it. Perhaps surprisingly, the performance improved significantly without this additional fan hour and all years reached target bed temperature. Using the average bed temperature to drive the differential thermostat control was still the best option, and cutting out the extra hour greatly reduced the average fan hours over the four years from 466 h to 255 h. Even though the temperature at 4 a.m. is likely to be cool, there must have been many occasions when the bed warmed during this hour.

For the rest of Phase 2 investigation of strategy, a diffstat set at 4°C and based on the average bed temperature was not improved on but for reasons of simplicity a single sensor may be preferable. In that case the sensor location of 1.5m was better.

Effect of diffstat setting. A setting of 4°C had been found effective in exploratory runs, not reported, but to check this, settings of 2, 3, 4, 5, and 6°C were compared in runs 10, 11, 5, 12 and 9, respectively. In each case, average grain bed temperature was used for the diffstat signal. Settings of 5°C and 6°C performed poorly, with only 25 and 0 % of years achieving target. Fan running hours were low (the values in Table 3 for run 12 are for the one successful year) which suggests that the differential was too demanding and so not enough ventilation time was permitted by the strategy. Runs with the diffstat at 2°C and 3°C performed better in that target maximum temperature was achieved in all four years, but at a cost of greater fan average running time than for 4°C (329 h for 3°C versus 255 for 4°C). So 4°C was the best setting, given the other parameters (location, range of years, starting date etc.)

Because using average bed temperature would require more sensors, a single sensor at 0.5 m below the bed surface was used for Runs 17–19 which explored if changing the diffstat setting during the cooling process could improve cooling or energy use. All three runs used an initial differential of 4°C, but when the bed temperature at 0.5 m below the surface dropped below 9^{o}C, the differential was increased to 6°C, 5°C and 7°C, respectively, to reduce the likelihood of the fan turning on. Results showed that increasing the differential to 7°C only gave success in 50% of years but using a diffstat setting of 6°C or 5°C gave 100% success with fewer fan hours compared with run 7 in which the differential was not changed from 4^{o}C (329 h for run 17 vs 358 h for run 7).

The bed temperature at which the diffstat setting was altered was explored. The strategy of run 17 was used, i.e. 4°C differential changed to 6°C, but the change was done when the temperature at 0.5 m depth reached 7°C, 11°C, 8°C and 10°C, in runs 30–33. When the differential was changed at a bed temperature of 11°C, only 75% of years succeed and when at 7°C, only 50% of years (though the target was missed by only fractions of one degree). The three settings 8°C, 9°C and 10^{o}C gave very similar results to each other, showing that the reduction in fan running hours was not very sensitive to the temperature at which the differential was changed. All three used the fan for fewer hours than run 7 where 4°C was used throughout so the results point to a benefit in increasing the differential by 2°C at a sensed bed temperature of between 8 and 10°C.

In run 20, the fan was forced to stay on for the first 72 h of elapsed time and then a diffstat setting of 4°C was used. Compared with run 5, in which the 4°C differential was used throughout, this strategy used more fan hours on average (292 vs 255) in the same elapsed time.

To check that adjusting the differential upwards was correct, in run 29, it was reduced from 4°C to 2°C once bed temperature reached 7°C. Compared with leaving the diffstat setting unchanged (run 7) this strategy increased the average fan running time from 358 to 396 h so was not effective.

Effect of airflow. The airflow used was 0.0032 m^3/(s.tonne dry matter) which for grain at 14.5% moisture content is close to 10 m^3/(h.tonne wet matter), recommended in the HGCA Grain Storage Guide. Runs 14 and 15 used airflows of 150% and 75% of this figure. At 75% flow, 7.5 m^3/(h.tonne wet matter), cooling was successful in only 50% of years, despite increased fan time, showing the risks of having too low an airflow. At 150%, all years were successful (as would be expected given that was the case with 100% airflow) and cooling was achieved with fewer fan hours on average than when using the recommended airflow (181 vs 255 h) but because the fans were forcing more air through the bed a higher pressure was needed and so about 35% more energy was used. Although cooling was completed in less elapsed time (a maximum of 3129 vs 3150 h), the difference was small and did not justify the additional energy. Hence, within the range of airflow used here, the recommended value of 10 m^3/(h.tonne wet matter) is confirmed as optimum for the location, years and other conditions used.

Night-time only operation of fan. Operating the fan only in the period when off-peak electricity prices applied was examined in run 16, using the simple 4°C diffstat strategy with a timeclock allowing the fan to run only between 0:00 and 7:59 a.m. This strategy did not allow the fan to use any opportunities between 8 a.m. and midnight that could achieve useful cooling so, if successful, cooling would be expected to take more elapsed hours but to cost less. Simulation showed none of the years succeeded in cooling to the 5°C target, the worst case being 7.1°C. This did not appear to be a promising strategy.

Effect of bed depth. All the runs in Phase 2 to this point have been simulated on a bed 4 m deep so for comparison, cooling of beds 6 m (run 22) and 2 m (run 23) deep were carried out. Run 22 succeeded in 75 % of years (the failed case failed by 0.1°C), run 23 in 100 %, and the average fan hours, 259 and 256 h, were close to the 255 h for a 4 m bed. The similarity between these runs of the fan hours required and the elapsed time needed to reach the target shows how much the cooling is dependent on the opportunities presented by the weather and selected by a good strategy, and how little on depth. This is because it is relatively quick to propagate a cooling front through the bed, whether shallow or deeper, once suitably cool air is available. Cost (max of the 4 years) per tonne rose from 0.9 to 1.5 to 2.7 p/tonne when depth was increased from 2 to 4 to 6 m. For the 6 m bed compared with the 2 m, the fan running time was similar but the volumetric flow rate would be 3 times higher and the pressure drop against which the fan delivered the air would be more than 3 times greater, so the energy required, proportional to the product of pressure and

flow, would be about 10 times greater. The 6 m bed contained 3 times the grain per unit area so the cost per tonne would come down from 10 times to 3.3 times greater for a 6 m bed compared to a 2 m bed. The above figures are in line with this explanation. It was concluded that 4 m was a suitable depth to use for the rest of this study.

Effect of start date. Runs 24 and 25 simulated cooling starts on 20 July and 20 September each year, for comparison with the 'standard' start date of 20 August used in run 5. Again the simple 4°C diffstat strategy was applied and an initial grain temperature of 25°C was assumed in all three runs. All the runs were successful in reaching the target temperature in the bed of 5°C but more fan hours were needed on average for a July start (290 h) than for August or September (255 h and 257 h). The increase in elapsed time from July – August – September of about 750 h each month simply arises because the cold weather to complete cooling was not available until December whenever cooling started.

Effect of initial grain bed temperature. The bed in run 5 was at 25°C initially so a bed at 35°C, run 26, was simulated for comparison. A similar time elapsed, for the same reason as for the previous point, but more fan hours were run (279 h vs. 255 h on average) as would be expected with a hotter bed. The insect concentration shows that bed temperature was reduced fast enough to control insect concentration, even starting at 35°C.

Effect of pest species. All the runs to this point used models of growth and cold mortality of *O. surinamensis*. A model for growth of *Acarus siro* became available late in this work so there was a brief opportunity to look at control of that pest species. Unfortunately, no cold mortality model was available so the assumption was made that the concentration of mites was unaffected by cold. The growth model predicted that growth would stop at a temperature between 10°C and 15°C depending on relative humidity. Results from run 21 for cooling time and cost were the same as for run 5, being unaffected by the activity of the pests in the concentrations used here. (For high concentrations of mites, heat and moisture released would be significant but those physical effects were not relevant here and were not modelled.) From an initial concentration of 1/kg, mite final concentration was on average 1.18/kg and the peak numbers in the layer slowest to cool (probably the uppermost layer) was 2.5/kg. This result shows that the mites would continue to breed when the insects had been stopped by cooling, but the increase in numbers was predicted to be modest.

Effect of weather as represented by location. Insufficient resource was available to carry out a wide range of runs using the second weather data set from Wittering, Cambs, but given the success of the simple diffstat strategy with Odiham weather data, simulation at Wittering focused on testing this strategy. As Wittering is further north and likely to be a little cooler, one would expect the strategy to succeed and perhaps with less elapsed time and fan time. Table 32 shows the three

runs, 34–36, in which the diffstat settings were 4°C, 3°C and 5°C, respectively. All other variables were as Table 30, run 5.

Table 32. Results for simulation runs at Wittering, Cambs, using diffstat settings 4**°C**, 3**°C** and 5 °C, respectively. Other variables were as Table 29, run 5.

Run	**% years when target reached**	**Closest approach when target missed, °C**	**Elapsed time, h**		**Fan run time, h**			**Total electricity, p/tonne**		**Average *O. surinamensis* concentration in bed at end of cooling, /kg**		**Max peak *O. surinamensis* concentration in bed during cooling, /kg**	
			min	**max**	**min**	**avg**	**max**	**min**	**max**	**min**	**max**	**min**	**max**
34	100	5.0	2914	3119	225	257	297	1.1	1.6	0.41	0.75	1.00	1.12
35	100	5.0	2321	3078	274	332	404	1.5	2.3	0.67	0.83	1.00	1.09
36	25	5.9	3136	3136	234	234	234	1.2	1.2	0.72	0.72	1.00	1.00

The 4°C diffstat setting produced good results at Wittering (run 34), with the target being met in 100% of the four years, and with almost identical fan running time (257 h) to that at Odiham (255 h) averaged over the four years. The average temperature of the air at Wittering was lower than at Odiham but only by 0.13°C, averaged over the four years weather from mid-August to end November. This temperature difference was probably less significant that variations in the occurrence of cool weather between locations.

As at Odiham, the settings of 3°C (run 35) and 5°C (run 36) were less successful than 4°C. At 3°C, cooling was successful in all years but 75 h more fan time was used. At 5°C, only 25% of years succeeded, though the target was missed by less than 1°C even in the worst case. Therefore, the same strategy, the 4°C diffstat, was best at both locations.

The insect species modelled here (*O. surinamensis*) was controlled quite readily by all the cooling treatments explored, but in practice, other species of pest would be present in the grain store. Also, uneven cooling caused perhaps by dust cones, hot spots in the grain, warmer parts of a structure and other factors may all make cooling in practice more difficult. Recommendations for target temperatures and times for cooling given in the HGCA Grain Storage Guide (HGCA, 2003) (GSG) have been developed from practical trials to ensure all species of insect and mite pest are controlled. Therefore, the grain temperatures predicted by simulation using the best strategy from Phase 2 were compared with the GSG targets (Table 33). For this comparison, the year 1998 at Odiham was selected as typical (one of four years in run 5 of Table 31). The simulated temperatures differed between layers of the bed so both the average and the maximum value anywhere in the bed could be examined. The GSG is not specific, simply specifying a single value for bed temperature because in practice distinctions between locations in a grain bed would be less, owing to factors not simulated such as variations in cooling between parts of the store and convection-driven air currents when the fan is off. However, since both the maximum and average temperatures were available from the simulation, both were included in Table 33.

When the average temperature was used for comparison with the GSG targets, Table 33 shows all three targets were easily met. The first GSG target, that the grain bed should be cooled to 15°C within 2–3 weeks of harvest, was not quite met if the maximum grain temperature in the bed calculated by the simulation was used. Grain maximum temperature fell to 17.3°C within 10 days and remained there for 12 days until conditions were cool enough to cause the fan to run. Maximum temperature then fell to target within 28 h so the target was only just missed. The 12°C and 5°C targets were comfortably met. It was concluded that the strategy of using a diffstat with a setting of 4°C was successful in meeting the GSG targets for this example year. Figure 17 shows the time course of the grain temperature. The temperatures at top, middle and bottom of the bed fell in steps when conditions were cold enough to trigger the fan operation. In between such

periods, the grain bed temperature remained constant because there was no ventilation. (Respiratory heating of the wheat was included in the simulation but the rate is very low because of the low moisture content and temperature so the heat released was too small to cause significant increase in temperature.)

Table 33. Comparison of simulated cooling with recommendations in the HGCA Grain Storage Guide (HGCA, 2003) for Odiham, 1998, with 4°C diffstat cooling strategy

Target in Grain Storage Guide	**Time for <u>maximum</u> bed temperature to achieve target**	**Time for <u>average</u> bed temperature to achieve target**
15°C within 2–3 weeks of harvest	3.4 weeks	1 week
12°C within 4 months	2.0 months	1.5 months
5°C by end December	Mid-December	Mid November

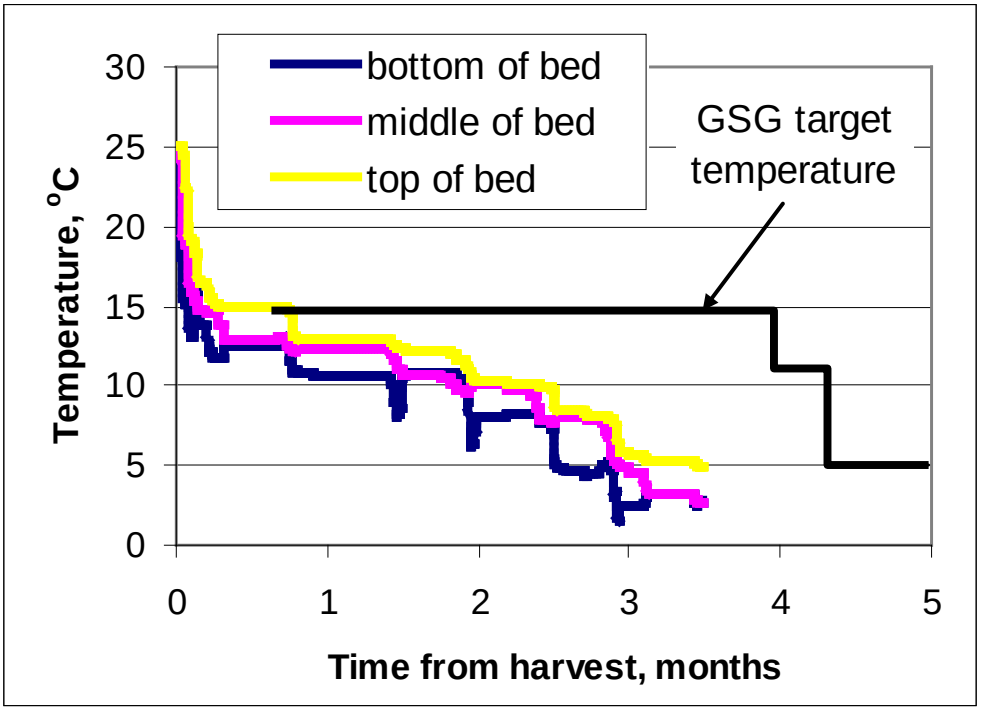


Figure 17. Time course of temperature at bottom, middle and top of a 4 m deep bed at Odiham in 1998, simulated with a differential thermostat set at 4°C comparing ambient temperature and average bed temperature.

Conclusions from Phase 2

1. Control of pest species *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. Because control was straightforward, the cooling target of the GSG was used as a more sensitive test of cooling.

2. A differential thermostat set at 4°C was the most effective method of controlling cooling fan operation, both at Odiham and at Wittering locations. It was clear that this device took full advantage of cool periods whenever they occurred.

3. Using the average bed temperature as the signal with which ambient temperature was compared resulted in the fewest fan running hours to achieve the cooling target, 255 h at Odiham and 257 h at Wittering. Using a single temperature sensor at 1.5 m or 0.5 m depth gave equally good cooling success, but required more fan hours.

4. Compared with using a 4°C diffstat setting throughout cooling, cooling could be achieved with fewer fan hours by increasing the setting to 5°C or 6 ^{o}C when the temperature at the sensor location reached 10°C – 8°C as cooling progressed.

5. The airflow recommended in the GSG 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.

Phase 3. Risk of insects under climate change scenarios

The objective of this phase was to examine the extent to which predicted climate warming would affect the success of controlling insect pests by cooling with ambient air. The most successful strategy for fan operation that emerged from the work of phase 2 was applied to weather data that captures changes in temperature and relative humidity likely due to climate change.

Weather data

In July 2009, the UK Climate Projections 09 (UKCP09) became available, including a facility to allow users to generate weather data predicted for a range of future scenarios, locations and other options. The Weather Generator (WG) included the facility to produce hourly data, as required by the Storedry model. Hourly data values are formed by disaggregating daily data to a statistically established pattern and although this relationship between the hourly data and its daily data is based on historical patterns that may not continue unchanged, it is, nonetheless, a useful facility for this work. The options for running the WG allow the user to select one of three 30-year time periods (2020–2049, 2050–2079 or 2080–2109), to choose an emissions scenario describing greenhouse gas emissions in the future (high, medium or low) and a location in the UK resolved to a 5 km square. A percentile parameter must be chosen, for reasons explained below. Other selections are also required but these need not be described here. Once the user has made the selections, a set of simulations is performed by the Hadley Centre for Climate Prediction and Research, and output values are produced for the user to download.

For any selection of location and climate change scenario selected, 100 simulations are run for each of the 30 years in the period of interest, as well as a similar set of control scenarios based on the period 1960–1990, i.e. with a 'baseline historical' level of greenhouse gases. Each 30-year period is calculated assuming a stationary climate, i.e. there is no change in level of greenhouse gases over the period. In effect, the 30 years are simply 30 individual years after the appropriate change scenario that represent normal variability of climate. The 100 runs populate the spectrum of scenarios resulting from the uncertainties in the climate change models and their parameters.

The change embodied in the data sets produced by the WG is, in addition, controlled by the 'percentile' parameter, selectable by the user. So, if the user selects the 90 percentile, the data produced by the WG is such that the change of weather based on the control situation is 90% likely. In the descriptive terms approved by UKCP09, it is 'very likely' to occur with the assumptions and parameters selected. A 50 percentile gives a set of 100 runs where the data values represent a change to the climate that is 'as likely as not' to occur. It is clear that a choice of percentile value of 50 will result in greater changes in predicted weather, specifically more warming, than those produced with a percentile value of 90. Both of these percentiles were used in the cooling work reported below.

For this work, the following parameters were selected for hourly output from the WG.

Location – Odiham airfield, Hampshire (lat. 51.2656, long. -0.9555)
Emissions scenario – High
Timescale – 2020–2049*
Percentile – 90%, 50%
Monthly temperature averages
2 sampling variables – mean air temperature
Sampling temporal average 1– Summer
Sampling temporal average 2– Autumn
* As Storedry was only able to handle 20 years of weather data, only the years 2020–2039 were used; however, as the data were stationary, this did not introduce a bias.

The 100 runs produced, each of 30 years, could not all be used for simulation because not enough time was available in the project so 10 of the runs were selected. As the 100 runs were done with randomly selected variables, the choice of which years to use did not matter so the last 10 were used, runs 91–100.

Simulations

The assumption was made that the best strategy for control of the cooling fan would be the strategy determined in Phase 2 to be the best one when using historical weather data at Odiham, i.e. a differential thermostat set at 4°C comparing the plenum temperature with that at 0.5 m depth in the grain bed. Other parameters of the simulation were also retained from Phase 2, the most important of which were as follows.

Bed depth	4 m
Airflow	0.0032 m^3/s/t dry matter [10 m^3/(h.t wet matter)]
Target maximum temperature	5°C
Start date for cooling	20 August
End date	31 December
Initial moisture content	14.5% wet basis

Simulations were run which used the weather data first for the control set of 10 runs, each of 20 years, and then for the climate change scenario for the same. Hence, cooling was simulated over a total of 200 years for each of control and scenario. This exercise was carried out using the 90 and then the 50 percentile values. The control data sets for both percentile selections would be expected to be the same (within the bounds of randomness of the weather) as they both assumed no climate change. It was considered better to use the control scenarios from the WG to compare with the climate change scenarios rather than use the 4 years historical data used in phase 2 of the work.

As was expected, cooling was less effective under the climate change scenarios so the simulations were re-run allowing cooling to continue until the end of February in the following year. This gave more periods of cool weather and thus, more opportunities for the target maximum grain temperature in the bed to be reached.

Some problems were encountered during some simulations, in that one of the iterative routines in the simulation failed to converge on a stable value. This routine concerns the grain equilibrium moisture and air relative humidity. On closer inspection of the problem, it was found that in some of the climate change scenario runs relative humidities of 100% occurred every hour for a 24 h period, and sometimes for more than one day in succession. Though this was not very realistic, it arose from the way the data was generated. (The UKCP09 helpdesk explained that the r.h. was predicted based on the chance of precipitation, and sometimes this prediction lead to a prediction of 100% r.h. for a particular day.) To achieve the average of 100% for the day, every hour of that day had to have an r.h of 100%. The persistent high values of r.h. led to the non-convergence problem in Storedry. To overcome it, a limit was placed on the weather data when read in such that

if a value of relative humidity of over 95% was read, it was reduced to 95%. In all but one run this avoided the problem. Tests were done to check how much this change affected the results using runs that worked with the 100% as well as 95% values and results were satisfactory.

