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Improved detection and monitoring of beetle pests in stored grain through use of a multi-species lure (LK0929)

by

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ABSTRACT

All grain harvested in the UK will at some stage be stored, whether on farms, in central stores or at mills. In all these premises grain is at risk to infestation by a range of insect pests. Effective detection of pest presence is essential in protecting harvested crops by revealing whether control is necessary and whether it has been successful. Compliance with the Assured Combinable Crops Scheme requires that steps be taken to monitor for the presence of beetle pests, yet many feel that they cannot rely on the results as monitoring methods have not been optimised.

A previous Defra-funded project identified a mixture of materials which showed promise as a multi-species lure for the saw-toothed grain beetle, the grain weevil and the rust-red grain beetle in laboratory conditions. The purpose of this project was to develop the first multi-species attractant lure for invertebrate pests of the cereals food chain by establishing a simplified mixture of easy-to-obtain compounds and proving activity against a wide range of beetle pests of the grain and milling trades.

Effects on insects of a mixture of volatiles obtained from peanuts and kibbled carob were evaluated. All individuals of a particular species detected less than 10% of the 180 compounds present and different species responded to different compounds. Behavioural bioassays of these compounds, together with other compounds identified from the literature as potential attractants, found that five of the 14 compounds tested against the grain weevil, seven of the ten compounds tested against the saw-toothed grain beetle and five of the ten compounds tested against the rust-red grain beetle were attractive. From these a total of 11 compounds were chosen for further investigation, of which only two were attractive to all three species and five were attractive to at least two species. A six-component mixture consisting of hexanoic acid, 3-methylbutanol, 4-ethylacetophenone, 3-octen-2-one, nonanal and E-2-nonenal was attractive in laboratory bioassays. In addition, this six-component mixture was attractive to an additional four species of stored product pest. This mixture was formulated into a range of dispensers and a polyethylene vial was the most effective dispenser type for this formulation.

The lure was evaluated in a research grain store and in a range of commercial premises (a maltings, a flour mill, a grain store and an import/export warehouse) over a six-week period in both PC™ traps and PC™ floor traps. There were some significant increases in insect numbers caught in traps with the lure, particularly for the rust red grain beetle, but in general these were small.

This project made significant progress towards the development of a food-based multi-species lure and in developing techniques to examine release rates. Further refinement and validation is required prior to commercialisation. Optimisation of monitoring methods will provide more effective detection and interpretation, enabling strategic application of control measures and reduction in pesticide usage.

SUMMARY

Introduction

Grain stored on farms, in commercial stores or at mills is at risk of infestation by a range of insect pest species. These may be primary pests, for example, the saw-toothed grain beetle, grain weevil and rust-red grain beetle, that cause serious damage to the grain; or secondary pests, which are associated with poor hygiene. These pests not only damage grain, but also may be responsible for the costs of rejection.

Early detection of insect pest presence is therefore key to ensuring that stored grain is protected by revealing whether control measures are necessary and, once these have been implemented, to indicate whether they have been successful. This requirement has been recognised within industry standard schemes such as the Assured Combinable Crops Scheme where compliance requires that steps are taken to monitor for beetle pests.

Although significant developments have been made in the design and validation of monitoring tools for crawling beetle pests, these methods have yet to be optimised. This could be achieved by the addition of an attractant lure. Lures may be based on pheromones or food attractants. Pheromones are chemicals produced naturally by an insect species which affect the behaviour of other insects, usually only from the same species. Sex pheromones tend to be produced by females and aggregation pheromones by males. The identity of the aggregation pheromones produced by the three major UK beetle pest species, the saw-toothed grain beetle, the grain weevil and the rust-red grain beetle are known, but some have multiple components, are not easily synthesised and therefore are prohibitively expensive. Lures based on food attractants have the potential to attract more than one species and therefore have an advantage over pheromone-based lures. However, the food attractant based lures used to date have tended to use natural products resulting in batch-to-batch variation in composition and hence performance. Also, many volatile compounds are usually present and effects of the individual components and their contribution to the overall effect are not known. The use of the natural product may also be expensive and labour intensive. Ideally the lure would contain only a few easily obtainable, inexpensive components shown to consistently produce an attractive effect.