Results and Discussion

Each scenario enabled the Storedry simulation program to produce output for 20 years for each of 10 weather generator data sets, i.e. for 200 years of cooling simulation. In a few cases, Storedry would not run correctly with some of the 10 scenarios because of numerical convergence problems. There was not enough time available in the project to resolve the difficulties for these problem datasets so the problem sets were omitted. There was a great deal of results material produced so this had to be reduced for presentation. Each series of 20 years had certain parameters and assumptions in common so key values for those 20 years were analysed to give minima, averages and maxima, forming a single row in the results Tables 34 and 35 below. Each of the sets (usually 10) was analysed in this way to give 10 rows in the tables. Finally, results for the climate scenario were derived by averaging the 10 rows. Values tabulated in Tables 34 and 35 are otherwise as explained for previous tables.

Table 34. Results for control and 90 percentile climate change scenario. Runs are all at Odiham for 4 m deep bed of wheat of initial m.c. = 14.5 % with initial insect concentration =1/kg, initial wheat temperature 25^{o}C. Target maximum temperature = 5^{o}C, airflow 0.0032 m^{3}/s/tdm. Cooling using differential thermostat set at 4 ^{o}C with sensor at 0.5 m depth. Each row of the table is for 20 years, 2020–2039, starting cooling on 20 Aug with ambient relative humidity limited to 95%.

Scenario and runs	**% years when target reached**	**Closest approach when target missed, ^{o}C**	**Elapsed time, h**		**Fan run time, h**			**Total electricity, p/tonne**		**Average *O. surinamensis* insect concentration in bed at end of cooling, /kg**		**Max peak *O. surinamensis* concentration in bed during cooling, /kg**	
Control			min	max	min	avg	max	min	max	min	max	min	max
37	80	6.2	2122	3196	317	387	449	1.6	2.4	0.24	0.92	1.00	1.00
38	90	6.7	1851	3054	293	378	462	1.5	2.4	0.23	0.99	1.00	1.06
39	85	6.7	1863	3050	340	378	421	1.7	2.2	0.39	0.96	1.00	1.08
40	90	5.5	1904	3078	297	377	449	1.5	2.3	0.28	0.99	1.00	1.00
41	90	5.7	1910	3083	273	363	432	1.4	2.3	0.30	0.96	1.00	1.06
42	95	5.1	1882	3063	328	387	465	1.6	2.3	0.14	0.95	1.00	1.06
43	80	6.4	1859	3184	277	361	399	1.5	2.3	0.13	0.98	1.00	1.00
44	90	6.7	1907	3151	322	377	459	1.6	2.5	0.26	1.00	1.00	1.10
45	90	5.5	1957	3074	247	373	448	1.4	2.3	0.24	0.96	1.00	1.07
46	90	5.3	1787	3086	296	369	449	1.5	2.4	0.21	0.99	1.00	1.07
Average	88	6.0	1904	3102	299	375	443	1.5	2.3	0.24	0.97	1.00	1.05
Climate change, cooling to end December													
47	60	6.3	2432	3011	285	364	429	1.5	2.2	0.33	0.99	1.00	1.17

48	65	6.5	1910	3176	288	409	488	1.5	2.5	0.27	1.15	1.00	1.00
49	70	7.0	2459	3135	340	412	460	1.7	2.5	0.70	1.04	1.00	1.27
50	85	5.3	2026	3088	355	409	494	1.7	2.6	0.43	1.06	1.00	1.17
51	25	8.8	2217	3065	342	387	437	1.8	2.2	0.57	1.03	1.00	1.00
52	55	7.0	2167	3103	338	414	479	1.8	2.4	0.28	1.03	1.00	1.00
53	65	7.3	1881	3195	381	429	483	1.9	2.5	0.32	1.07	1.00	1.20
54	25	10.4	2530	3085	367	457	512	2.0	2.6	0.35	1.03	1.00	1.00
55	80	5.9	2239	3078	358	399	459	1.7	2.3	0.44	0.99	1.00	1.14
56	80	6.0	2121	3113	330	432	499	1.7	2.5	0.61	1.01	1.00	1.20
Average	61	7.0	2198	3105	338	411	474	1.7	2.4	0.43	1.04	1.00	1.11
Climate change, cooling to end February													
57	95	5.7	2432	3927	277	362	429	1.4	2.2	0.08	0.99	1.00	1.17
58	95	5.8	1910	4239	288	409	488	1.5	2.5	0.16	1.15	1.00	1.28
59	95	5.9	2459	3569	329	412	466	1.7	2.5	0.41	1.04	1.00	1.27
60	100	5.0	2126	3464	355	417	494	1.7	2.6	0.17	1.06	1.00	1.17
61	79	6.5	2217	4255	290	406	493	1.6	2.6	0.18	1.03	1.00	1.18
62	100	5.0	2171	3827	338	406	479	1.7	2.5	0.10	1.02	1.00	1.11
63	95	5.4	1881	4024	362	422	483	1.8	2.5	0.13	1.07	1.00	1.20
64	89	5.2	2530	4429	326	424	512	1.6	2.6	0.22	1.03	1.00	1.00
65	100	5.0	2239	4378	358	400	459	1.7	2.3	0.13	0.99	1.00	1.14
66	100	5.0	2121	3558	316	426	499	1.7	2.5	0.15	1.01	1.00	1.20
Average	95	5.4	2209	3967	324	408	480	1.6	2.5	0.17	1.04	1.00	1.17

Referring to Table 34, the first block shows results for the control set of no climate change. Cooling to the GSG target temperature of 5°C by end December was successful for an overall average of 88% of the 200 years simulated, no lower than 80% and no higher than 90% for any set of 20 years. In the years when cooling was unsuccessful, the closest approach temperatures averaged 6.0°C and the worst were 6.7°C. Overall average fan running time was 375 h.

As this control scenario is effectively the historical situation it would be expected to be in line with the results found in Phase 2 for 4 years of historical data at Odiham; although, those 4 years, 1998–2001, are beyond the UKCP09 baseline 'no warming' time window of 1960–1990. Those results showed 100% success, and an average fan running time of 358 h, which are reasonably in line with the results of 88% and 375 h with the WG control data.

As in Phase 2, the insect concentration was well controlled by the temperatures reached so the concentration was not a sensitive indicator of the success or otherwise of the cooling. The GSG targets were therefore used, as before.

Turning to the 90 percentile climate change scenario, the second block of results in Table 34, the percentage of years in which cooling successfully reached GSG target was reduced to 61%. In the two most difficult sets of 20 years, success was only 25%, i.e. 5 of the 20 years were successful. In other sets of 20 years, success was as high as 85%, showing that the predicted weather was very variable between 20 year sets. The worst year was in run 54, where in one year the lowest temperature reached was 10.4°C. However, even in this worst case, the concentration of *O. surinamensis* only reached a peak of 1.27 so control was maintained. Control of *A. siro* may not have been achieved but unfortunately these simulations were not done for mites or any other pest species that may be active at cool temperatures.

Fan running time required to reach target, in the years where this was possible, was 411 h on average compared with 375 h for control and electricity consumption reflected this increase. The maximum bed-average concentration of *O. surinamensis* insects was 1.04/kg, compared with the control case where it fell to 0.97/kg. The fact that one is greater than unity while the other is less is not significant because it does not mean the insects were multiplying at that stage, just that cooling had been slower so cold mortality had been less.

Extending the time for cooling from end December to end of February, the third block of Table 34, resulted in 95% of years being successful, with the worst case being 79% of 20-year set run 61. Although more time elapsed before the target temperature was achieved, the average concentration of *O. surinamensis* insects in the bed at the end of cooling did not increase from end December to end February because the bed was already below the growth temperature for that

species Pest species active at lower temperatures than the saw-tooth grain beetle may not be sufficiently controlled if the time for cooling is extended to end of February.

Fan running time for end of February finish was 408 h, up from 375 h for the control. The control and end of February scenarios had similar success rates (88 and 95%) so it is reasonable to compare fan running times, which excluded unsuccessful years. At first, it appears odd that the average fan running time did not increase between cooling to end December (411 h) and cooling to end February (408 h). This is as a result of unsuccessful years not being included in the calculation of running time. Runs that did not succeed by end December must have had too little fan running time owing to too warm weather. Allowing two more months gave cold weather opportunities for the diffstat, and final cooling to target took the total average running time to 408 h.

Table 35. Results for control and 50 percentile climate change scenario. All other parameters are as Table 6.

Scenario and runs	**% years when target reached**	**Closest approach when target missed, ^{o}C**	**Elapsed time, h**		**Fan run time, h**			**Total electricity, p/tonne**		**Average *O. surinamensis* concentration in bed at end of cooling, /kg**		**Max peak *O. surinamensis* concentration in bed during cooling, /kg**	
Control			min	max	min	avg	max	min	max	min	max	min	max
67	95	5.3	1904	3121	337	395	454	1.7	2.6	0.28	0.97	1.00	1.00
68	95	5.5	1974	3134	302	375	435	1.6	2.2	0.40	0.93	1.00	1.07
69	85	6.0	1757	3134	230	362	464	1.2	2.4	0.20	0.96	1.00	1.06
70	100	5.0	1840	3080	286	380	464	1.5	2.3	0.23	0.95	1.00	1.07
71	85	7.3	1884	3111	267	372	462	1.5	2.3	0.17	0.97	1.00	1.07
72	80	5.8	1951	3134	282	347	451	1.4	2.3	0.10	0.94	1.00	1.00
73	95	6.7	2007	3017	280	359	474	1.4	2.5	0.21	0.94	1.00	1.07
74	100	5.0	1815	2916	290	364	452	1.6	2.2	0.27	0.94	1.00	1.07
75	95	5.1	1930	3086	306	388	459	1.6	2.4	0.18	0.99	1.00	1.06
76	85	6.6	1954	3125	293	368	433	1.4	2.3	0.49	0.97	1.00	1.06
Average	92	5.8	1902	3086	287	371	455	1.5	2.4	0.25	0.96	1.00	1.05
Climate change,													

cooling to end December													
77	50	8.7	2550	3054	319	394	523	1.7	2.7	0.71	0.99	1.00	1.09
78	50	8.4	2528	3112	318	375	426	1.5	2.4	0.43	1.02	1.00	1.23
79	40	9.2	2818	3102	410	446	494	2.1	2.7	0.62	1.09	1.00	1.27
80	55	9.6	2584	3183	389	434	507	1.8	2.7	0.68	1.10	1.00	1.33
81	25	8.6	2151	3060	367	409	430	1.8	2.3	0.77	1.14	1.00	1.00
82	30	9.0	2477	3146	362	396	442	1.8	2.5	0.96	1.08	1.00	1.00
83	20	9.9	2272	3102	277	368	436	1.5	2.3	0.47	1.06	1.00	1.00
84	35	9.1	2428	3153	342	401	424	1.7	2.4	0.72	1.05	1.00	1.23
Average	38	9.1	2476	3114	348	403	460	1.8	2.5	0.67	1.07	1.00	1.14
Climate change, cooling to end February													
85	90	5.4	2550	4091	319	409	523	1.7	2.7	0.26	0.99	1.00	1.09
86	95	5.0	2500	3390	403	462	555	2.0	3.0	0.30	1.12	1.00	1.39
87	65	6.7	2528	4022	318	386	429	1.5	2.4	0.31	1.02	1.00	1.23
88	95	5.0	2818	4425	402	463	564	2.0	3.1	0.22	1.09	1.00	1.27
89	95	5.0	2584	4354	375	435	507	1.8	2.7	0.32	1.10	1.00	1.33
90	85	5.7	2151	3972	367	423	465	1.7	2.4	0.17	1.14	1.00	1.33
91	95	5.0	2477	4239	293	420	487	1.7	2.8	0.23	1.08	1.00	1.18
92	75	5.8	2272	4550	277	380	464	1.5	2.3	0.05	1.06	1.00	1.26
93	90	6.0	2428	4229	342	410	494	1.7	2.5	0.36	1.05	1.00	1.23
Average	87	5.5	2479	4141	344	421	499	1.7	2.7	0.25	1.07	1.00	1.26

The 50 percentile scenarios also had a control case which should be statistically indistinguishable from the control set of the 90 percentile case above. In the first block of Table 35, the control, cooling was successful for an overall average of 92% of the 200 years, which is close to the 88% for control in Table 34. In the years when cooling was unsuccessful, the closest approach temperatures averaged 5.8°C and the worst was 7.3°C. Overall average fan running time was 371 h, very close to 375 h for control in Table 34.

In the 50 percentile case of climate change, the second block of Table 35, again with the GSG target of cooling to 5°C by end December, the cooling success fell to an average of only 38% of the years simulated, the worst case being 20%. The average temperature in the worst years that failed to reach the target was 9.1°C, which was 2.1°C higher than in the 50 percentile scenario. The worst case, in the 20 years of run 83, was 9.9°C slightly lower than for the 90 percentile scenario of climate change, though not significantly so. Fan running time was 411 h but this figure is of limited use as it is only for the 38% of years in which cooling was successful.

If the cooling window was extended to end February, Table 35 third block, the success rate climbed from 38 to 87%, and the average of the closest approach temperatures fell from 9.1 to 5.5°C. The control and end February scenarios had similar success rates so it is reasonable to compare the average fan running time, which was 421 h, up 13.5 % from 371 h for the control, with electricity consumption up in proportion.

Insect bed average concentration in the worst layer of successfully cooled beds was 1.07, showing that there was lower cold mortality in the 50 percentile scenario of Table 34.

Conclusions from Phase 3

1. Two climate change scenarios and their controls were simulated at the Odiham 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.

2. 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.

3. 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.

Overall conclusions

1. Simulation with historical weather data showed that the risk posed by *O. surinamensis*, in stored wheat in south east England could be controlled by cooling with ambient air. Provided the fan gave an airflow rate recommended in the HGCA Grain Storage Guide, controlling its operation with a differential thermostat set at 4°C was highly effective in achieving the cooling rate required for this pest. Though data for the mite species *Acarus siro* was more limited, that species was also controlled by such a cooling arrangement.

2. Because of the relative ease of control of the target species of insect, the cooling targets in the GSG were adopted. The main target, cooling to 5°C by end of December, was more challenging to achieve. A differential thermostat using the average bed temperature to compare with ambient was the best approach, *i.e.* met the target in all years simulated and used fewest fan running hours. Its performance could be improved by increasing the differential from 4°C to 6°C once the bed had cooled to between 10°C and 8°C.

3. Rises in temperature owing to climate change are likely to reduce very significantly the likelihood of being able to meet the current HGCA cooling target for temperature and time. The insect model suggests O. surinamensis would still be well controlled by cooling with ambient air using a diffstat but the effect on other, more cold-tolerant, pests should be determined. Extending the time for cooling from the end of December to the end of February allowed the target temperature to be achieved but the slower cooling means that some pest species may not be controlled. Models are needed of growth and cold mortality of the full range of pest species active at low temperatures.

3.3.4. Conclusions for Objective 2

- Direct comparison with previous insect surveys is difficult due to the use of different detection methods. However, it would appear that the presence of psocid species in stores has increased and there may be a difference in the presence of *Tyrophagus* spp. A more extensive survey is required to confirm this.
- Resistance to pirimiphos-methyl in *O. surinamensis* and *A. 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.
- Hygiene measures, including the use of chemical insecticides, still result in the presence of primary insect and mite pests in stores. A larger study is required to determine the most effective hygiene methods.
- UVC is effective at reducing egg hatch and spore germination in storage pests. 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 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. Rises in temperature owing to climate change are likely to reduce very significantly the likelihood of being able to meet the current HGCA cooling target for temperature and time.

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

Hazards in grain storage include fungi and mycotoxins, insects and mites as well as loss of quality below food safety thresholds. All of these hazards are related to temperature and moisture. By identifying and not exceeding moisture and temperature thresholds, it is possible to halt or prevent deterioration by these causal agents and, if done in the shortest possible time frame, it is possible to prevent unacceptable levels of damage. Monitoring of temperature and moisture content can also provide an indirect method to detect arthropod presence. It is, therefore, essential that monitors are available to record these physical conditions accurately so as to enable critical limits to be set. Although temperature both in-store and in-bin is easy to measure and its distribution can be predicted, accurate in-bin moisture or available water content measurement still needs to be developed. Arthropod presence can also be measured directly through the use of insect and mite monitors (traps). It is essential to understand the best way to deploy and interpret monitors for arthropod presence to enable population levels to be assessed enabling preventative or control actions to be triggered. Monitoring of physical condition of the stored grain and for the presence of arthropod infestations is key to the development of the HACCP approach and must be reliable for critical limits to be set. Insects and mites are 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 (Hubert *et al*., 2003) and may therefore, also spread

mycotoxin-producing fungi throughout stores. This is an important aspect of mycotoxin origin that has yet to be investigated.

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

3.4.1. Step 3.1 Monitors and interpretation

3.1.1 Interpretation of trap catch in surface and buried PC traps

Introduction

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. If a model could be produced to predict population size from the number of insects found, this would aid in the establishment of thresholds that could be incorporated in an HACCP approach. 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 PC trap catches. Preliminary studies were undertaken and showed that it is necessary to test a wide range of population densities: 1–7 insects/kg of each species. The numbers of insects captured at each population density at combinations of three temperatures (10°C, 15°C and 20˚C) and two grain moisture contents (13% and 17%) was examined in a laboratory study. A study in the Fera grain store was then conducted to determine the robustness of the data from the laboratory study under fluctuating conditions of temperature and moisture content likely to be found in UK grain stores.

Materials and Methods

Laboratory study of interpretation of PC trap catch.

Insects

Recently collected field strains of both *O. surinamensis* and *S. granarius* were used to avoid any behavioural changes that might be associated with long-term laboratory culture. The *O. surinamensis* Hartley strain was collected on 2nd November 2006 and cultured on a mixture of whole and kibbled wheat (75% and 25%, respectively). The *S. granarius* Witham strain was collected on 27th June 2007 and cultured on whole wheat. Mixed ages and genders of adults were used for all tests. Both species were cultured at 25˚C and 60% r.h. in constant darkness.

Test conditions

Whole wheat was used for all tests. The moisture content (mc) of the wheat was adjusted by adding water or drying the wheat at the temperature at which the test was carried out until the

required moisture content (13% or 17%) was obtained. The moisture content was maintained by adjusting the relative humidity in the controlled environment room to match the equilibrium relative humidity (e.r.h.) expected at that mc and ambient temperature for whole wheat. All of the tests were carried out in constant darkness.

Test method

Black buckets (depth 26 cm, dia. 31 cm) were filled with 5 kg of wheat at the appropriate moisture content. The buckets were coated with Fluon around the inside at the top to prevent the insects from escaping. The appropriate number of insects was added to give the required population level (1, 2, 3, 4, 5, 6 or 7 insects/kg) and left to acclimate for one week. Two PC traps were placed in each bucket; one at the surface and one buried to a depth of 5 cm. The contents of the traps were counted after one week. Five replicates were set up for each combination of species, population, temperature and moisture content. The mean trap catch with 95% confidence intervals was calculated for each set of conditions and regression lines were produced. The software used was GenStat Release 11.1. A linear relationship was forced between insect density and moisture content after an increasing trend was found in the data. The continuous variable was insect density.

Large scale interpretation of PC trap catch

This study was initially undertaken in the winter of 2008–2009 using an insect density of 1 insect per kg for both species. However, during the study there was a cold snap when temperatures fell rapidly to an uncharacteristic low. The temperature during the study was then below those used in the laboratory study. This is referred to as “Study 1”. Therefore, the study was repeated during summer 2009 when temperatures were higher and within the range tested in the laboratory. The latter study tested a range of population densities: 1, 4 and 7 insects per kg for *S. granarius* and 1, 3 and 5 insects per kg for *O. surinamensis*. This is referred to as “Study 2”.

Grain Bins

The studies were carried out in six square 30 tonne capacity bins. Each has a surface area of 9 m^2. Each bin is 4 m deep and has an additional 1 m deep hopper at the bottom. Each bin was filled with 25 tonnes of feed wheat to a level approximately 1 m below the top of the bin. The grain was dried to 13% for safe storage.

Insects

Laboratory susceptible strains of both *O. surinamensis* and *S. granarius* were used to facilitate breeding up of the large numbers required. The more recently collected field strains used in the laboratory study do not increase in numbers in the laboratory in sufficiently reliable numbers to set up a large-scale study. Mixed age adult insects of both species were used.

Test method Study 1

Insect populations of 1 insect/kg for the top 1 metre depth of grain in the bin were set up in each bin. The insects were measured by volume into batches of 790 insects of each species and held in 115 x 150 ml round plastic pots with holes pierced in the lids. Kibbled wheat (50 g) was added to each pot. The insects were acclimated to test conditions (15ºC, 60% r.h.) for two days in a controlled environment room then introduced into the grain in a nine point grid pattern at a depth of 0.5 m to replicate the location of the bulk of the population reported in the literature.

Test method Study 2

Insect populations of 1, 4 and 7 *S. granarius*/kg and 1, 3 and 5 *O. surinamensis*/kg were set up sequentially in each bin (Table 36). The insects were measured by volume into appropriate batches for the population density which was introduced. The insects were held in 115 x 150 ml round plastic pots with holes pierced in the lids. Kibbled wheat (50 g) was added to each pot. The insects were held overnight at 20ºC, 60% r.h. in a controlled environment room. These conditions approximated ambient conditions in the grain store at the time of insect introduction. The insects were introduced into the grain in batches in a nine-point grid pattern at a depth of 0.5 m. Insects were added to the original populations to increase them to the density required for the subsequent population. The entire study was of shorter duration than the life cycles of the insects so the population could not increase during the study.