As a result of a previous Defra-funded project a mixture of materials was identified which showed promise and long lasting activity as a multi-species lure for the saw-toothed grain beetle (*Oryzaephilus surinamensis*), the grain weevil (*Sitophilus granarius*) and the rust-red grain beetle (*Cryptolestes ferrugineus*) in laboratory conditions. The purpose of this LINK project was to:

- determine which components of this mixture produced the attractant effect;
- establish how these could be obtained;
- determine the range of species for which it was effective;
- formulate the simplified mixture in an easy-to-use lure dispenser;

- ensure that this was effective for a minimum of six weeks and
- validate its use in monitoring devices in industry premises.

The research required to achieve these objectives was undertaken under seven milestones.

Milestone 1. Identify chemical structure of those components which will comprise the simplified attractant mixture

Previous studies have shown that a mixture of volatiles derived from kibbled carob and peanuts is attractive to the saw-toothed grain beetle, the grain weevil and the rust-red grain beetle in laboratory studies. This mixture was used as the basis for identifying the key components that would need to be included in a multi-species lure. Identification and screening of all of the volatile compounds present in the mixture is very time-consuming. One way in which the number of compounds to be tested can be reduced is to assess which of the chemicals the target insects can detect. If the insect does not detect the chemical then no behavioural effect can result. Detection of volatile compounds by an insect can be determined using electroantennography in which the change in potential across the antenna generated by the interaction of volatile compounds and their corresponding chemoreceptor cells is measured. If this process is coupled to analysis of the mixture by a gas chromatogram (gas chromatography-electroantennography (GC-EAG)) it is possible to assess exactly which compound in a complex mixture is causing the effect. GC-EAG was used to determine the compounds that *O. surinamensis, S. granarius* and *C. ferrugineus* were able to detect. The compounds were then identified using gas chromatography-mass spectrometry (GC-MS).

Using GC conditions for optimal separation of the volatiles in the peanut/carob mixture, it was found that over 180 compounds were present. Female *S. granarius* responded to a significantly larger number of compounds than male *S. granarius.* There were no significant differences between the numbers of compounds responded to by females and males of *O. surinamensis* or *C. ferrugineus.* The total numbers of compounds responded to by both males and females varied between species: both sexes of *O. surinamensis* responded to 12 peaks, *S. granarius* to 43 peaks and *C. ferrugineus* to 32 peaks. The total number of peaks to which all ten tested individuals of *O. surinamensis*, *S. granarius* and *C. ferrugineus* responded was 4, 10 and 12 respectively. Differences in the physiological status of insects used in experiments may explain why individual insects within a species may respond to different chemicals. The structures of 18 of the compounds that all 10 test insects of a species responded to were tentatively identified and authentic standards were available for 14 of these.

Milestone 2. Agree which additional species of beetle should be tested and identify premises at which trials should be undertaken

The laboratory studies focussed on the three principal beetle pests of grain storage premises in the UK, *O. surinamensis, S. granarius* and *C. ferrugineus*. However, it is important that a lure in a monitoring device will attract as many different species as possible. Key industry representatives were therefore consulted to determine the species considered to be of greatest importance that should be included in tests. In addition it was important to identify the type of premises that could be used in the latter stages of the project for validation of the lure.

A letter was sent to four industrial bodies requesting that eleven insect species were ranked in terms of importance from high to zero. Using this information the following additional species were chosen for testing: *O. mercator* (merchant grain beetle), *Tribolium castaneum* (rust-red flour beetle), *T. confusum* (confused flour beetle), *S. oryzae* (rice weevil), *S. zeamais* (maize weevil), *Rhyzopertha dominica* (lesser grain borer), *Ahasverus advena* (foreign grain beetle), *Stegobium paniceum* (biscuit beetle), *Typhaea stercorea* (hairy fungus beetle), *Ptinus tectus* (Australian spider beetle) and *Liposcelis bostrycophila* (psocid). Where possible recently-collected field strains were tested to ensure that behavioural traits were as close as possible to real-world responses.

In consultation with industrial partners it was agreed that to represent different industry sectors the following types of premises would ideally be used in the latter stages of the project for validation of the lure and development of a protocol: maltings, grain store (floor storage), grain store (bins), flour mill, animal feed mill and port.

Milestone 3. Complete behavioural assessment of components to include in simplified attractant mixture and obtain in sufficient quantity for trials

Milestone 1 determined the identity of compounds in the carob/peanut mixture that the three species tested were able to detect. However, the GC-EAG technique does not give any information on the nature of the behavioural effect (e.g. attraction or repellency), if any, that is elicited by these compounds. Therefore, it was necessary to undertake behavioural bioassays to determine whether any of the compounds identified were attractive. In addition, so that potential attractants were not overlooked, compounds identified in the literature as attractants for storage beetles were included in the compounds tested. The behavioural bioassay chosen was the pitfall bioassay that has previously been identified as the most effective technique for this type of investigation. The following investigations were made to determine the compounds to be included in the lure:

1. Identification of the optimum amount of carob-peanut extract to use as a positive control (standard reference).
2. Initial screening of individual compounds for a behavioural response. All of the compounds were tested at two concentrations in 5 µl of pentane. The amounts were chosen were equivalent to the concentration in the

carob-peanut extract used for GC-EAG and equivalent to the total amount of volatiles in the carob-peanut extract necessary to elicit a behavioural response as determined in test 1. For compounds identified from the literature the amounts tested were based on those reported in the literature as being necessary to elicit a behavioural response.

3. To establish the response thresholds with the three species further pitfall bioassays were carried out with a number of the compounds which gave a positive response in the initial screening pitfall bioassays. The criteria used to prioritise the testing of these compounds were toxicity, efficacy, availability and the extent to which the compounds occur naturally in the commodities in which traps are used. The compounds chosen for testing were: hexanoic acid, 3-methylbutanol, 4-ethylbenzaldehyde, 4-ethylacetophenone, 2-phenylethanol, E-3-octen-2-one, hexanal, nonanal, 3-methylbutyrolactone, E-2-nonenal and γ-nonanoic lactone.

4. Studies were made testing mixtures of the 10 compounds above to determine the most effective mixture for the three species.

The response thresholds for the carob-peanut extract compared with a pentane control were 100 ng for *O. surinamensis* and 1 μg for *S. granarius* and *C. ferrugineus*. These amounts were used as positive controls in subsequent tests with individual compounds. Five of the compounds tested in the first screen elicited a positive response from *S. granarius*, seven of the compounds tested elicited a positive response from *O. surinamensis* and five of the compounds tested elicited a positive response from *C. ferrugineus*. The response thresholds for the 10 compounds identified and chosen for further testing ranged from 10 ng to 100 μg. Two of the compounds, 4-ethylacetophenone and E-2-nonenal, elicited positive responses from all three species tested. Five of the compounds tested elicited a response from two of the species tested: 2-phenylethanol, 3-methylbutanol, hexanoic acid, 3-octen-2-one and nonanal.

Each species was tested with the mixture of compounds found to elicit the greatest effect for that species. Therefore the mixture for each species was initially different. Further testing and refinement of the mixture determined that all three species were attracted to a six-component mixture consisting of hexanoic acid, 3-methylbutanol, 4-ethylacetophenone, 3-octen-2-one, nonanal and E-2-nonenal. This mixture was used subsequently for testing of the additional species and for formulation in a lure dispenser.

Milestone 4. Develop and conduct laboratory tests of formulation to ensure that lure will work well in empty premises and in presence of grain

Once the simplified mixture of compounds had been shown to result in attraction of the three species in the laboratory it was necessary to determine that this effect would still be observed when grain was present. Previous studies with food-based lures had shown that response was dramatically decreased in the presence of grain. There were three possible reasons for this: (1) the lures do not release sufficient material (2) the presence of grain volatiles interferes with the attraction of the insects and (3) the grain absorbs the volatiles released from the lure. Prototype lure dispensers constructed from polythene tube were used in both

laboratory and grain store studies to determine whether response was likely to decrease in the presence of grain. Headspace sampling by solid phase micro-extraction (SPME) was used to quantify the volatiles released from the lure.