Table 36. Sequence of events for Study 2

Week	**Action**
0	Set up population of 1 insect/kg for both species
1	Place traps
2	Count insects in traps and set up population of 4 *S. granarius*/kg and 3 *O. surinamensis*/kg
3	Place traps
4	Count insects in traps and set up population of 7 *S. granarius*/kg and 5 *O. surinamensis*/kg
5	Place traps
6	Count insects in traps

Monitoring

Five pairs of PC traps were deployed as described in the manufacturer's instructions: one at the surface and one 5 cm deep. Monitoring points in each bin were at the centre and 0.50 m from each corner of the bin.

In Study 2, in addition to the PC traps, spear samples of approximately 1 kg of grain were taken from one bin only. These were taken from the surface and at depths of 10 cm and 50 cm in the grain. Samples were taken at nine points across the bins and the number of insects (dead and alive) found in the samples was converted to insects per kg. These samples were taken at the end of the study when the maximum numbers of insects were present.

In Study 1, the temperature in each bin was recorded at 30 min intervals at the surface of the grain and at a depth of 0.10 m halfway between the edge and the centre of the bin using a Squirrel data logger and thermocouples. Samples were taken weekly for moisture content analysis from the surface and a depth of 5 cm adjacent to the temperature monitoring point. In Study 2, temperatures were recorded hourly: ambient, grain surface and at depths of 0.10 m and 0.50 m using a combination of TinyTalk and TinyTag data loggers. Samples were taken for moisture content analysis from the surface of the grain, at depths of 0.10 m and 0.50 m, weekly throughout the experiment.

Statistical analysis

Comparisons of trap catch were made with the laboratory results to test predictions. The skewness of the field data (which are count data) was taken into account when calculating the 95% confidence intervals of the means. The log-transformed data were fitted to a Generalized Linear Model (Poisson) and means and 95% confidence intervals were calculated and back-transformed.

Results and discussion

Laboratory study

Buried traps were a better predictor of population size than surface traps for *O. surinamensis* (Figures 18a and b). 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* (Figures 19a and b). 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.

Equations for the lines are shown in Figure 20.

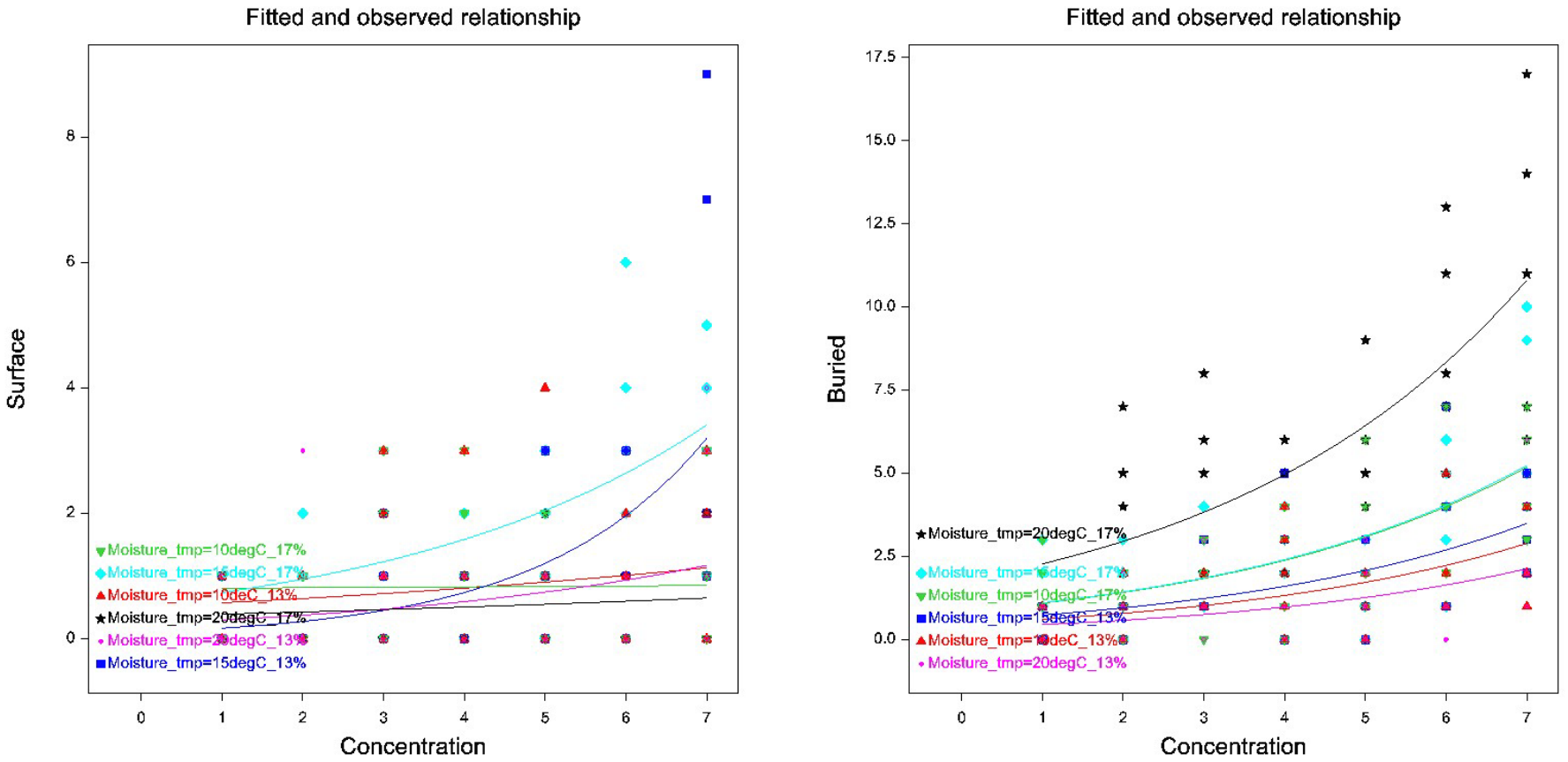


Figure 18a. *O. surinamensis* fitted lines for surface and buried traps

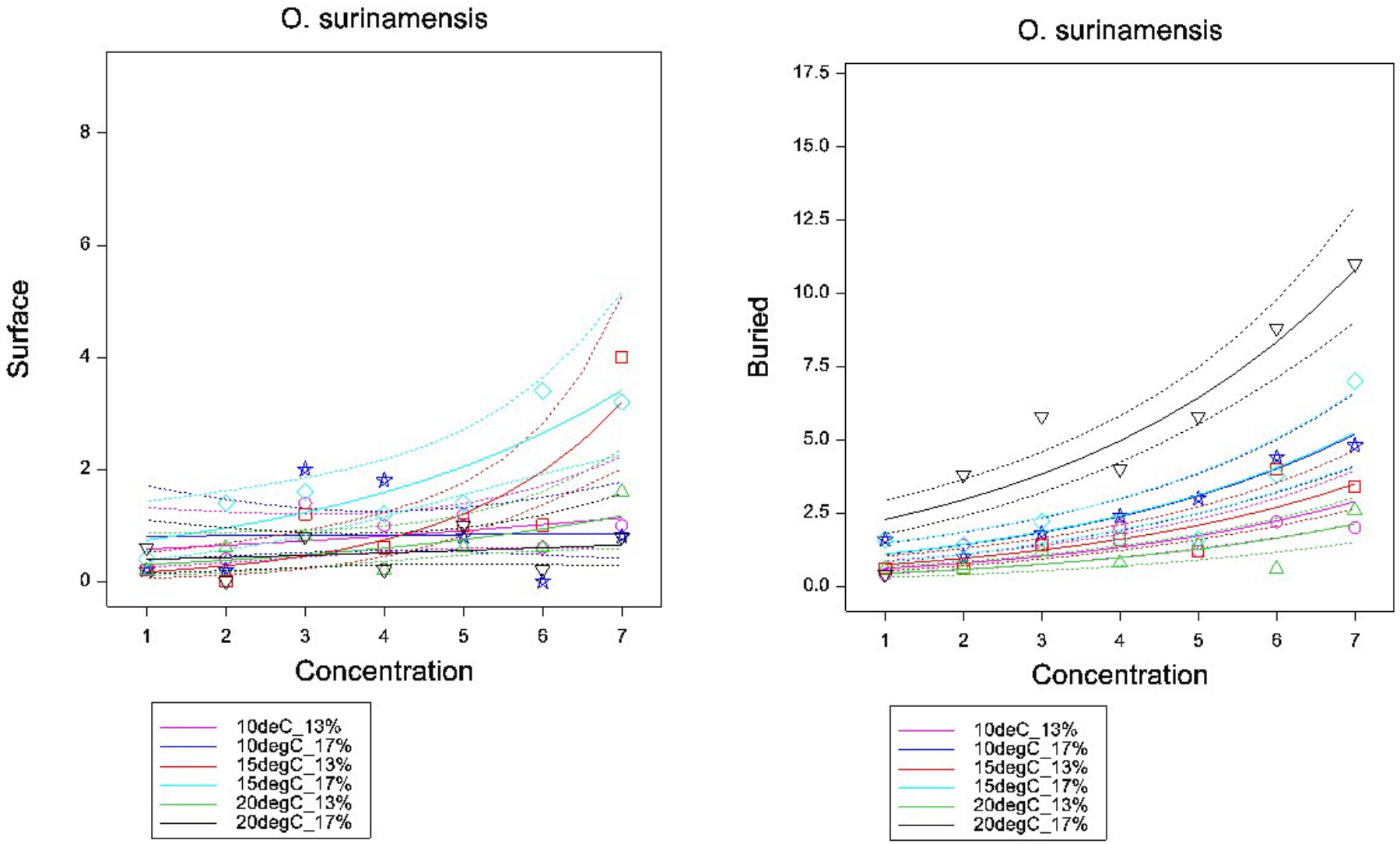
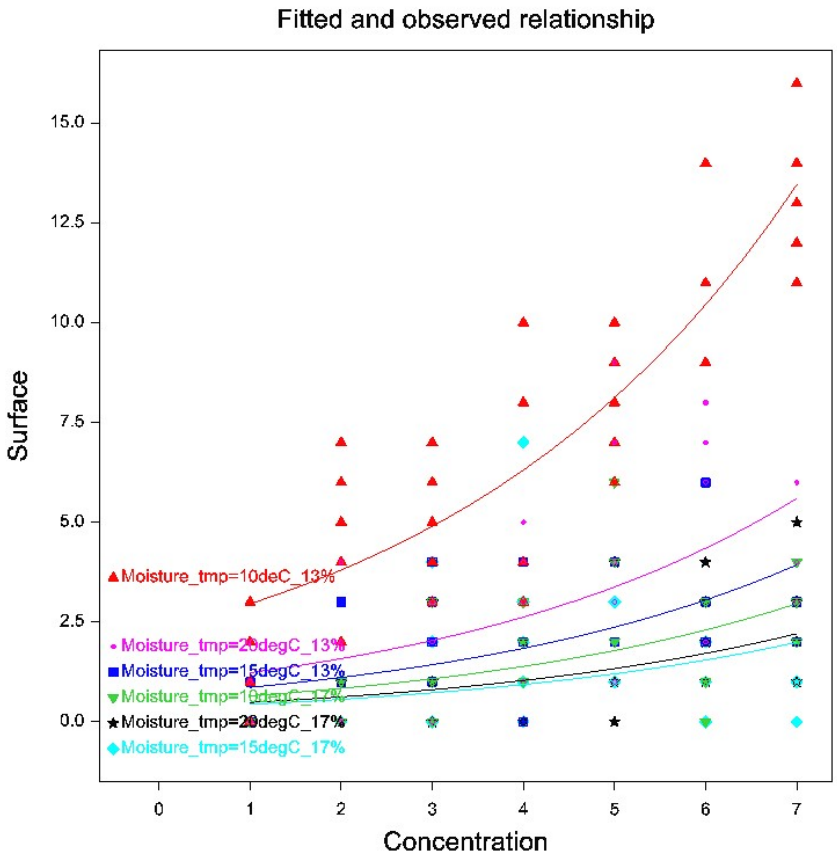


Figure 18b. *O. surinamensis* fitted lines for surface and buried traps with means and 95% confidence intervals



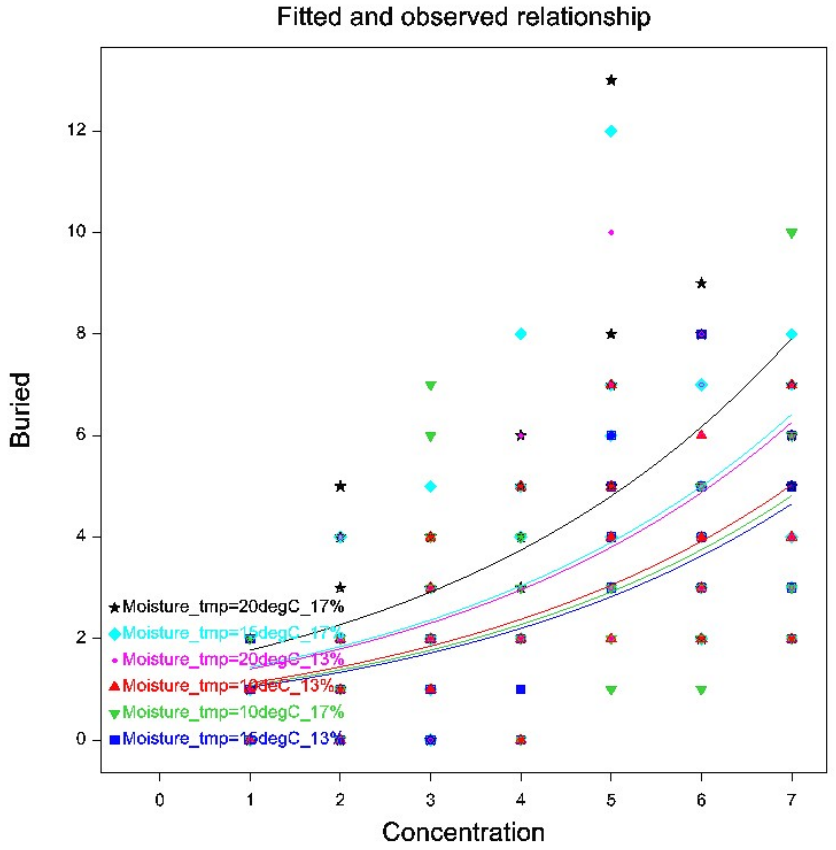


Figure 19a. *S. granarius* fitted lines for surface and buried traps

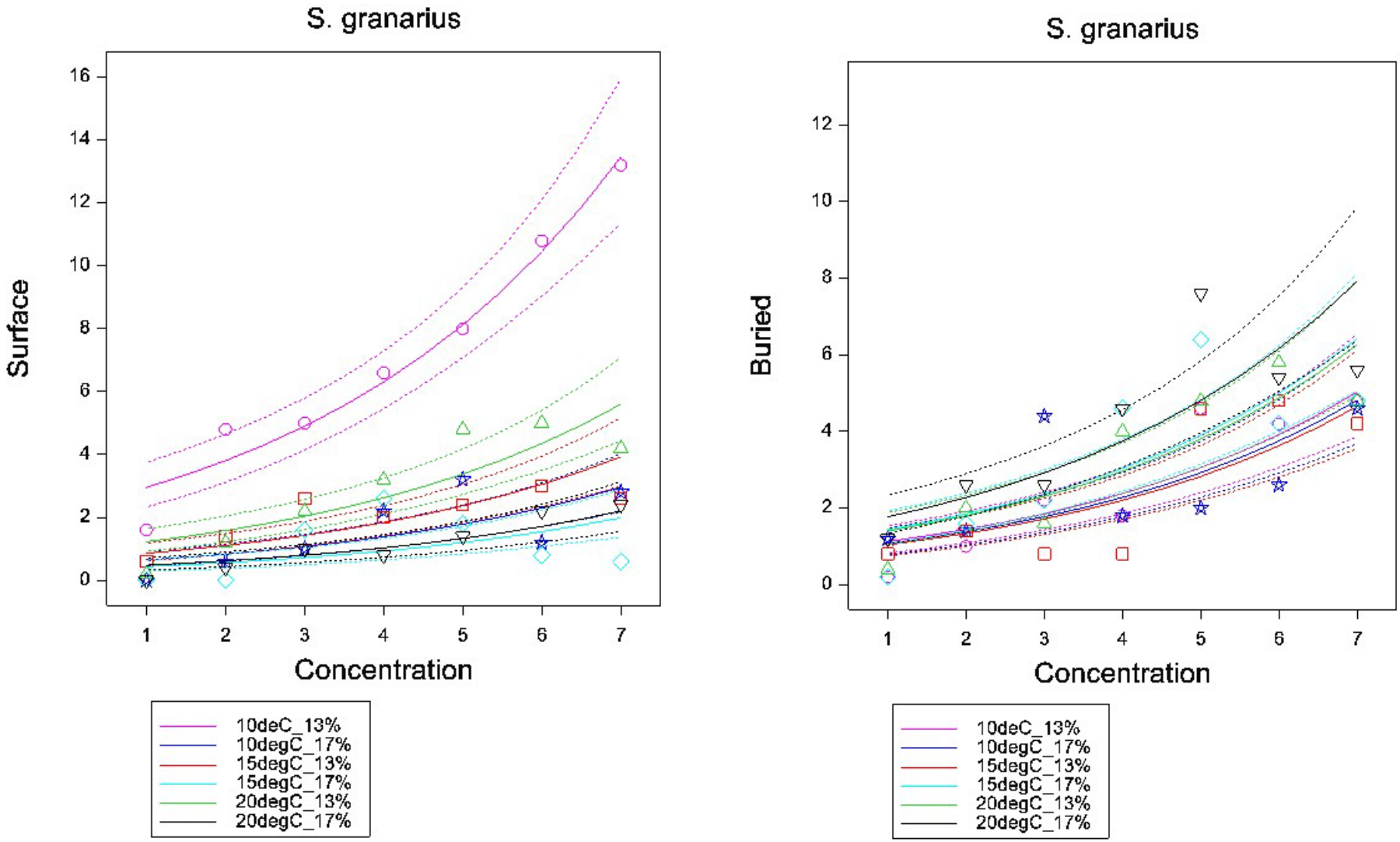


Figure 19b. *S. granarius* fitted lines for surface and buried traps

O. surinamensis

Buried

10°C-13%: $\log(count) = 0.259\,(0.025)\,concentration - 0.753\,(0.194)$

10°C-17%: $\log(count) = 0.259\,(0.025)\,concentration - 0.170\,(0.167)$

15°C-13%: $\log(count) = 0.259\,(0.025)\,concentration - 0.565\,(0.184)$

15°C-17%: $\log(count) = 0.259\,(0.025)\,concentration - 0.159\,(0.166)$

20°C-13%: $\log(count) = 0.259\,(0.025)\,concentration - 1.060\,(0.213)$

20°C-17%: $\log(count) = 0.259\,(0.025)\,concentration + 0.565\,(0.146)$

Surface

10°C-13%: $\log(count) = 0.113\,(0.106)\,concentration - 0.667\,(0.515)$

10°C-17%: $\log(count) = 0.009\,(0.104)\,concentration - 0.223\,(0.469)$

15°C-13%: $\log(count) = 0.489\,(0.115)\,concentration - 2.259\,(0.671)$

15°C-17%: $\log(count) = 0.255\,(0.077)\,concentration - 0.558\,(0.406)$

20°C-13%: $\log(count) = 0.227\,(0.124)\,concentration - 1.427\,(0.649)$

20°C-17%: $\log(count) = 0.084\,(0.133)\,concentration - 1.014\,(0.635)$

S. granarius

Buried

10°C-13%: $\log(count) = 0.249\,(0.025)\,concentration - 0.131\,(0.175)$

10°C-17%: $\log(count) = 0.249\,(0.025)\,concentration - 0.175\,(0.177)$

15°C-13%: $\log(count) = 0.249\,(0.025)\,concentration - 0.209\,(0.178)$

15°C-17%: $\log(count) = 0.249\,(0.025)\,concentration + 0.113\,(0.165)$

20°C-13%: $\log(count) = 0.249\,(0.025)\,concentration + 0.087\,(0.166)$

20°C-17%: $\log(count) = 0.249\,(0.025)\,concentration + 0.322\,(0.158)$

Surface

10°C-13%: $\log(count) = 0.257\,(0.025)\,concentration + 0.831\,(0.142)$

10°C-17%: $\log(count) = 0.257\,(0.025)\,concentration - 0.683\,(0.193)$

15°C-13%: $\log(count) = 0.257\,(0.025)\,concentration - 0.400\,(0.178)$

15°C-17%: $\log(count) = 0.257\,(0.025)\,concentration - 1.080\,(0.218)$

20°C-13%: $\log(count) = 0.257\,(0.025)\,concentration - 0.046\,(0.164)$

20°C-17%: $\log(count) = 0.257\,(0.025)\,concentration - 0.977\,(0.211)$

Figure 20. Regression equations

Large scale Study 1

A late harvest due to the weather resulted in mean temperatures recorded during the 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 (Figure 21). Moisture content of the grain at the surface increased from 13% to approximately 15.5% (Figure 22a). There was a smaller increase in m.c. at 5 cm deep (Figure 22b). The total numbers of insects caught in traps decreased with time (Figure 23). 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.