The concentration of volatiles released from lures in sealed beakers was markedly lower at 7^0C compared to 20^0C. For the most volatile component, ethyl isobutyrate the concentration in the headspace was approximately 3-fold lower at 7^0C than at 20^0C. For the less volatile components, such as 4-ethylacetophenone, the drop in concentration was far greater (130 fold). When lures were sampled at 7^0C in an open beaker the concentrations were reduced still further, with the 5 least volatile components not being detected at all and the 5 most volatile components detected in only trace amounts. The open beaker mimics a pitfall cone (PC™) trap which is also open at the top. Studies conducted in the grain store showed similar results to the laboratory studies. The solubility of volatiles in the non-polar polythene tubing declines with decreasing temperature, reducing the rate of diffusion of volatiles through the walls of the polythene lure. The vapour pressure of the compounds in the air also declines with decreasing temperature.

The most likely explanation of why the prototype lures performed relatively poorly in the presence of grain in tests was that at low temperatures and in an open headspace (e.g. a grain silo in winter), the release of volatiles from the lures was too low to build up enough to attract more insects into the traps. In grain stores the volume of headspace above the traps is much larger than in bioassay arenas in the laboratory and this will prevent the volatiles building up to the same levels as in a bioassay arena. Air currents above the grain and through the grain (if ventilated) will also carry volatiles away from the trap preventing their build-up. All these factors will markedly reduce the concentrations of volatiles in the air above monitoring devices in the grain store at low temperatures compared to bioassays conducted at 20^0C. This study gave useful information on the amount of volatiles that the lure would need to release in a practical situation.

An appropriate lure dispenser that would release the volatiles in appropriate concentrations and over a period of at least six weeks was required. This device also needs to be easy-to-use and affordable. Initial trials used the same dispenser that is currently used in the PC™ floor trap. However, this was not found to be suitable and other types of dispenser were evaluated. These studies were carried out in the laboratory using pitfall bioassays. The six component mixture was formulated together with butylatedhydroxytoluene (BHT) to prevent oxidative breakdown of the compounds and a matrix to stabilise release at different temperatures into 5 types of dispenser. These consisted of a polythene tube, a polyethylene vial, the ring dispenser as used in the PC™ floor trap, the ring lure in a lightweight polythene bag and the ring lure in a heavy weight polythene bag. The effectiveness was evaluated 24 h after removing the lures from a freezer at -18°C (Week 0), after one week, three weeks and six weeks. All lures were left at room temperature between tests. The most effective lure dispenser for the three species tested was the polythene vial. There was a significant

increase in response compared to the control at week 6 for all three species. This dispenser was chosen for use in trials in the CSL grain storage facility and in commercial premises.

The laboratory studies had produced a mixture of easily obtainable compounds that could be formulated in an easy-to-use dispenser that was effective for six weeks. The performance of this prototype lure was evaluated in the grain storage facility at CSL. The lure was used in PC™ traps placed both on the grain surface and buried 5 cm below the grain surface of a 116 tonne floor stored grain bulk and in PC™ floor traps placed around the periphery of the grain bulk. Known numbers of *O. surinamensis, S. granarius* and *C. ferrugineus* were introduced to the grain bulk and allowed to disperse for one week prior to introduction of the traps. Traps were monitored for six weeks and the number of insects found was recorded. The first trial, prior to evaluation in the laboratory of different types of dispenser, used the rubber ring lure dispenser that is the same type of dispenser as currently supplied with the PC™ floor trap. Over the whole trial period, significantly more *O. surinamensis* were found in PC™ traps containing the lure on the surface in the grain bulk and *C. ferrugineus* in PC™ traps containing the lure both on the surface and buried below the surface. However, with *S. granarius* significantly more insects were found in both surface and buried PC™ traps without the lure over the period of the trial. This indicated that there might have been a component in the formulated lure that was repelling rather than attracting this species. In addition, volatiles were lost rapidly from this type of dispenser so that attraction was not maintained over the whole six weeks.

A small-scale trial was carried out in the grain bulk to examine the problem with the formulation for *S. granarius.* This used the ring lure with the matrix and BHT added but without the 6-component mixture. Greater numbers of *S. granarius* were found in traps that did not contain the lure. Further laboratory assessments also indicated that the matrix within the formulation was repellent to *S. granarius*. The matrix did not stabilise the release rate of the volatiles from the lure at different temperatures. It was therefore concluded that the matrix and BHT should not be included in the lure formulation.