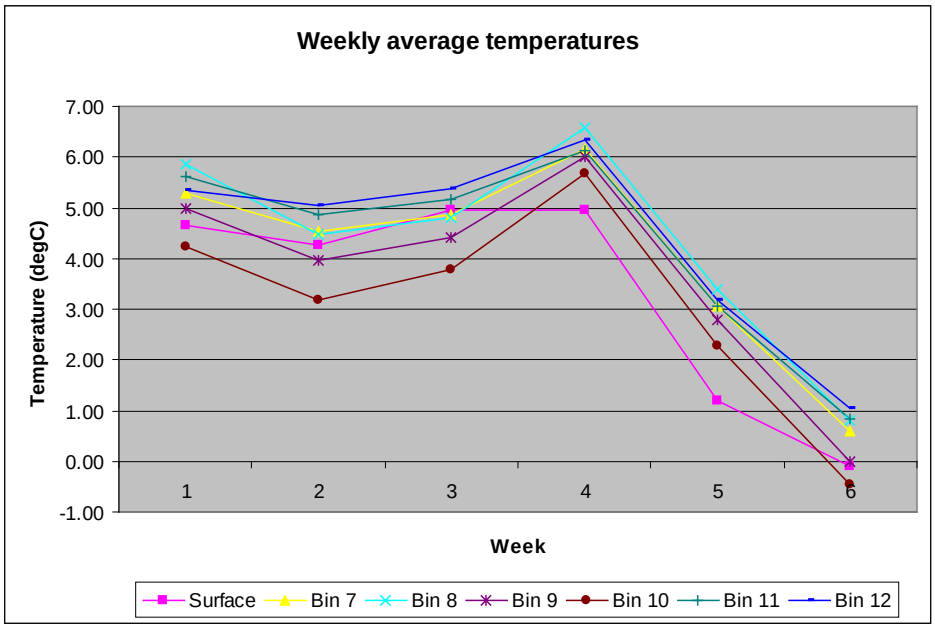
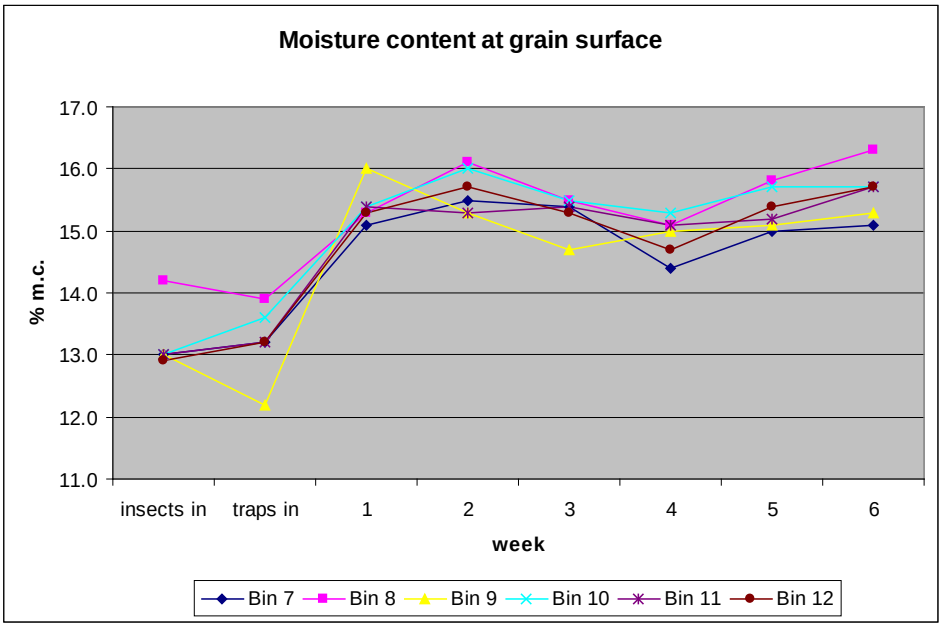


Figure 21. Mean weekly temperatures in the six bins during Study 1.



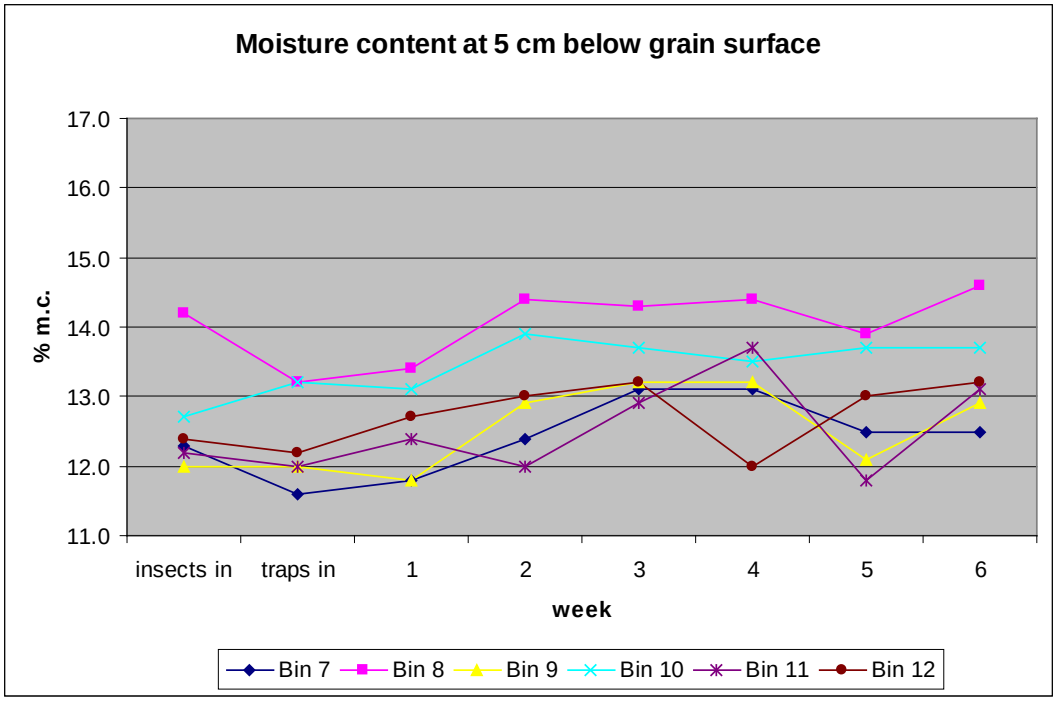
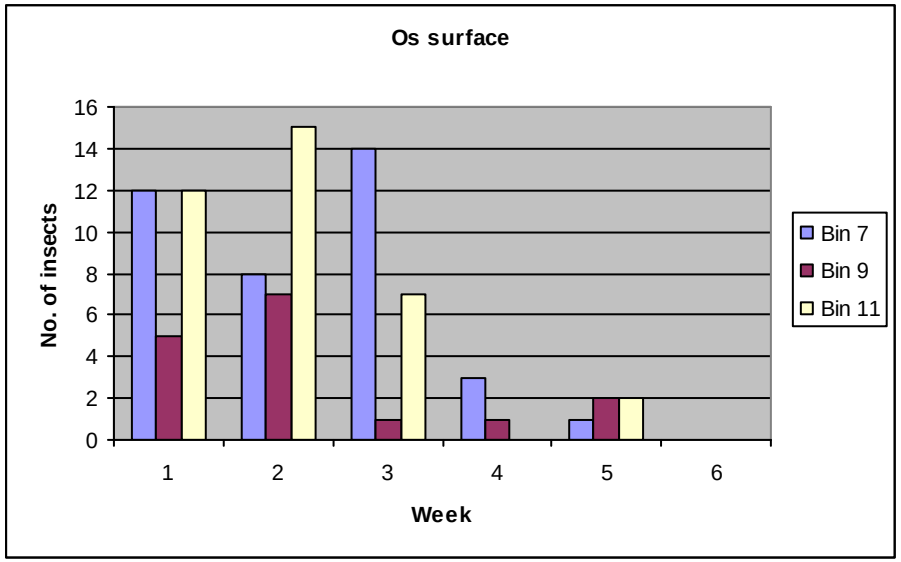
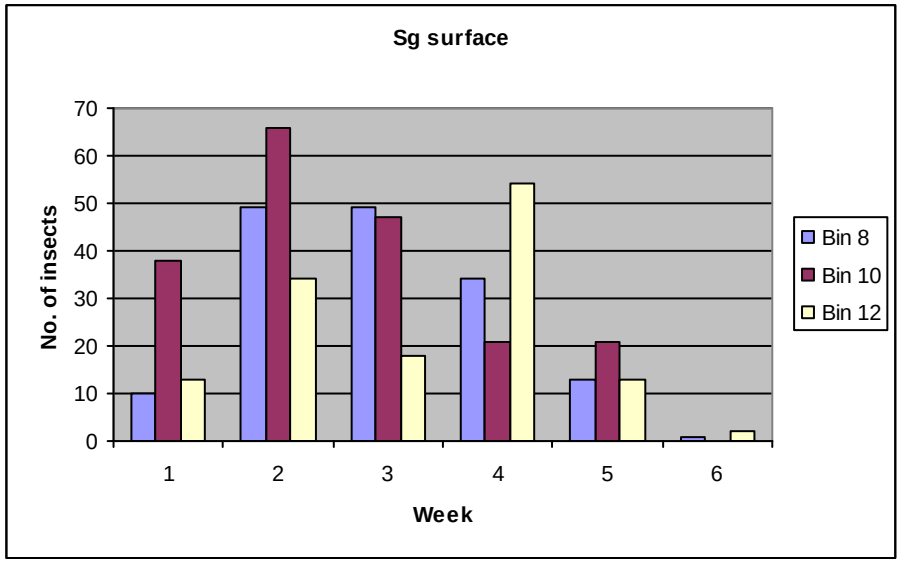


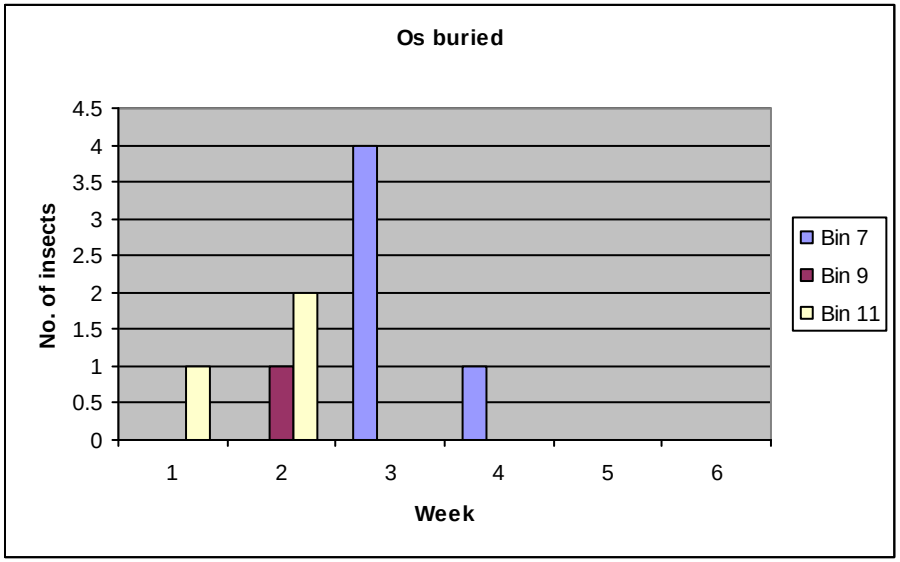
Figure 22. Mean moisture content at the grain surface and 5 cm below the grain surface in the six bins during Study 1.

Mean trap catches across all bins for each species and 95% confidence intervals were plotted on the graphs of laboratory data which best matched the conditions in the grain store over the six week study (Figures 24a and b). The best comparisons for surface traps were 10ºC and 13% and 15% m.c. and for buried traps 10ºC and 13% m.c. 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. Based on this data, surface trap catches may be better predictors of population size than buried traps for both species.

Figure 23. Total trap catches per bin.







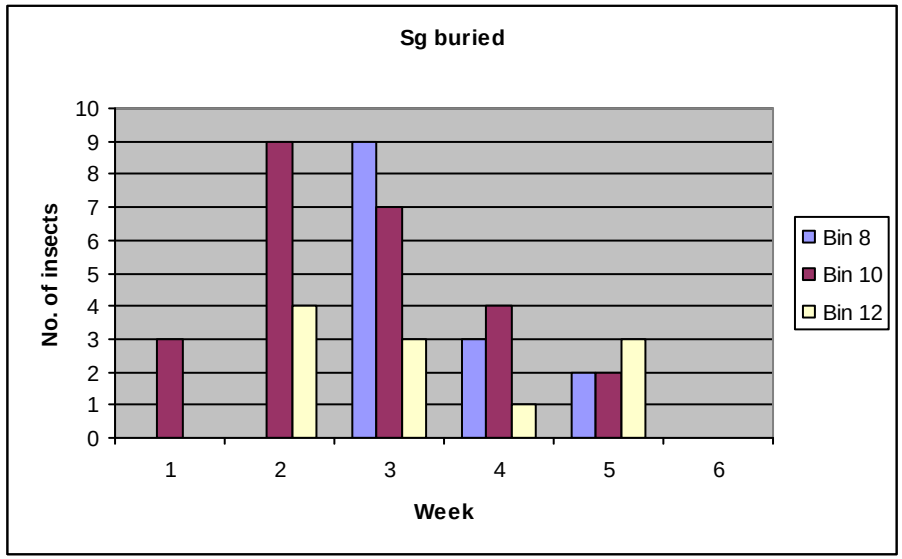


Figure 24a. Mean *O. surinamensis* trap catches overlaid on laboratory data (error bars and dotted lines = 95% confidence intervals)

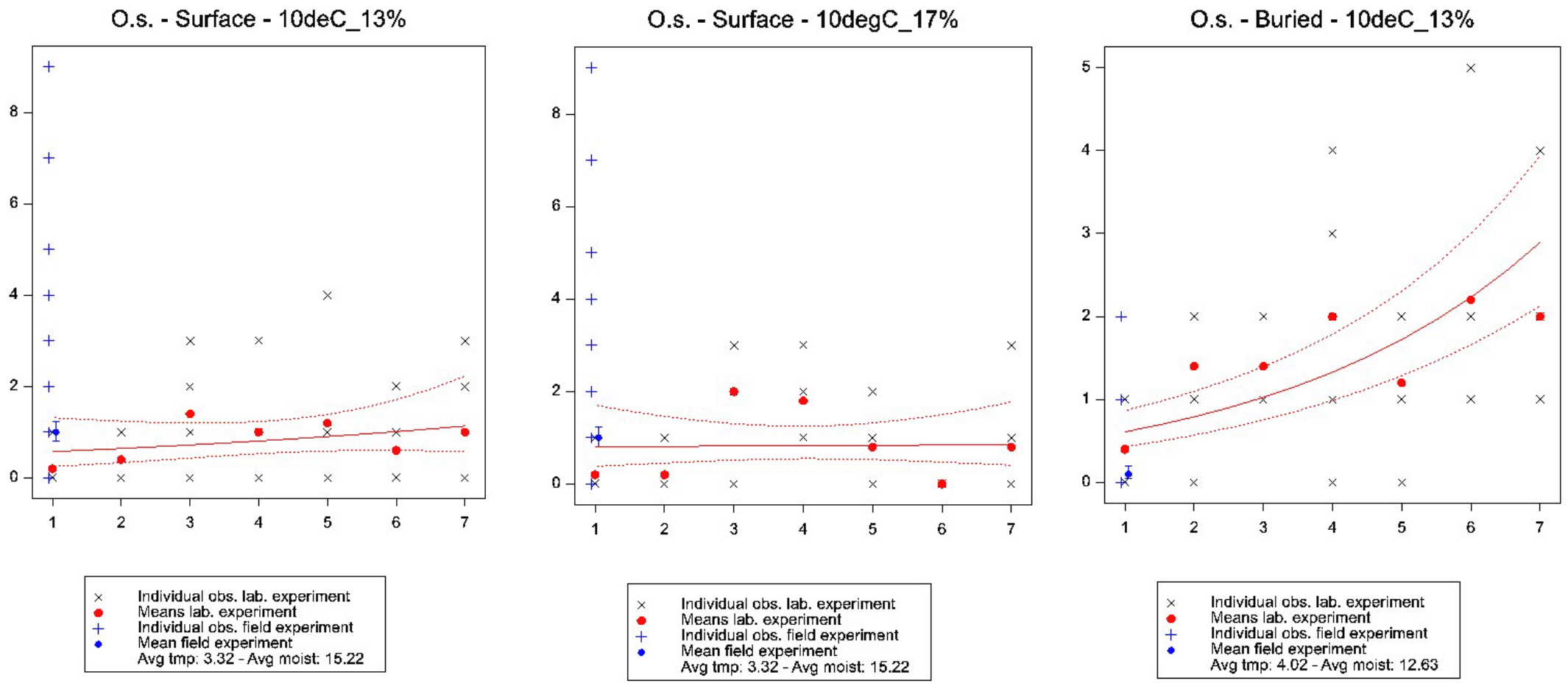
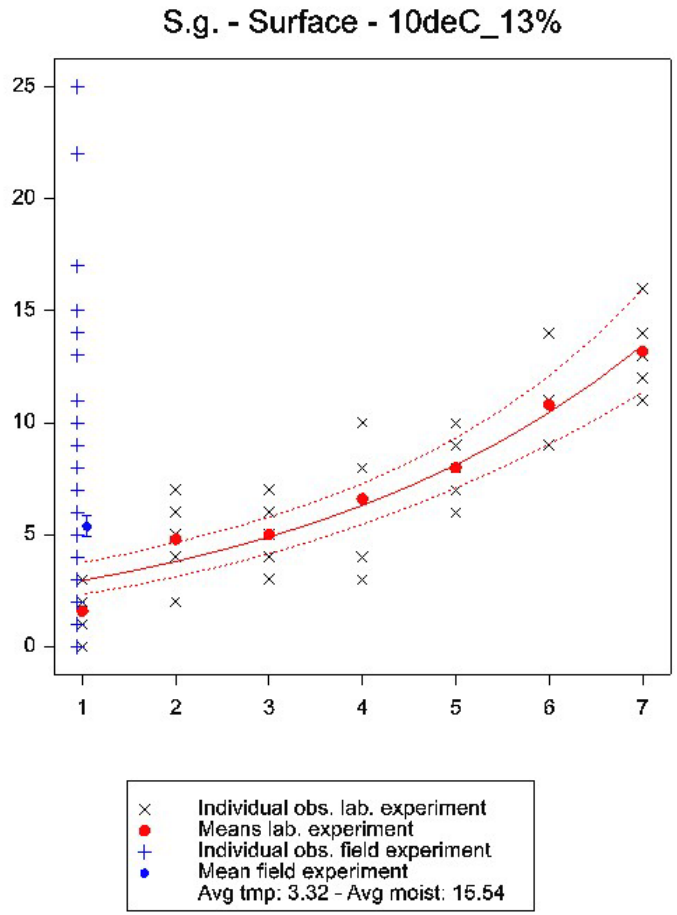
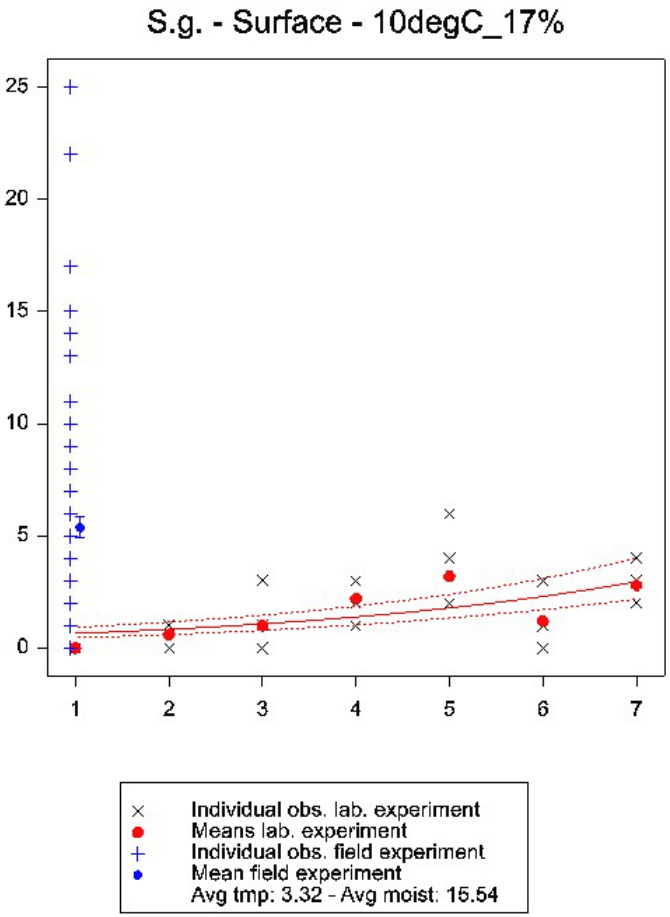
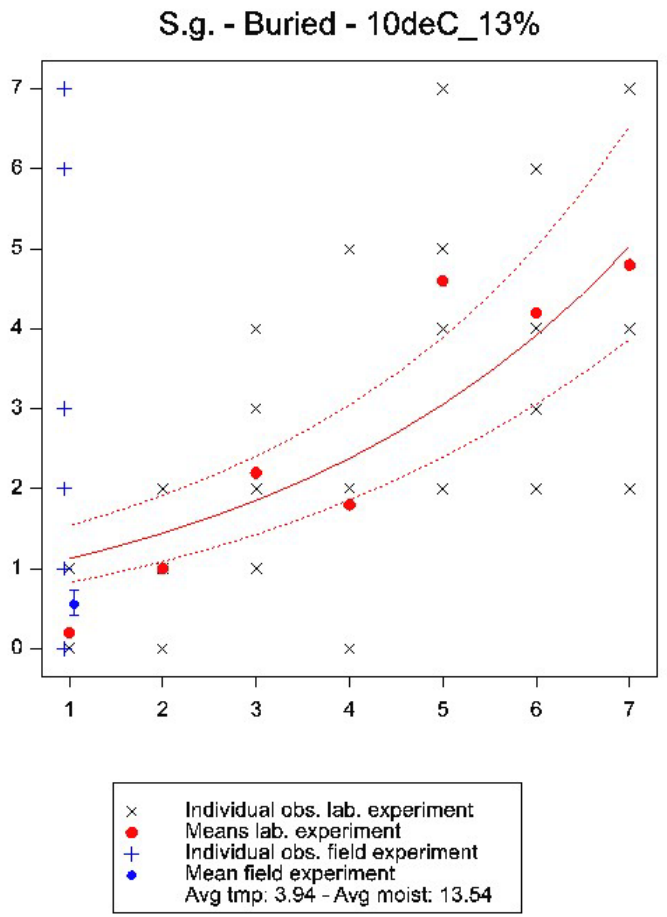


Figure 24b. Mean *S. granarius* trap catches overlaid on laboratory data (error bars and dotted lines = 95% confidence intervals)







Large scale Study 2

Mean temperatures recorded in the grain store during Study 2 were almost all between 15 and 20ºC. Temperatures were lower during trapping at the highest insect densities than during trapping at the lower two insect densities. Ambient temperature was recorded at two points at opposite ends of the bin store (Table 37).

Table 37a. Mean temperatures (ºC) during trapping – 1 insect/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Ambient			18.9	18.8		
10 cm	sensor failed	17.3	20.1	17.3	17.7	18.3
50 cm	14.8	14.7	15.9	16.0	15.0	16.3

Table 37b. Mean temperatures (ºC) during trapping – 3 *O. surinamensis*/kg, 4 *S. granarius*/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Ambient			17.6	17.7		
10 cm	sensor failed	17.7	19.6	18.0	18.1	18.5
50 cm	17.1	17.0	17.6	17.9	17.2	18.7

Table 37c. Mean temperatures (ºC) during trapping – 5 *O. surinamensis*/kg, 7 *S. granarius*/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Ambient			17.0	16.9		
10 cm	sensor failed	17.2	15.0	17.5	17.6	17.8
50 cm	17.0	17.0	17.2	17.5	17.2	18.3

Grain moisture content ranged from 11.6% to 15.0% and varied between bins (Table 38). There was little difference between grain moisture contents at the three depths sampled, although the grain at the surface of each bin was drier than grain at 50 cm.

Table 38a. Moisture content (%) during trapping – 1 insect/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Surface	13.1	12.4	11.6	11.4	12.9	11.6
10 cm	14.6	14.1	12.7	13.9	13.2	12.7
50 cm	14.7	13.7	13.4	12.9	13.4	12.9

Table 38b. Moisture content (%) during trapping – 3 *O. surinamensis*/kg, 4 *S. granarius*/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Surface	12.7	13.0	12.0	12.2	12.5	12.5
10 cm	14.4	13.6	13.1	12.7	13.4	13.1
50 cm	15.0	12.8	13.2	13.0	12.5	12.7

Table 38c. Moisture content (%) during trapping – 5 *O. surinamensis*/kg, 7 *S. granarius*/kg

	O. surinamensis			*S. granarius*		
Bin	8	10	12	7	9	11
Surface	13.6	13.4	12.5	12.7	12.2	12.2
10 cm	14.0	13.2	13.0	12.7	13.6	13.2
50 cm	14.2	13.2	13.2	12.8	12.8	13.1

Mean trap catches across all bins for each species and 95% confidence intervals adjusted for overdispersion were plotted on the graphs of laboratory data which best matched the conditions in the grain store over the six week study (Figures 25a and b). The best comparison for both surface and buried traps at the lowest two insect densities was 20ºC and 13% m.c. At the highest insect densities the closest comparative laboratory data was 15ºC and 13% m.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 (Figures 26a and b).

Very few *O. surinamensis* were found in spear samples. None were found in surface samples, one at 10 cm and seventeen at 50 cm. The only dead insect was one of those found at 50 cm. The distribution of *O. surinamensis* across the bins was also very patchy in spear samples (Figure 27).

The distribution of *S. granarius* found in spear samples was also very patchy (Figure 27). The mean numbers of live *S. granarius* found in spear samples was greater than 7/kg at each depth sampled, even though approximately 10% of the insects found were dead (Tables 39a–c). Therefore, more insects must have dispersed upwards than downwards in the grain from their points of introduction. Given that spear sampling is known to be an inefficient method of sampling insects in grain, it is likely that the density of insects near the surface of the grain was very much higher than that which was intended.

The major difference between the first and second grain store studies is that the temperature was much lower during the first study. This would have meant that the insects moved much more and travelled larger distances during the second study. This may account for the larger numbers of insects moving towards the surface of the grain and being trapped.

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. It is already known that moisture content gradient ventilation affect the movement and distribution of *O. surinamensis* in bins of wheat (Collins and Conyers, 2009).

Figure 25a. Mean *O. surinamensis* trap catches overlaid on laboratory data with overdispersion adjustment to 95% confidence intervals (error bars and dotted lines = 95% confidence intervals)

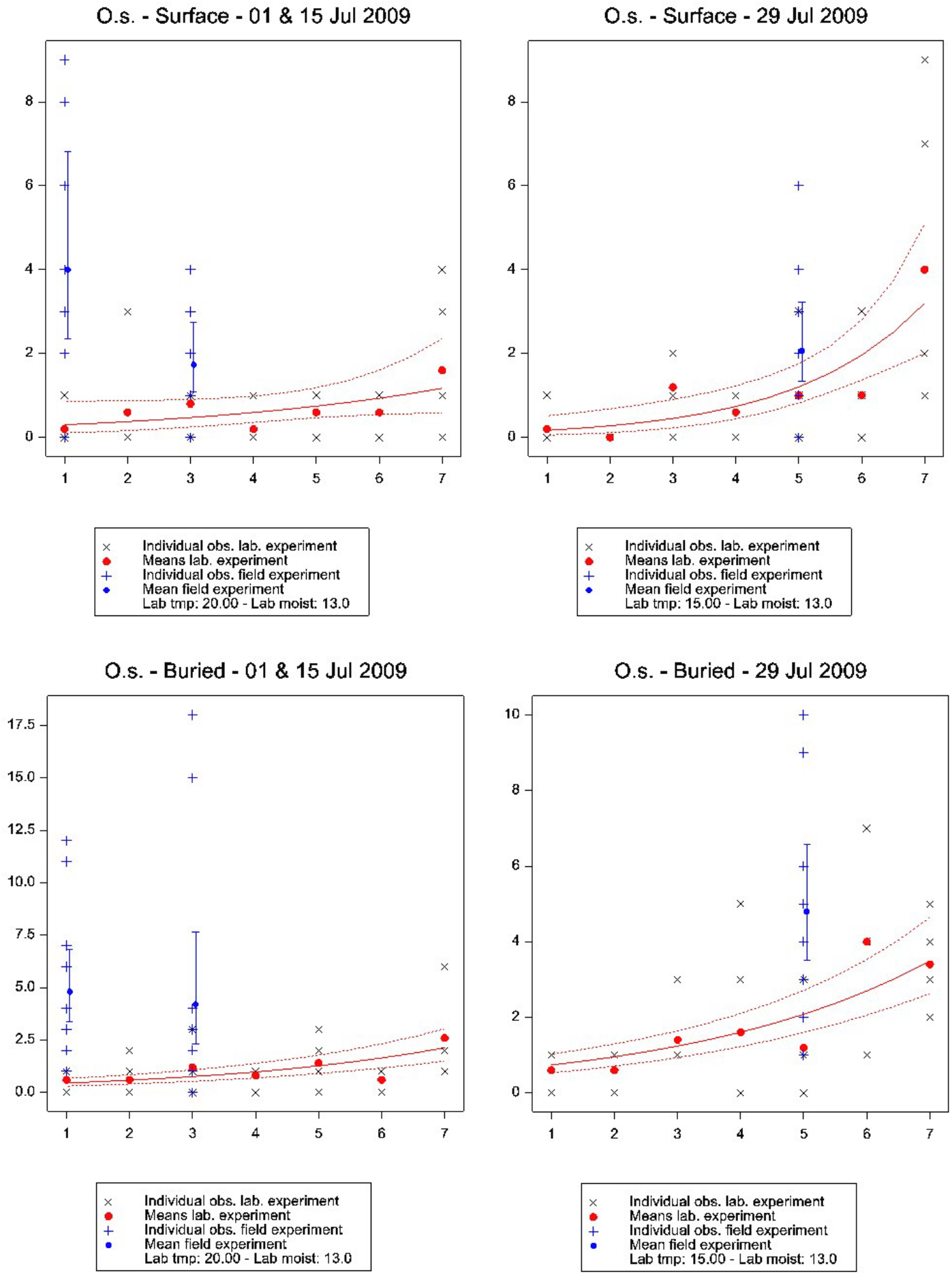


Figure 25b. Mean *S. granarius* trap catches overlaid on laboratory data with overdispersion adjustment to 95% confidence intervals (error bars and dotted lines = 95% confidence intervals)

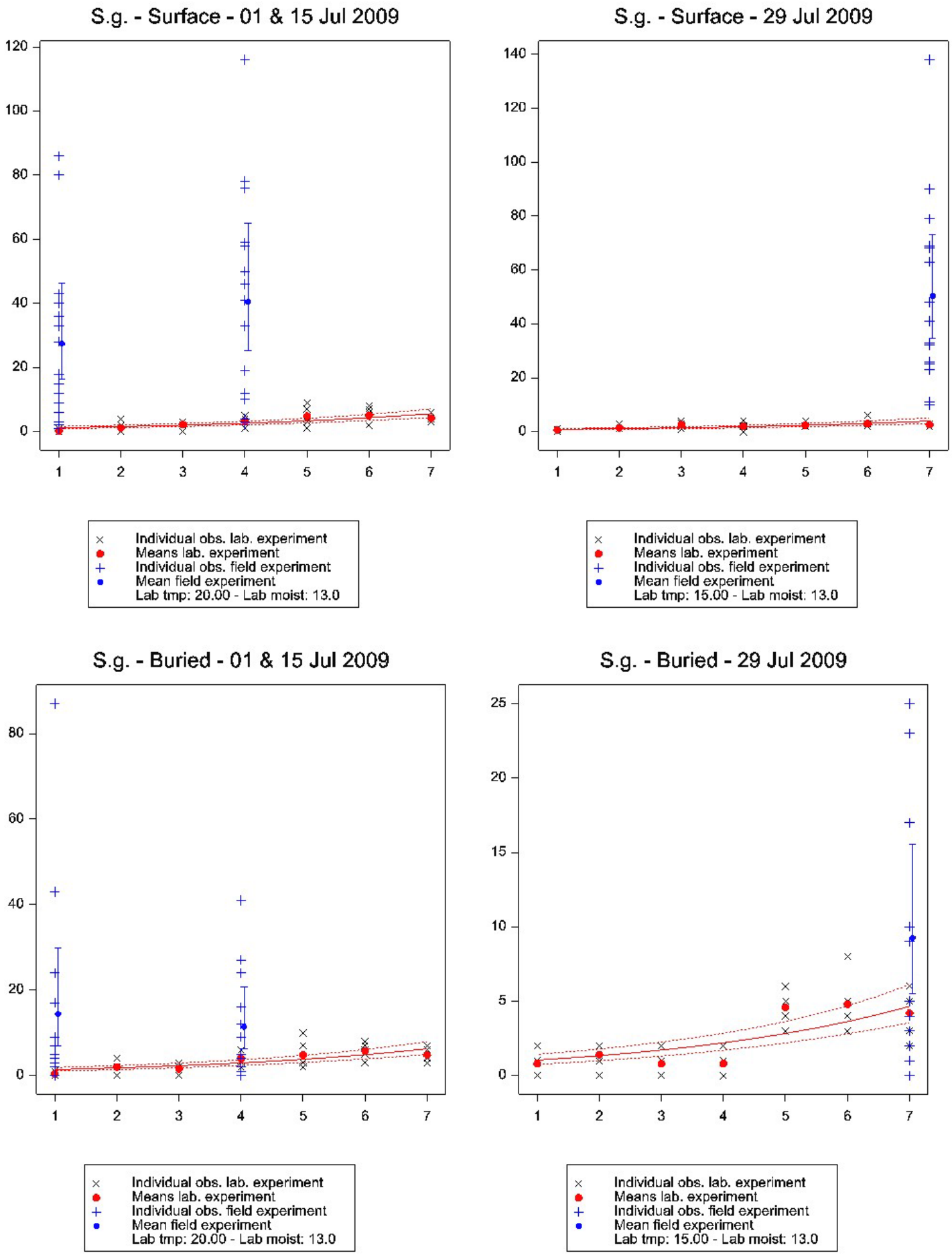


Figure 26a. Distribution of *O. surinamensis* trap catches across bin areas at the surface and 10 cm

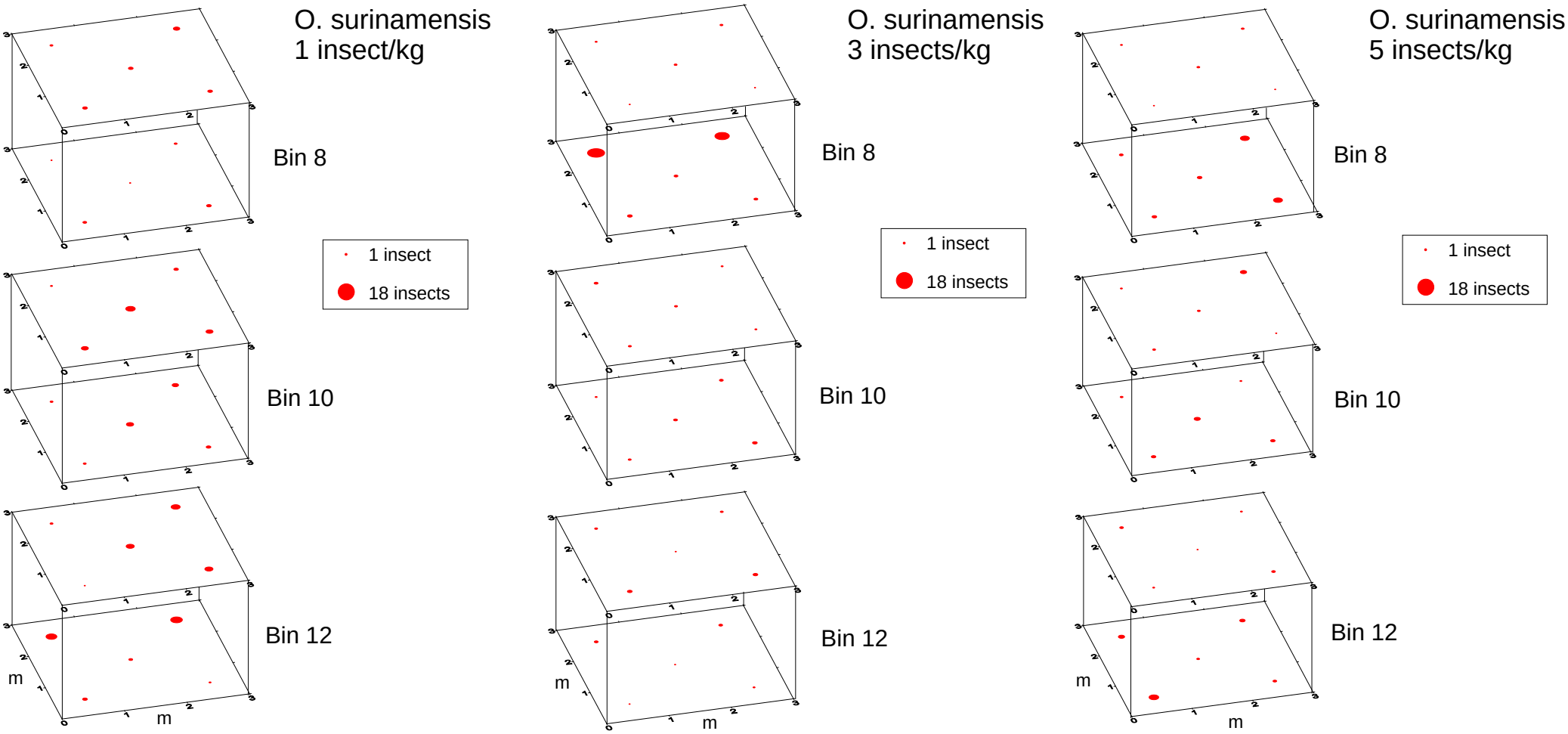


Figure 26b. Distribution of *S. granarius* trap catches across bin areas at the surface and 10 cm