Based on the laboratory comparison of the lure dispensers and the results from the trials described above, the polythene vial was chosen for testing in the Storage Research Unit. Known numbers of *O. surinamensis, S. granarius* and *C. ferrugineus* were introduced to the 116 tonne grain bulk and allowed to disperse. PC™ traps were used both on the grain surface and buried below the surface and PC™ floor traps were positioned around the periphery of the grain bulk. A comparison was made between traps with and without the lure. Significantly more *C. ferrugineus* were found in PC™ traps containing the lure both on the grain surface and buried below the surface. With *O. surinamensis* and *S. granarius* greater numbers of insects were generally found in PC™ traps with the lure although these differences were not significant. Significantly more *O. surinamensis* were found in PC™ floor traps containing the lure compared to those without the lure. A final trial was undertaken in the Storage Research Unit to examine the effect of reducing the amount of compounds in the lure but fewer insects were caught. On the basis of these trials it was concluded that in

general, the increases in trap catches were small and the lure was more effective in the PC™ Floor Traps than in the grain bulk.

Milestone 5. Conduct laboratory tests to confirm that simplified lure is effective against the agreed additional species

The effect of the six-component mixture identified for attraction of *O. surinamensis, S. granarius* and *C. ferrugineus* was evaluated for the eleven additional species identified by industry for milestone 2. This was examined using the standard pitfall bioassay in laboratory studies. Four of the additional species tested responded to both the six-component test mixture and the carob-peanut extract. These were: *O. mercator, T. confusum, S. oryzae* and *L. bostrychophila.* Together with *O. surinamensis, S. granarius* and *C. ferrugineus* this brings the total number of species found to respond to the lure volatiles to seven. Four of the species responded to the carob-peanut extract but not to the test mixture. These were: *R. dominica, S. zeamais, A. advena* and *T. stercorea.* Therefore, there is potential to identify the components of the carob-peanut extract which are responsible for this behavioural effect. Three of the additional species tested, *T. castaneum, S. paniceum* and *P. tectus,* did not respond to either the test mixture or to the carob-peanut extract. Identification of attractive compounds for these species would require a return to the more basic search for attractive volatile extracts of foodstuffs followed by identification of the attractive volatiles by EAG and behavioural bioassays. However, of these three species trade representatives prioritised only *T. castaneum*. All of the field strains of the three primary pests of major importance (*O. surinamensis*, *S. granarius* and *C. ferrugineus*) responded to both the test mixture and to the carob-peanut extract.

Milestone 6. Complete trials of chosen trap and lure combination in chosen premises

Using the type of premises identified from milestone 2, four different premises were identified for validation of the lure in commercial premises. The premises were a maltings, a flour mill, import/export warehouse and a premises with a floor stored bulk of barley. The aim of this milestone was to compare monitoring devices with and without the formulated lure over a six-week period and to examine differences in numbers caught and the numbers of positive traps.

Three of the premises used PC™ floor traps and the fourth used PC™ traps both on the grain surface and buried below the surface. Traps, with and without the lure, were placed in alternate positions and checked each week for a six-week period. The number of insects found and the number of positive traps (ie. traps containing one or more insects) were recorded.

Insects were found in traps at all four premises. The range of species found varied at each site but representatives of the major storage beetle pest species were found at all sites. Comparison of traps with or without the lure showed that no significant differences were found, either in terms of the number of insects caught or the number of positive traps at the three sites where comparisons were possible. At premises 4 a

total of only 2 individuals were caught in two different traps neither of which contained a lure. The effectiveness of the use of these monitoring devices for early detection of insect presence was confirmed. Further optimisation of the lure is required before a clear benefit for its inclusion can be shown on a consistent basis.

Milestone 7. Refine protocol for use of trap and lure combination, validate in experimental grain storage facility and promote to trade

The results obtained from the trials in commercial premises and in the CSL Grain Storage Research Unit demonstrated that in general only a small increase in the number of insects found in traps with the lure was observed and the degree of enhancement was not the same for the different species. Further refinement of the lure and validation of this is therefore required, so it was not possible to produce and refine a protocol for trap and lure use.