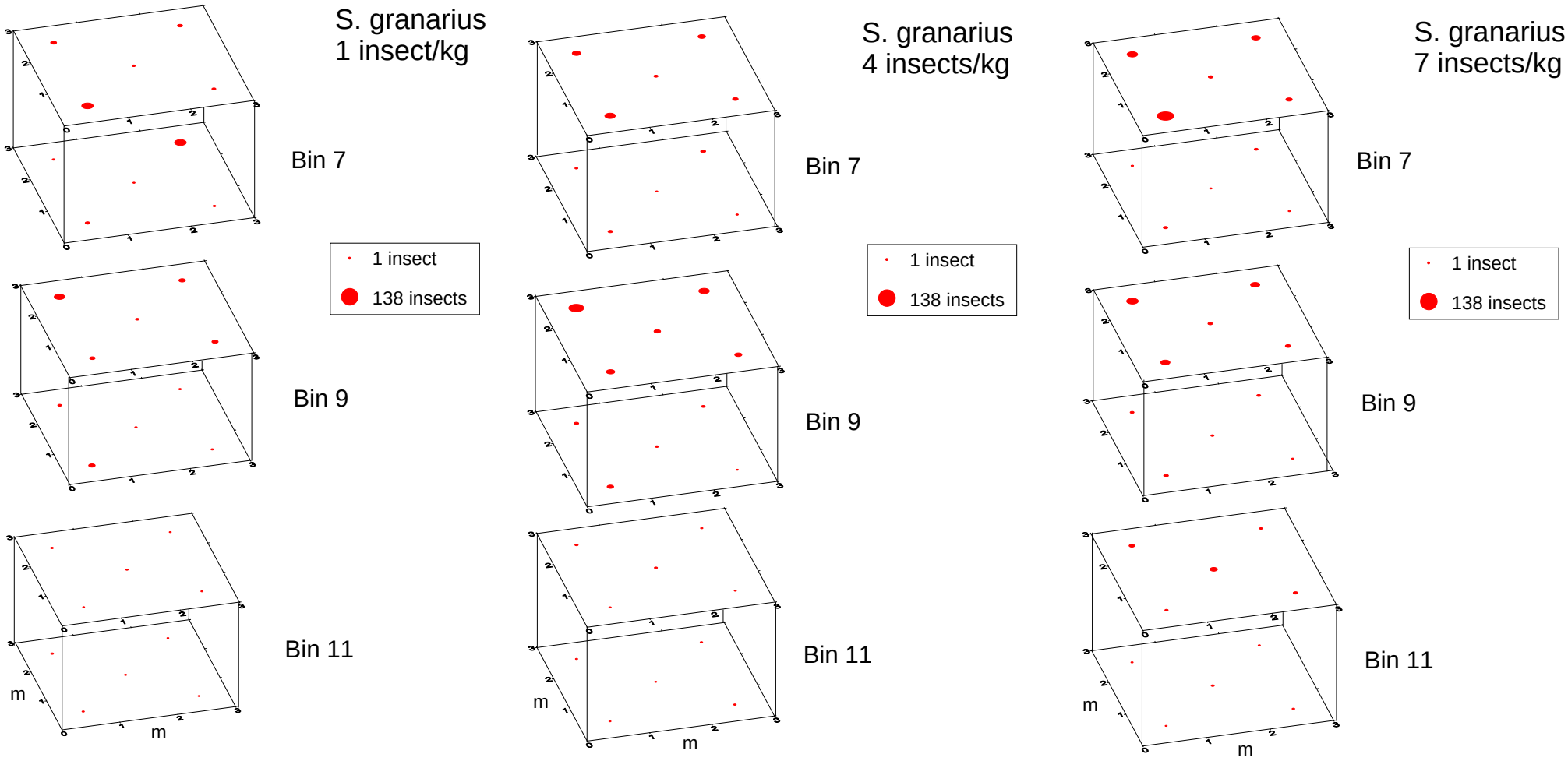
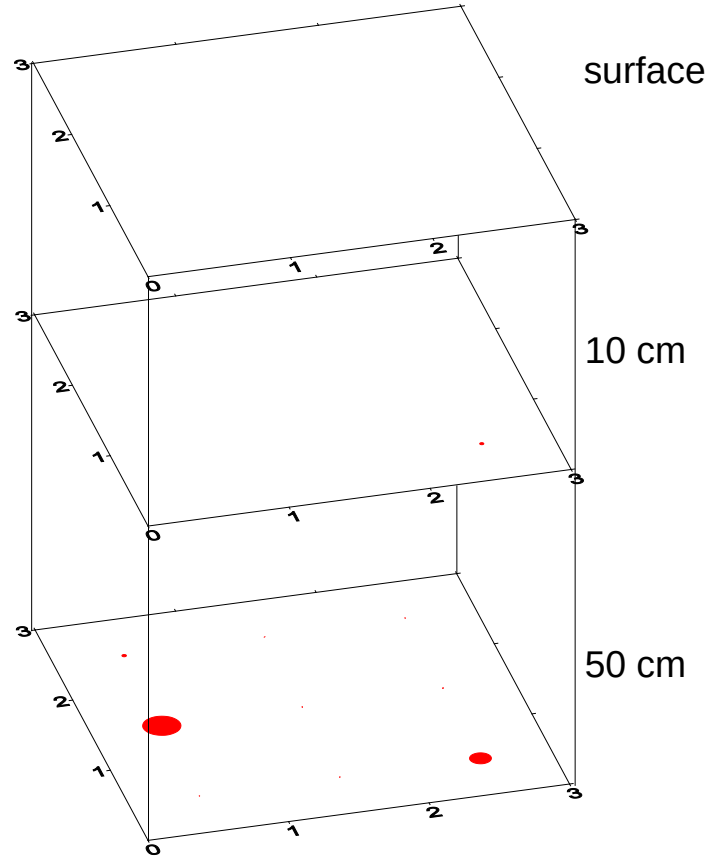
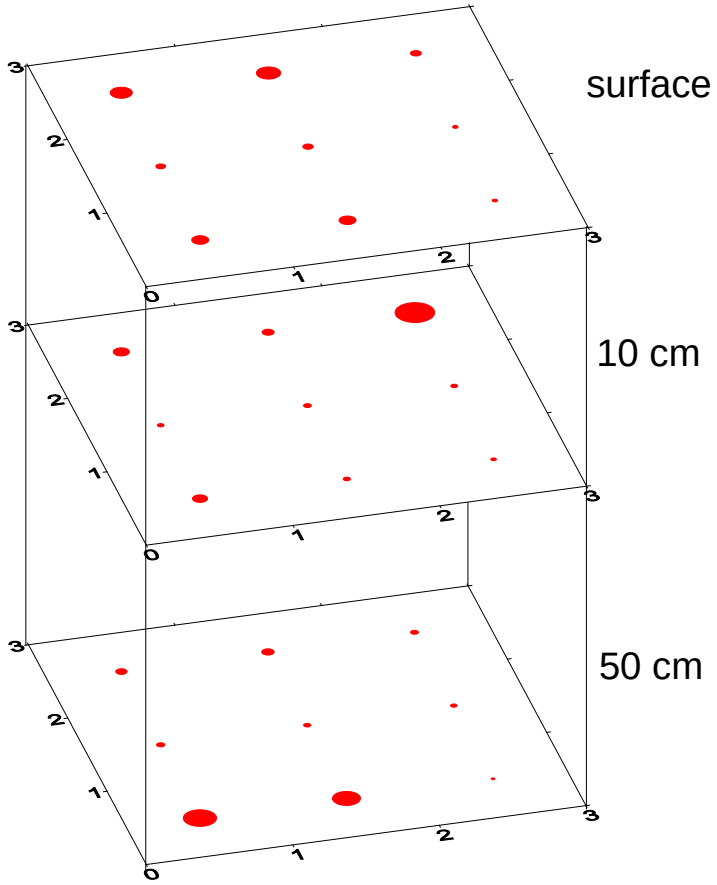


Figure 27. Distribution of insect trap catches across bin areas at the surface, 10 cm and 50 cm



O surinamensis spear samples



S. granarius spear samples

Table 39a. Number of *S. granarius* at each spear sampling point as equivalent number per kg – surface

Equivalent insects/kg	Equivalent dead insects/kg	% Dead insects/kg	Equivalent live insects/kg
13.7	0.0	0	13.7
15.3	0.0	0	15.3
6.7	1.3	20	5.4
7.5	3.0	40	4.5
5.1	0.0	0	5.1
1.4	0.0	0	1.4
13.3	3.3	25	10.0
9.9	0.0	0	9.9
1.3	0.0	0	1.3
Mean 8.3	Mean 0.9	Mean 9.4	Mean 7.4

Table 39b. Number of *S. granarius* at each spear sampling point as equivalent number per kg – 10 cm

Equivalent insects/kg	Equivalent dead insects/kg	% dead insects/kg	Equivalent live insects/kg
11.7	2.3	20.0	9.4
8.8	2.5	28.6	6.3
28.2	1.3	4.8	26.9
2.3	0.0	0.0	2.3
3.1	0.0	0.0	3.1
2.4	0.0	0.0	2.4
10.0	1.4	14.3	8.5
2.6	0.0	0.0	2.6
1.5	0.0	0.0	1.5
Mean 7.8	Mean 0.8	Mean 7.5	Mean 7.0

Table 39c. Number of *S. granarius* at each spear sampling point as equivalent number per kg – 50 cm

Equivalent insects/kg	Equivalent dead insects/kg	% Dead insects/kg	Equivalent live insects/kg
5.7	0.0	0.0	5.7
6.6	0.0	0.0	6.6
3.1	0.0	0.0	3.1
4.6	1.2	25.0	3.5
4.0	1.3	33.3	2.7
3.2	1.1	33.3	2.1
22.1	0.0	0.0	22.1
19.4	1.1	5.9	18.2
0.0	0.0	NA	0.0
Mean 7.6	Mean 0.5	Mean 12.2	Mean 7.1

3.1.2 Development of a moisture sensor

Introduction

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. It should then be possible to calculate the moisture content of the grain from temperature and humidity measurements using Integrated Grain Store Manager (Knight and Wilkin, 1997).

Method

An experimental computer controlled grain management system, Storecheck RH, was installed in six 30 tonne bins in the Fera grain store by Robydome Ltd. Temperature and humidity were monitored using probes inserted in the grain. Bin 4 had eighteen probes, nine 2 m in length and nine 0.5 m in length. The other five bins had two probes, one of each length. Each bin was fitted with a Woods 6F8430 air movement cooling fan. Ambient conditions were monitored using a probe fixed near the inlet of one of the fans. When ambient temperature was less than 2°C below the average temperature in the bins the fans were switched on automatically.

Humidity readings from the grain were more variable than expected and moisture contents calculated from probe readings using Integrated Grain Store Manager (Knight and Wilkin, 1997) 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:

- A new tip was designed to prevent contamination of the sensor by grain entering the tip.
- Sealing the top of the probes with silicone to prevent the movement of air.
- Sealing the bottom of the probes with silicone to prevent the movement of air.

Results and discussion

The temperature in Bin 4 reduced over time to reach 5°C as expected (Figure 28).

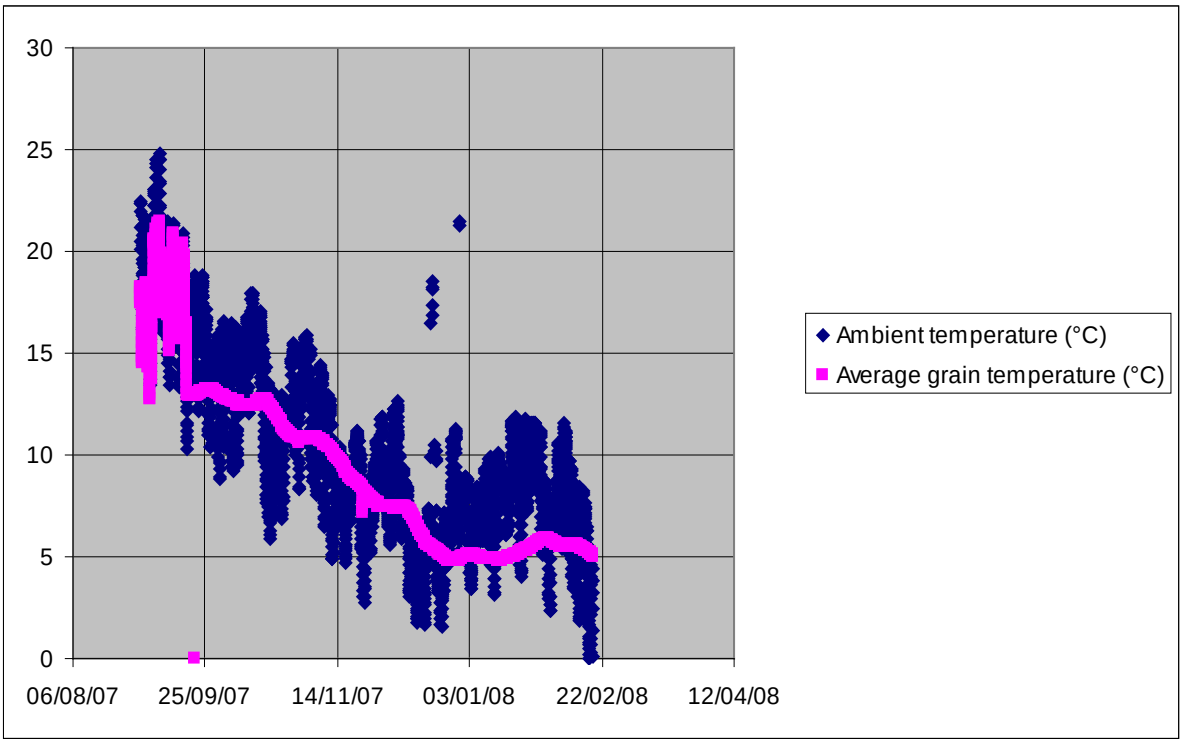


Figure 28. Temperature change in Bin 4 over time

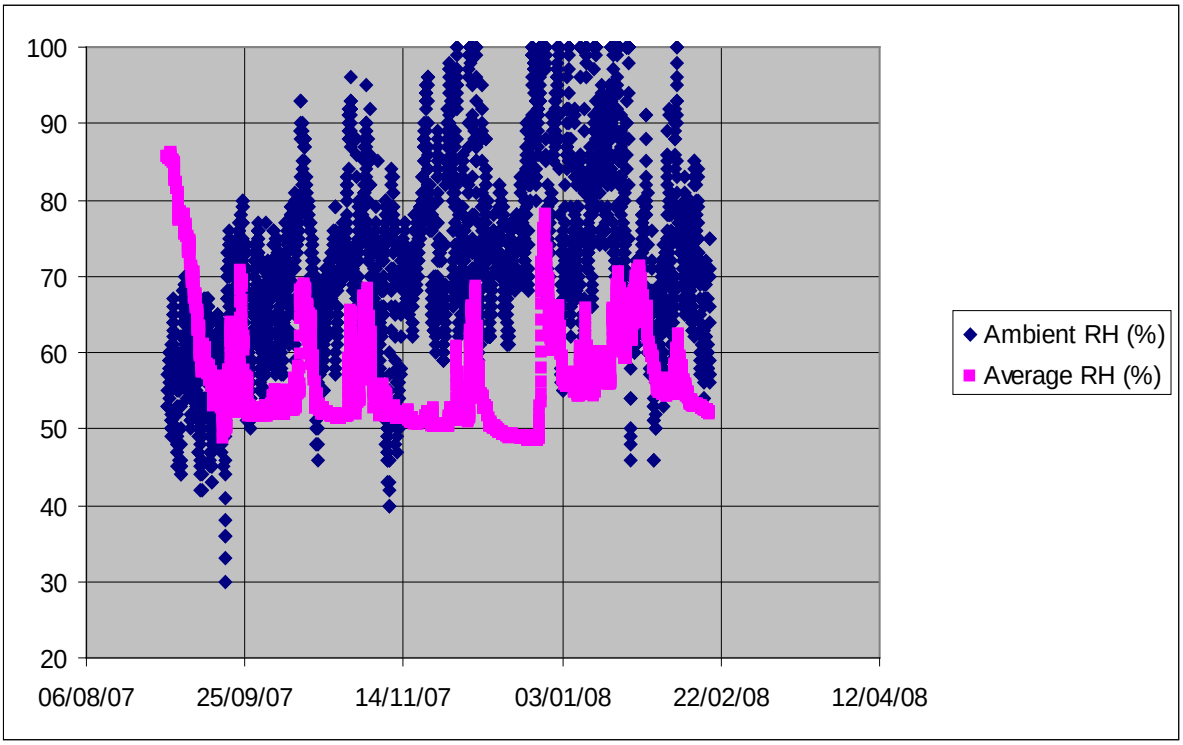


Figure 29. Change in relative humidity over time

Atmospheric humidity varied as expected (Figure 29). However, humidity readings from the grain were more variable than expected (Figure 29) and moisture content calculated from probe readings using Integrated Grain Store Manager did not agree with moisture contents determined using the oven method. The average moisture content between 0.5 and 2 m in Bin 4 measured using the oven method was 13.8%. The moisture content calculated from probe readings varied between 12.9% and 21.6%.

When the tip of one of the probes was examined it was found to be full of grain that had been forced through an orifice at the bottom of the probe when the probe had been pushed into position. A new tip was designed to prevent contamination of the sensor by grain entering the tip. The tip of one of the probes was changed for a tip of the new design at 0 days and the other was not altered. Both probes were 2 m in length and positioned in bin 4 where the moisture content determined by the oven method was 13.5 %.

Examination of the predicted moisture content readings gained from the humidity probe with the modified tip showed that there was no improvement over the sensor with the unmodified tip (Figure 30). The predicted moisture content given by readings from the two probes varied with atmospheric temperature. Whenever atmospheric temperature was more than a few degrees above the grain temperature, high moisture contents were predicted. Under these conditions, air will travel down through the grain. When atmospheric temperature was less than the grain temperature, the

predicted moisture content was similar to the oven moisture content. Under these conditions, the flow of air through the grain would be upwards.

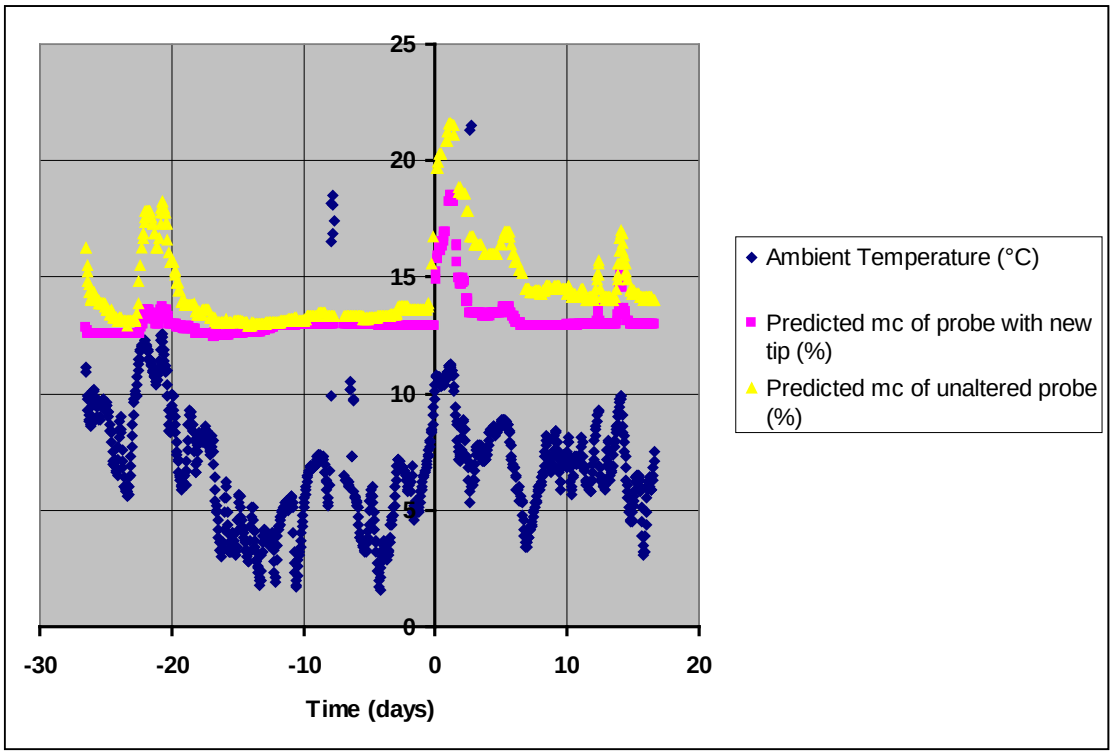


Figure 30. Predicted moisture content from modified and unmodified probes.

These results suggested that air was leaking through the top of the probe leading to false humidity readings. When atmospheric temperature was higher than the grain temperature, atmospheric air moved down the hollow probes so that the sensors read atmospheric humidity rather than the humidity in the grain. This would explain predicted moisture contents higher than the oven moisture content. When atmospheric temperature was lower than grain temperature, air moved in the opposite direction and so the sensor read the humidity of the air from the grain bulk. In this case, the predicted moisture content was more accurate.

To test this theory, the top of a third probe from the same bin was sealed using a blob of silicone. Figure 31 shows moisture content predicted from later readings given by the two probes from Figure 30 and the third probe, sealed at 0 hours. The predicted moisture content of the third probe varied in the same way until the top was sealed. Once the top had been sealed, the predicted moisture content dropped over the course of 10 days to give a steady prediction close to the oven moisture content.

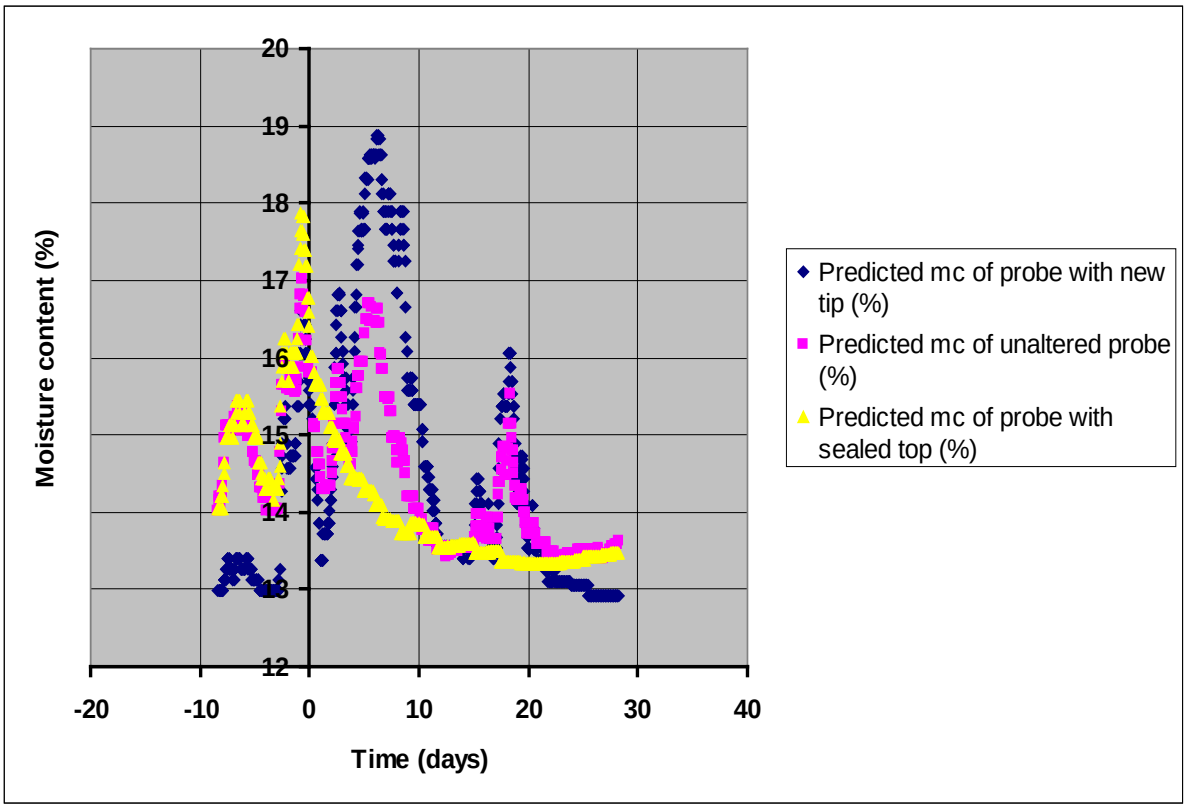


Figure 31. Modified probes sealed with silicone.

The bottom of two probes were further modified to make them faster reacting. This was done by drilling small holes in the shaft of the probes just above the sensors and then filling the shaft with silicone so that the sensors were sealed in a small chamber at the bottom of the probe.

Figure 32 shows the moisture content predicted by readings from two probes sealed above the sensors and two unaltered probes. The sealed probes settled over the course of 4 days to give steady predictions close to the oven moisture content.

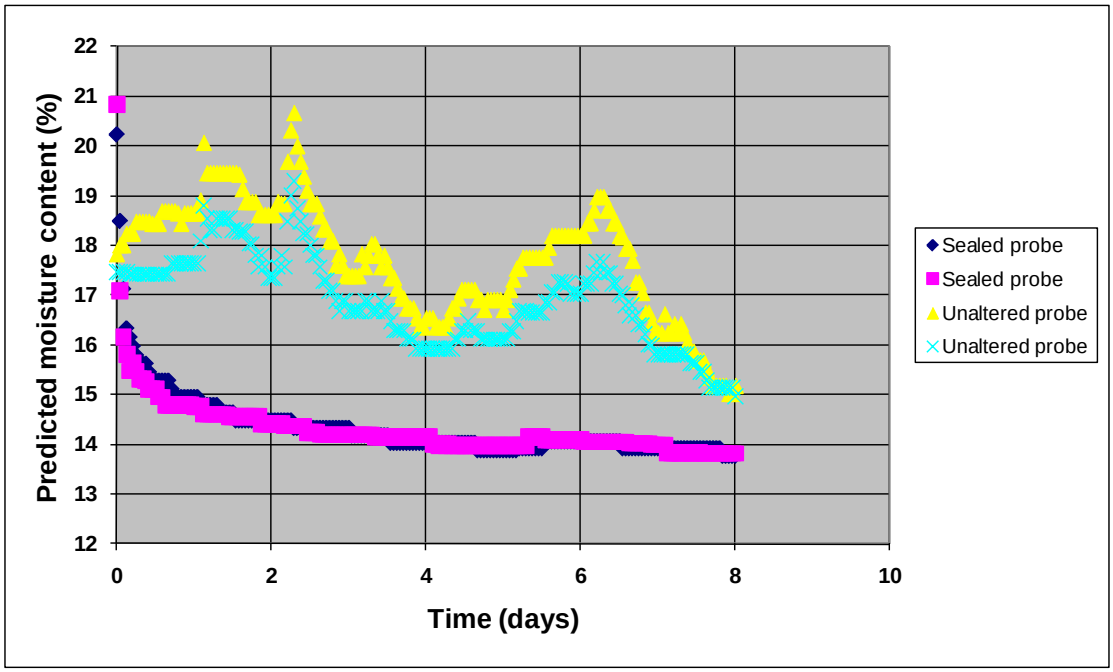


Figure 32. Predicted moisture content from unmodified probes and probes with the final modification

Conclusion

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.

It was planned that a more comprehensive study would be undertaken in a commercial facility, but unfortunately, due to ownership changes of one of the project partners this was not possible. Available resource was therefore, used in other areas of the project.

3.4.2. Step 3.2 Arthropods as vectors of mycotoxin-producing fungi

Introduction

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 (Hubert *et al*., 2003) and may, therefore, spread mycotoxin-producing fungi throughout stores.

Mites have long been considered as fungal vectors (Jacot, 1930) and fungal dispersion via mites has been documented in stored food habitats (Griffiths *et al*., 1959; Armitage and George, 1986). The relative importance of storage mites as vectors is unclear; however they are capable of dispersing toxigenic fungi in certain conditions (Franzolin *et al*., 1999). Mites can act as passive vectors with spores and conidia dispersed randomly by adhering to the surface of mites as they

move through commodities, although their capacity to act as random passive vectors is relatively low due to their small size. They can also act as selective fungal carriers, transferring a high load of specific fungal species (Hubert *et al*., 2003). Storage mites feed on fungi and are selective in their feeding habits (Griffiths *et al*., 1959). The majority of fungal species are found actively by mites and ingested, with spores adhering to the mite bodies during grazing (Hubert *et al*., 2003). Spores can also be carried in the digestive tract, which are then shed with the faeces and can serve to distribute fungi around stores (Griffiths *et al*., 1959; Hubert *et al*., 2003).

Insects are also known to harbour micro-organisms, including pathogenic bacteria, and be capable of transferring them from contaminated to uncontaminated commodities (Channaiah *et al*., 2010; Husted *et al*., 1969). Vectoring of entomopathogenic fungi is also known to occur by insects that are neither susceptible to the fungi or the target prey species (Baverstock *et al*., 2010).

Storage pests are able to survive and multiply on fungi, which allows for survival in empty stores, thereby preventing starvation during periods when more suitable food sources are not available (Sinha, 1979). Storage pests in grain stores may therefore, act as vectors for mycotoxin-producing fungi by either active transmission during feeding or by passive transmission as they migrate within a store. The potential to act as vectors will depend on what fungi are naturally present on or in the pests and whether these fungi can be transferred onto clean commodities.

The aims of these experiments were twofold:
1. To determine the natural fungal load carried externally and internally by storage insects and mites.
2. To determine the ability of storage insects and mites to vector mycotoxin-producing fungi.

To determine the natural fungal load carried externally and internally by storage insects and mites.

Materials and Methods

Provision of samples

Sixty farmers, maltsters, commercial stores and pest control operators were initially contacted to request samples of insect and mites from grain stores. Of those contacted, 9 provided a total of 77 samples. On receipt at the laboratory, the samples were inspected and the contents identified. The samples were then put into a freezer prior to analysis.

Where a large number of insects were present in the samples (>20), internal and external extracts were prepared. If there were <20 insects then only external samples were prepared.

Preparation of samples from external surfaces of pests

Where large numbers of insects were present in the samples, extracts were obtained from 50 insects. Where there were lower numbers of insects in the samples, all those in the sample were analysed or combined with other samples from the same location. Where mites were present, a small spatula-full was analysed per sample.

The pests were put into 1.5 ml microcentrifuge tubes and 200 µl of 0.05% sterile Tween 80 was added. The tubes were then vortex mixed for 30 seconds to release any spores from the external surfaces of the pests.

Preparation of samples from inside pests

The pests were put into 1.5 ml microcentrifuge tubes and surface sterilised with 0.5 ml of a 1% solution of sodium hypochlorite. The tubes were shaken for 2 minutes, the sodium hypochlorite solution was pipetted off and 0.5 ml of a 0.05% solution of sterile Tween 80 was added. The Tween 80 solution was then removed and replaced with another 0.5 ml of Tween 80. This rinsing procedure was repeated two more times. Sterile Tween 80 (0.05%; 100 µl) was then added to each tube and the contents of the tubes were macerated with a small plastic pestle.

Incubation and identification of fungal spores

Serial dilutions were made in sterile distilled water of the extract and spread onto DG18 agar (Oxoid) with chloramphenicol (Oxoid). The agar plates were incubated at 25°C for 5–7 days in the dark. Only the main mycotoxin producing fungi (*Aspergillus flavus*, *Aspergillus ochraceus, Aspergillus niger* and *Pencillium verrucosum*) were identified and quantified.

Results

Of the 77 samples received at the laboratory, 47 contained storage pests. The most common insect species were *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus* and *Ahasverus advena*. The most common mite species were *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus* spp. and *Cheyletus* spp.

From the samples that contained pests, 29 external and 18 internal samples were prepared (Table 40). Twenty one samples contained mycotoxin-producing fungi, of which 20 originated from external samples and one from an internal sample (Table 40). Eighteen samples contained *P. verrucosum* (ranging from 0.5×10^2 to 2×10^5 CFU/ml), 8 contained *A. flavus* (ranging from 1×10^2 to 1.5×10^5 CFU/ml), 1 contained *A. ochraeus* (1×10^3 CFU/ml) and 3 contained *A. niger* (ranging from 102 to 0.5×10^2 CFU/ml) (Table 40).

Table 40. Samples analysed for mycotoxin-producing fungi

Sample number	Sample type	Fungal species (CFU/ml)				Pests in samples
		P. verrucosum	*A. flavus*	*A. ochraeus*	*A. niger*	
S09-004426	External Internal	0 0	1×10^3 0	0 0	0 0	Insects
S09-004427	External	0.5×10^3	1×10^5	0	0	Insects
S09-004428	External Internal	1.5×10^2 0	1×10^3 0	0 0	0.5×10^2 0.5×10^2	Insects
S09-004853	External Internal	1.5×10^4 0	0 0	0 0	0 0	Insects and mites
S09-004873	External Internal	2.5×10^2 0	1×10^3 0	0 0	0 0	Insects and mites
S09-010120	External Internal	0 0	0 0	0 0	102 0	Insects and mites
S09-022137	External	0.5×10^2	0	0	0	Insects
S09-003738/1	External Internal	3.5×10^4 0	1×10^2 0	0 0	0 0	Mites
S09-003738/2	External Internal	0 0	0 0	0 0	0 0	Mites
S09-003738/3	External Internal	0.5×10^5 0	0 0	1×10^3 0	0 0	Mites
S09-003738/4	External Internal	2.5×10^3 0	0 0	0 0	0 0	Mites
S09-003738/5	External Internal	1.5×10^3 0	0 0	0 0	0 0	Mites
S09-003738/6	External Internal	0 0	0 0	0 0	0 0	Mites
S09-006979	External Internal	0 0	0 0	0 0	0 0	Insects and mites
S09-006980	External	0	0	0	0	Insects and mites
S09-005761 & S09-005762	External	2×10^2	0	0	0	Insects
S09-004078	External Internal	1.1×10^3 0	1.5×10^3 0	0 0	0 0	Mites
S09-004079	External	0	0	0	0	Mites
S09-004080	External Internal	0 0	0 0	0 0	0 0	Mites
S09-004106	External	1.1×10^5	0	0	0	Insects and mites
S09-004107	External	0	0	0	0	Insects and mites
S09-004108	External	1.5×10^4	2.5×10^2	0	0	Insects
S09-004110	External Internal	2×10^5 0	1.5×10^5 0	0 0	0 0	Insects
S09-004111	External	6×10^3	0	0	0	Insects
S09-004481, S09-004482, S09-004483, S09-004484, S09-004490	External Internal	1.5×10^5 0	0 0	0 0	0 0	Insects
S09-004491	External Internal	0 0	0 0	0 0	0 0	Mites
S09-004492, S09-004493, S09-004494, S09-004495, S09-004496, S09-004497	External	0.5×10^2	0	0	0	Insects
S09-021378	External Internal	0 0	0 0	0 0	0 0	Insects and mites
S09-004792	External	1×10^4	0	0	0	Insects

To determine the ability of storage insects and mites to vector mycotoxin-producing fungi

Materials and Methods

Pesticide-free wheat (15 and 18% moisture content for the insect and mites, respectively) was used in the experiments. The wheat was autoclaved prior to use in the experiments. The wheat was sealed in autoclavable plastic containers and sterilised at 110^{o}C for 15 minutes. The process was repeated a further 2 times over 3 consecutive days. After each cycle in the autoclave the containers were shaken to ensure the grains in the centre had a chance to be closer to the edge of the box and, therefore, a more even exposure to the high temperature.

Six metal tubes (7.5 cm long and 1.6 cm in diameter) were joined together using hot wax to form a 45 cm column. Each tube held approximately 10 g of wheat, which comprised of about 200 grain kernels. Prior to each experiment the tubes were autoclaved at 121^{o}C for 40 minutes to prevent fungal contamination from previous tests. In-between each tube, a 3 x 3 cm square of 2000 µm nylon mesh was sealed to the conjoining tubes with hot wax.

One of the metal tubes (sealed at one end with black paper) was half filled with sterilised wheat (approximately 5 g) and one *P. verrucosum* inoculated grain was added to the centre. The tube was then filled with sterilised wheat and 10 beetles of laboratory strains of *O. surinamensis*, *S. granarius* and *C. ferrugineus* or approximately 50 mites of a mixed population of *A. siro* were added. Each species was assessed separately. The tube was sealed with black paper, laid horizontally in a fluoned tray and put into the experimental conditions (20^{o}C ± 2^{o}C and 70% ± 5% r.h.) for 24 hours. This was to allow the pests to move around the grain and encounter the single fungal infected grain.

At the same time, the other 5 tubes were prepared by filling each tube with approximately 10 g of sterilised wheat. In-between each tube, the nylon mesh was sealed to the conjoining tube using hot wax. The mesh allowed the pests to pass freely between the different tube sections but retained the grain within each tube. The ends of the outer tubes were sealed with black paper. The sealed tubes were laid horizontally in a fluoned tray and put into the experimental conditions for 24 hours. Three replicates were prepared comprising of tubes containing *P. verrucosum* but no pests, pests but no *P. verrucosum* and *P. verrucosum* plus pests. An original sample of grain containing no *P. verrucosum* and no pests was also assessed.

After 24 hours, the tube containing the fungi and pests was opened at one end and a piece of nylon mesh was sealed to the tube with hot wax. The column of 5 tubes was then opened at one end and the tube containing the fungi and pests was joined to the other tubes with hot wax. The tubes were again laid horizontally in a fluoned tray and returned to the experimental conditions for 24 hours (for insects and mites) and 7 days (for mites only).

Each section of tube was then disconnected from the column and the numbers of insects in each tube were counted. For the mites, the grain from each section was sieved over a 710 µm mesh, the sievings were examined under a low power binocular microscope and the numbers of live mites were counted. The grain from each tube was put into separate bags to await fungal analysis.

The grain was assessed for fungi using DG18 media. A template was made with 30 dots and placed under every plate to make counting grains and placing grains easier. Thirty grains were randomly selected from each sampling bag and placed onto the media using sterile forceps, one at a time. The forceps were not sterilised between each grain taken from the bag, but were sterilised between each sample. The grain was also taken from the bag furthest away from the initial site of inoculation first to further reduce the risk of contamination. Plates were read after 6 days incubation initially to check for growth from the grains, then again at 12 days so that the fungi had a chance to develop into distinguishable colonies. The initial test was in case the plates became too overgrown and not easy to read at 12 days.

Results and Discussion

In preliminary experiments, it was found that grain in all sections of the column was infected with *P. verrucosum* when there was:

- 1%, 10% and 100% by weight of infected grains and 100 *O. surinamensis* exposed for 5 days.
- 1% by weight of infected grains and 10, 50 and 100 *O. surinamensis* exposed for 5 days.
- 1% by weight of infected grains and 10 *O. surinamensis* exposed for 3 days.

In the main experiments, insects were found in all sections of the columns (Table 41), including the tube the furthest away from initial inoculation tube, indicating that they were able to move freely between the different sections during the 24 hour experimental period. Insect mortality in the tubes was <10%.

Mites were mostly found in the initial inoculation tube after 24 hours, although a small number (<4) were found in the next two sections of the columns (Table 41). After 7 days, mites were found in all tubes, other than the tube furthest away from the inoculation tube, although most were in the first tube (Table 41).

Table 41. Numbers of insects and mites in each tube after exposure period

Experimental details	Tube no.	Numbers of pests in each tube				
		O. surinamensis (24 hours)	*S. granarius* (24 hours)	*C. ferrugineus* (24 hours)	*A. siro* (24 hours)	*A. siro* (7 days)
No Pv & pests	1	1	5	3	21	11
	2	1	0	2	0	2
	3	3	0	2	0	10
	4	0	1	3	0	9
	5	1	0	0	0	10
	6	1	4	0	0	0
Pv & pests (Replicate 1)	1	2	5	1	41	46
	2	1	2	4	0	9
	3	4	0	0	1	9
	4	0	0	5	0	3
	5	1	0	1	0	6
	6	0	3	1	0	0
Pv & pests (Replicate 2)	1	2	4	2	24	16
	2	1	4	1	1	2
	3	3	1	4	2	5
	4	1	0	0	0	0
	5	0	0	1	0	2
	6	3	1	2	0	0
Pv & pests (Replicate 3)	1	4	9	2	21	14
	2	3	0	1	4	14
	3	0	0	3	0	9
	4	0	0	1	0	3
	5	1	0	2	0	3
	6	1	1	0	0	0

Pv = *Pencillium verrucosum*

It is not known whether the pests moved continuously up and down the column or whether they remained fairly static in certain sections. However, the results show that *P. verrucosum*-infected grains were present in sections where pests were not detected during the assessments, indicating that some movement back and forth between sections may have occurred.

Table 42 shows the % *P. verrucosum* infected grains following exposure to the pests. Tube 1 contained the fungal inoculum and tube 6 was the tube furthest away from the fungi (approx. 34cm away). With the insects, most of the randomly selected grains were infected with *P. verrucosum,* irrespective of species and distance from the initial inoculated grain. With the mites, the percentage of *P. verrucosum* infected grains decreased as the distance from the initial inoculated grain increased, with none of the selected grains in the furthest tube found to be infected.

Table 42. % Pv infected grains following exposure of pest species

Experimental details	Tube no.	% Pv Infected grains (out of 30 grains)				
		O. surinamensis	*S. granarius*	*C. ferrugineus*	*A. siro* (24 hours)	*A. siro* (7 days)
No Pv & pests	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Pv & no pests	1	100	100	100	100	100
	2	0	16.7	16.7	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Pv & pests	1	100	100	100	90	100
(Replicate 1)	2	100	100	100	23.3	96.7
	3	100	100	100	10	80
	4	73.3	100	100	0	23.3
	5	100	100	100	0	0
	6	93.3	100	93.3	0	0
Pv & pests	1	100	100	100	100	100
(Replicate 2)	2	100	100	100	23.3	86.7
	3	100	100	100	0	30
	4	100	100	100	0	10
	5	100	100	86.7	3.3	0
	6	86.7	100	96.7	0	0
Pv & pests	1	100	100	100	100	100
(Replicate 3)	2	100	100	100	50	93.3
	3	100	100	100	10	76.7
	4	100	100	100	3.3	3.3
	5	100	100	100	0	0
	6	100	100	100	0	0

Pv = *Pencillium verrucosum*

Although the results indicate that, in some cases, all of the 30 sampled grains in the sections were inoculated with *P. verrucosum*, it is likely that contamination occurred during the assessments rather than all the grains being infected as a result of transmission by the pests. It does however, indicate that the pests were able to transmit fungal spores to the previously uncontaminated grain, as there was no other way this could have occurred i.e. there were no air movements through the columns, the grains were retained in their individual sections by the mesh and the initial grain sample was *P. verrucosum*-free. When no pests were present, most of the *P. verrucosum* was detected in the initial inoculation tube and not in the other sections (other than 5 grains in the second tube in experiments with *S. granarius* and *C. ferrugineus*).

Conclusions

The results of these experiments indicate 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.

These experiments indicate that 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.

3.4.3. Step 3.3 Establishment of thresholds

The findings from Objectives 1 and 2, together with the data and results from the studies in Objective 3 were examined to determine whether there should be any changes in current key recommendations for best practice and where new recommendations or thresholds should be established.

Studies under Objective 1 had shown that occurrence of *P. verrucosum* is widespread, but had also highlighted areas of the store where greater levels of the inoculums could be found. Recommendations could therefore, be made to pay particular attention to these areas. It had also been shown that *P. verrucosum* could develop in the surface layer of grain during the winter months and that this area should therefore, be monitored closely. As expected, development of the fungus occurred more slowly at 15°C and, therefore, cooling of grain prior to drying could be recommended.

Studies under Objective 2 had established limits of temperature and moisture content for development of common insect and mite pest species. These can be used in conjunction with monitoring of the physical parameters to assess risk. The finding that there is widespread resistance to one of the commonly used chemical control agents, pirimiphos methyl, leads to the need for recommendations to reduce the development of resistant populations. Although it was shown that hygiene methods can reduce the insect and mite populations present in empty stores, these were not completely eradicated, reinforcing the need for an integrated pest management approach. The modelling of cooling strategies established that current recommendations for best practice are robust and reinforce the benefit of the use of differential thermostats.

Objective 3 established that interpretation of 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.

These findings were taken forward to Objective 4 to be used in the production of an HACCP approach to grain storage establishing best practice.

3.5. Objective 4: Validation of the integrated approach by using data from a 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 resource 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.

3.5.1. Step 4.1 Defining assessment and management of risk using an HACCP approach

Terms of reference

This is a generic HACCP plan that covers the storage of grain on farm (for grain stored on the place of production) and in central stores (for grain produced at different sites of production). It starts at the intake of grain (grain produced on farm or grain delivered to a central store) through to unloading of store and dispatch of the grain to the customer. i.e. transferred to the next point in the food or feed supply chain. The HACCP plan covers food and feed safety issues and key quality attributes at the point of dispatch and will look at the following hazards:

Biological	Chemical	Physical
Salmonella and other vegetative pathogens (e.g. E.coli) Storage mites † Storage insects †	Mycotoxins ‡ Ochratoxin A (OTA) Fusarium mycotoxins (deoxynivalenol (DON), T-2 and HT-2 and zearalenone (ZER or ZON)) Pesticide residues ‡ Polycyclic aromatic hydrocarbons (PAHs)	Ergot Glass Stones Metal Rodent faeces †

† Quality issue ‡ Exceeding statutory or guidance MRLs

The HACCP is underpinned by a number of prerequisite programmes (defined as Good Storage Practice programmes (GSP) in this grain store HACCP), which are implemented to maintain a hygienic grain store environment. These are identified in the hazard analysis and detailed in the Good Storage Practice Programme table.

Critical Control Points (CCP) are derived using professional judgement based on risk assessment of hazards. A CCP is a step in the process where control is necessary to prevent or reduce to an acceptable level a significant (high risk) hazard. Low and medium risk hazards are identified as controlled by the GSP programme.

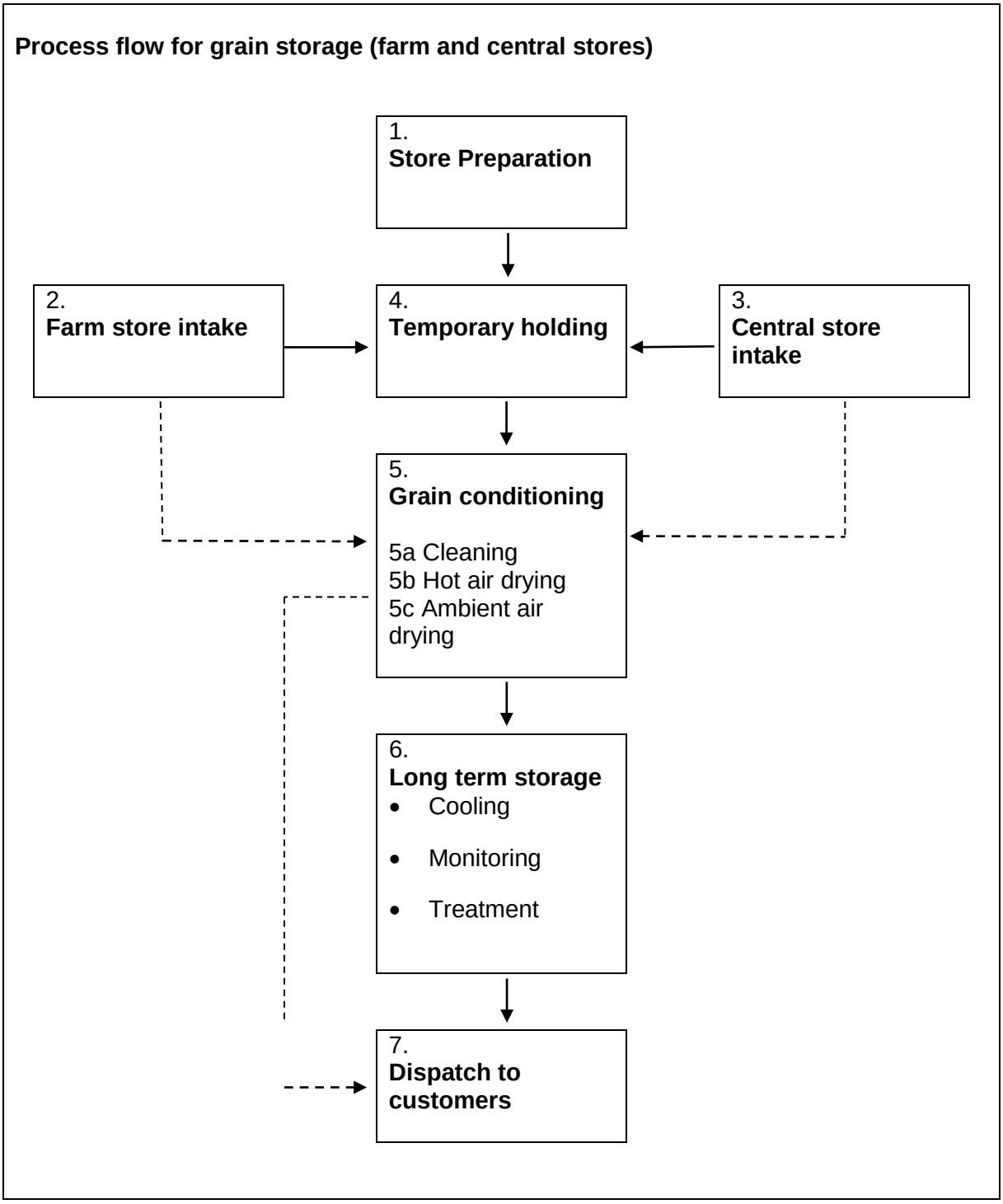
In this HACCP, the control and monitoring of hazards including remedial/corrective actions taken is linked to relevant legal requirements (e.g. pesticide regulations) and good practice as defined in recognised standards and codes of practice including, for example: ACCS, SQCS, TASCC assurance schemes. These standards may go into greater detail than is possible or appropriate in this HACCP plan.

Product description and intended use

- Product types: wheat, barley and oats
- Treatments: grain is conditioned (cleaned and dried). Insecticides may be applied for the control of storage pests (pesticide treatments).

- Storage conditions: grain is stored dry and cool (≤15% moisture content (mc) - target 14.5% and ≤15°C depending on storage time and market requirements)
- Intended use : for marketing as a raw material for further processing as a food or feed product (human and animal consumption).

Process flow diagram



Risk evaluation

The aim is to evaluate the risk of each hazard and determine the significant hazards and the uncertainty associated with the evaluation of risk.

Risk is a function of the likelihood of occurrence of the hazard and the severity of the consequence hazard being present in the grain at dispatch if the controls failed. Momentary failure of low risk hazard does not represent a significant food safety hazard. Whereas momentary failure of a high risk hazard represents a significant food safety risk (or failure to comply with legal requirements).

A four point scoring system is used

Likelihood		Severity †	
1.	Very low/unlikely	1.	Very low
2.	Low/possible	2.	Low
3.	Medium/probable	3.	Medium
4.	High/very likely	4.	High

† Food safety or failure to comply with legal requirements

Overall risk or significance of a hazard can be categorised into high, medium or low based on the risk rating (1-16 likelihood x severity score), e.g.

- Low risk (1-5)
- Medium risk (6-10)
- High risk (11-16)

Table 43. Hazard analysis

Process step	Hazard (and possible cause/source)	Risk score (LxS)	Control measure
1. Store preparation (farm and central stores) Preparation of empty store prior to loading with new crop, and includes where applicable cleaning and disinfection, maintenance and pesticide treatment of key equipment and the store structure. Storage structures and equipment are fit for purpose.	1.1 Introduction of mites, insects, pathogenic bacteria and mould/fungi to new crops due to contamination from previous crop residues or non-crop uses	6	Store cleaning and maintenance procedures and pesticide treatment (insect and mite infestation)
	1.2 Introduction of glass, stones and rodent faeces to new crop due to contamination from previous crop residues or non crop uses	2	Store cleaning, glass control, design and maintenance and pest control procedures
	1.3 Introduction of pesticide residues to new crop from incorrect application to store structures	1	Pesticide control procedures (including for example use of approved pesticides in the approved manner by trained operators using calibrated application equipment)
2. Farm store intake Grain ex combine from crops produced on farm and transferred to an on-farm store facility.	Presence of biological, chemical and physical hazards due to introduction at previous stages.	Not applicable	Control is pre-storage and relies on Good Agricultural Practice (GAP) during growing, harvesting and post-harvest handling on farm.
3.Central store intake Receipt of grain from outside sources at a central store facility, including intake checks and tipping of grain at intake point.	3.1 Presence of *Fusarium* mycotoxins and ergots due to contamination at previous stages (growing and post-harvest handling).	4	Grain intake procedures (approved suppliers and agreed grain quality specifications)
	3.2 Presence of pesticide residues due to introduction at previous stages (crop production and handling)	1	Grain intake procedures (approved suppliers and agreed grain quality specifications)
	3.3 Presence of physical hazards (e.g. glass, metal and stones) due to contamination at previous stages (incorrect growing and post-harvest handling)	1	Grain intake procedures (approved suppliers and agreed grain quality specifications)

Process step	Hazard (and possible cause/source)	Risk score (LxS)	Control measure
4.Temporary holding Temporary storage of grain pre-drying.	4.1 Introduction of mites, insects and OTA causal fungi from handling equipment and store structure	6	Store cleaning and deign and maintenance procedures Pesticide treatments of store structure
	4.2 Growth of mite and insect populations due to high grain moisture and prevailing temperature	4	Store grain dry and cool, dry grain with high moisture promptly Store design and maintenance procedures
	4.3 Introduction of OTA as a result of growth of causal fungi on grain due to high grain moisture, prevailing temperature and storage time	16	Store grain dry and cool, dry grain with high moisture promptly Store design and maintenance procedures
	4.4 Introduction of glass and rodent faeces from the environment (lights, rodent ingress, etc.)	1	Glass and pest control procedures
5a Grain conditioning - Cleaning Cleaning of grain and admixture of pesticides (if applicable), including grain handling (e.g. conveying)	5.1 Introduction of storage pests and OTA causal fungi from equipment	3	Store cleaning procedures
	5.2 Introduction of pesticides due to incorrect admixture to grain	3	Pesticide application procedures (including for example use of approved pesticides in the approved manner by trained operators using calibrated application equipment)
	5.3 Introduction of glass and rodent droppings from the environment (lights, rodent ingress etc.)	1	Glass policy and pest control procedures
	5.4 Introduction of metal from equipment	1	Equipment maintenance procedures

Process step	Hazard (and possible cause/source)	Risk score (LxS)	Control measure
5b. Grain conditioning - Hot air drying Drying grain (batch or continuous drying process) by heated-air (direct or indirect fired). Equipment is operated efficiently.	5.5 Introduction of HTAs from fuel oils in direct fired systems	6	Use appropriate grade of oils in compliance with equipment manufacturers recommendations (waste oils are not permitted)
5c. Grain conditioning - Ambient air drying Bulk drying of grain using high volume ambient air drying systems. Equipment is operated efficiently	5.6 Introduction of OTA mycotoxin as a result of growth of causal fungi due to slow drying, including deterioration of undried grain ahead of the drying front	9	Dry grain in correct time frame Equipment maintenance procedures (correct airflow)
6. Long term storage Storage of conditioned grain in cool and dry conditions including cooling, monitoring of grain condition and insect and mite population, and pesticide treatment where applicable (spot treatment or fumigation).	6.1 Introduction of OTA mycotoxin as a result of mould growth due to high grain moisture and temperature	16	Store grain dry and cool Store design and maintenance procedures
	6.2 Growth of insect and mite populations due to high grain moisture and temperature	16	Store grain dry and cool Store design and maintenance procedures
	6.3 Introduction of mould fungi (OTA) from growth and spread of insect populations .	9	Store grain dry and cool Store design and maintenance procedures
	6.4 Introduction of pesticide residues due to incorrect application to grain and short interval to unloading of grain from store.	6	Pesticide application procedures (including, for example, use of approved pesticides in the approved manner by trained operators using calibrated application equipment)
	6.5 Introduction of pathogens and faeces from pests (rodents, birds, etc.) glass from lights in the store environment.	1	Glass and pest control procedures

Process step	Hazard (and possible cause/source)	Risk score (LxS)	Control measure
7. Dispatch Unloading of store, and loading of transport vehicles.	7.1 Introduction of insects, mites and pathogens from grain handling equipment and transport vehicles including, for example, previous loads.	1	Cleaning procedures (grain handling equipment) and transport controls (haulier assurance procedures)

1 – 5 Low Risk

6 – 10 Medium risk

11 – 16 High risk (significant hazard)

Table 44. Good Storage Practice (GSP) programmes

Good Practice Programme (GPP)	Hazard(s) controlled by the GPP programme	Checking procedures	Remedial actions
Hygiene and housekeeping procedures All key equipment, store structures and environs are cleaned to a defined schedule and documented procedures to minimise the risk of product contamination. Storage facilities must be clean, dry and fit for purpose.	Introduction of Salmonella and other pathogenic bacteria, storage pests (mites and insects) and fungal moulds (OTA causal agents) from equipment, the store structure and previous crop debris.	Planned visual inspection to observe hygiene standards (e.g. pre loading with grain and at regular intervals during the storage period). Check stores for pests with traps prior to loading.	Take action to remedy defects and re-establish a hygienic environment. Consider whether additional cleaning, trapping and treatment is required. Review procedures to ensure appropriate hygiene standards are maintained.
Pesticide application procedures Personnel making decisions on use are competent to make the decision required. Personnel applying pesticides are competent to use the equipment required. Only approved pesticide products are used and any conditions of approval are complied with. Pesticide application equipment is maintained and calibrated to ensure it is fit for purpose.	Introduction of pesticide residues due to incorrect or inaccurate application of pesticide products (people and equipment) during treatment of store structure, admixing with grain and spot treating/fumigating stored grain, and short interval to unloading of grain from store.	Planned review of pesticide application records, e.g. prior to unloading of stores.	Review potential grain contamination risk and take necessary action, e.g. stock control measures. Review the pesticide application procedures and take action to ensure appropriate procedures are maintained.
Store environment standards The store structures are designed, constructed and maintained to control product contamination risks and are suitable for the intended purpose, including temporary and long term stores.	Introduction of hazards when store structure is compromised e.g. from water or pest ingress.	Planned visual inspection of the store structure to check it is sound and fit for purpose, e.g. for leaks and water ingress, pest proofing.	Take action to remedy defects and review potential grain contamination risk. Review store environment standards and take necessary action.

Good Practice Programme (GPP)	Hazard(s) controlled by the GPP programme	Checking procedures	Remedial actions
Equipment maintenance procedures Equipment is suitable for the intended purpose and maintained at all times to ensure it is working efficiently, including grain handling and drying equipment.	Introduction of metal form faulty equipment	Planned maintenance inspection of equipment to ensure fit for purpose.	Take action to remedy defects and review potential grain contamination risk. Review maintenance procedures
Glass control procedures Glass in areas with exposed grain is protected to prevent contamination and listed on a register. Glass breakage procedures are in place, including an incidence log.	Introduction of glass from the store environment) and grain handling equipment (lights, etc.).	Planned check of glass fixtures and fittings (on glass register) for damage and compliance with control procedures, e.g. at same time as store and grain condition checks.	Remedy any defects and review potential grain contamination risk and initiate appropriate stock control procedures. Review control procedures and their implementation.
Grain intake procedures (central stores) Grain is sourced from reputable suppliers who can provide a degree of assurance that hazards are not likely to be present in supplied grain through the implementation of appropriate standards of GAP (e.g. ACCS, SQCS). Grain is sampled at intake and checked for moisture content and presence of defects and that accompanying documentation is correctly presented (e.g. pestcide passport, mycotoxin risk assessment, assurance scheme certification)	Presence of chemical (e.g. pesticides, *Fusarium* mycotoxins) and physical (e.g. ergots and stones) hazards.	Trained operators check each batch for compliance with intake criteria (grain quality including moisture content and correctly presented documentation). Supplier approval and performance is evaluated according to schedule.	Review acceptability of load. Reject if appropriate. Where applicable, feed back individual food safety rejections via crop assurance schemes' complaint systems. Review suitability of suppliers.

Good Practice Programme (GPP)	Hazard(s) controlled by the GPP programme	Checking procedures	Remedial actions
Pest control procedures Inspection and treatment of premises to deter and eradicate pest ingress (birds, insects and rodents). Premises are designed and maintained to prevent entry and harbouring of pests.	Introduction of Salmonella and other-pathogenic bacteria from pests. Introduction of rodent faeces due to pest activity.	Planned check of pest control activities and inspection of premises for pest activity and observation of areas requiring proofing or hygiene measures.	Take necessary action to resolve any pest ingress, proofing or hygiene issues. Review procedures and their implementation.
Transport control procedures Transport of dispatched grain is contracted to competent haulage organisations who can provide assurance that hazards are not likely to be introduced through the implementation of appropriate controls (e.g. TASCC Haulage Code of Practice, GAFTA schemes)	Introduction of hazards from vehicle including previous loads Introduction of hazards due tor compromising grain condition during transport, e.g. pest and water ingress.	Condition of transport is checked prior to loading of grain.	Take action to remedy defects and establish suitability of transport.
Training procedures Personnel are trained, instructed and supervised commensurate with their activity and competent to undertake the tasks required.	Introduction of hazards due to failure by personnel to follow correct procedures.	Planned review of staff competence to carry out activities.	Review training needs.

Table 45. Hazard analysis table (significant hazards)

Process step	Hazard and cause	Control measures	CCP or GSP	Critical limit	Monitoring procedures	Corrective action
1. Store preparation	All identified hazards are covered by the GSP programmes.					
2. Crop harvest (farm stores)	All identified hazards are covered by the GSP programmes.					
3. Grain intake (central stores)	All identified hazards are covered by the GSP programmes.					

Process step	Hazard and cause	Control measures	CCP or GSP	Critical limit	Monitoring procedures	Corrective action
4. Temporary holding **Holding of grain prior to conditioning (cleaning and/or drying).**	4.1 Introduction of OTA due to mould growth when conditions are favourable to growth (moisture content, temperature and holding time)	Grain stock is managed to prevent mould growth depending on moisture content, temperature and holding time. Temperature is reduced by low volume aeration. Action to reduce mc below 18% before bulk drying is considered. GSP of Store design and maintenance procedures	CCP	Grain is cooled and dried with respect to condition and holding time based on the HGCA safe storage time calculator.	Planned checks of grain condition (mc and temperature).	Investigate any temperature rise, e.g. 2°C or more Review grain condition and consider need to remedy defects Review storage and drying practices and capacity options

Process step	Hazard and cause	Control measures	CCP or GSP	Critical limit	Monitoring procedures	Corrective action
5. Grain Conditioning **a. Cleaning, etc.** **b. Hot air drying** **c. Ambient air drying**	All identified hazards are covered by the GSP programmes.					

Process step	Hazard and cause	Control measures	CCP or GSP	Critical limit	Monitoring procedures	Corrective action
6. Long term storage– Storage of conditioned grain, including cooling, monitoring and pesticide treatment	6.1 Introduction of OTA mycotoxin due to mould growth when conditions are favourable to growth (temperature and moisture abuse)	Store grain dry and cool. Temperature is reduced by low volume aeration. GSP of Store design and maintenance procedures	CCP	≤15% mc † *Target: 14.5% mc* ≤15°C † *Target: <15°C within 2–3 weeks, <12°C within 4 months and <5°C by end December (minimum 10°C for malting barley)* Refer to the HGCA Safe Storage Time calculator for a safe storage risk assessment	Check temperature regularly (at least every 2/3 days) until target temperatures are reached and then weekly. Check mc each week until grain temperature stabilises. Then check monthly thereafter.	Investigate any significant rise in mc or temperature Review grain condition and consider need to remedy defects Review storage and drying practices.

Process step	Hazard and cause	Control measures	CCP or GSP	Critical limit	Monitoring procedures	Corrective action
	6.2 Growth of insect and mite populations when conditions are favourable to growth (temperature and moisture conditions)	Store grain dry and cool. Temperature is reduce by low volume aeration. GSP of Store design and maintenance procedures	CCP	≤15% mc † *Target: 14.5% mc* ≤15°C † *Target: <15°C within 2–3 weeks, <12°C within 4 months and <5°C by end December (minimum 10°C for malting barley)* Refer to the HGCA Safe Storage Time calculator for a safe storage risk assessment	Check temperature regularly (at least every 2/3 days) until target temperatures are reached and then weekly. Check mc each week until grain temperature stabilises. Then check monthly. Lay insect monitors. Check weekly early season and then monthly thereafter.	Investigate significant changes in grain condition or insect populations. Review grain condition and consider need to remedy defects including pest treatments Review storage and drying practices.
7. Dispatch	All identified hazards are covered by the GSP programmes.					

† 15% mc and 15°C are on the edge of safe storage; ideally, grain should be stored drier and cooler depending on storage period and market requirements. Although some commercial contracts may be made at 15% mc the safer target required for good storage practice based on established data for insect, fungal and mite growth is 14.5% mc otherwise the safe storage period becomes diminished.

Documentation and Record Keeping

A working HACCP system can generate many records, particularly of monitoring activities. The method of recording may be using purposely designed record forms, utilising existing systems including electronic recording systems, or a simple daily diary. The chosen method will depend on the nature and size of the grain store operation.

For small businesses, it may be appropriate for record keeping to be limited to recording by exception. For example, it may not be necessary to record the results of all routine checks; only record that the checks have been completed, e.g. in a diary. However, if anything different happens or something goes wrong, a note should be made including what remedial action was taken and the justification.

3.5.2. Step 4.2 Production of the new guide for best practice

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.

3.6. Overall conclusion

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

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