During the course of this project techniques in spatial analysis were used to examine insect distribution in the grain bulk in relation to traps with and without lures. This type of analysis can be used to determine optimal distance for the spacing of traps and can also be used to indicate positions of infestations and for the setting for thresholds for control. The techniques were used to examine whether the distribution of insects changed over the duration of the trial from the original uniform distribution at the introduction of the population to a patchy distribution. These results show that the distribution of the three species did not remain uniform over the duration of the trial and that *O. surinamensis* and *C. ferrugineus* clustered in the same areas whereas *S. granarius* formed smaller clusters which varied in location with time. The patches and gaps may have been due either to insect movement or differential insect mortality in different locations within the grain bulk.

These, and similar, techniques can be used to visualise the areas in which infestations are present and in this way can target areas for treatment. The same techniques can be used after treatment to identify any residues of infestation. Through the use of spatial models optimal spacing for traps can be determined. Effective monitoring tools are key if these techniques are to be used to their full potential and the progress made within this project in the development of a multi-species lure and techniques for examining release of volatiles from lures has provided a significant step towards this goal.

In conclusion this project has made significant progress towards the development of a multi-species lure. A simplified mixture of only six compounds has been identified and formulated in a practical easy-to-use dispenser. Important information on the release of volatiles from lures has also been obtained. However, further refinement of the lure mixture is required for optimisation. This will then be a valuable tool for monitoring for insect presence and the development of appropriate control measures when used as part of an integrated pest management strategy.

INTRODUCTION

The UK grain trade handles about 20 million tonnes of cereals *per annum* and a key element in protecting this commodity prior to reaching the consumer is good grain storage. Grain can be stored on farms, in central stores or at mills, but in all these premises it is at risk of infestation by a range of insect pests. These are primary pests which cause serious irreversible damage to grain and secondary pests associated with poor hygiene. There are significant economic costs associated with insect infestation not only in terms of direct damage to the commodity but also in terms of the costs associated with rejection. In normal years, less than 1% of loads are rejected from maltings or intervention as a result of insect infestation (Wilkin, 2003) but this can rise to 5 or even 10% in unusually warm years (Farmers Weekly, 1999, 2003). Effective detection of pest presence is an essential tool in protecting harvested crops by revealing whether control is necessary and whether it has been successful. There is increasing awareness among grain storage staff of the need for pest monitoring and the importance of the results, for example the HGCA Grain Storage Guide stresses that pesticides should not be used unless insects are found. Compliance with the Assured Combinable Crops Scheme requires that steps are taken to monitor for the presence of beetle pests yet many feel that they cannot rely on their current monitoring results as monitoring methods have not been optimised.

Work at the Central Science Laboratory (CSL) resulted in the invention of the first properly optimised and validated trap for storage beetles yet it still traps only a small percentage of the population present (Cogan and Wakefield, 1994; Wakefield and Cogan, 1999). A trap for use on flat surfaces in the grain storage and milling trades was also developed (Collins and Chambers, 2003). The first lure for the saw-toothed grain beetle, *Oryzaephilus surinamensis*, was also developed (Wakefield and Chambers, 1999). This increased trap catch by a ten-fold but this lure is species specific and the detection of the many other insect species which threaten stored grain remains to be improved. As a result of a Defra-funded project, in which a principal objective was to identify and test attractants which could act as multi-species lures, materials were identified which, in prototype lures, showed promising and long lasting activity in laboratory studies as attractants for the saw-toothed grain beetle (*O. surinamensis*), the grain weevil (*Sitophilus granarius*) and the rust-red grain beetle (*Cryptolestes ferrugineus*). This lure was based on food volatiles. If it were pheromone based, the pheromones for all three species would have to be included. Although these pheromones have been identified they are difficult and costly to synthesise. The use of food volatiles rather than the species-specific pheromones also allows for expansion of the number of target species to include those for which the attractant pheromones are unknown. A food attractant for the foreign grain beetle, *Ahasverus advena,* has been simplified recently (Wakefield *et al.,* 2005.) The advantages of identifying the key compounds in a complex mixture of food volatiles that are responsible for the attraction are consistency of the material, reduced costs and ease of production and elimination of materials that may potentially affect the attractiveness of the material.

The overall objective of this project was to develop the very first multi-species attractant lure for invertebrate pests of the cereals food chain. The basis for this work used a mixture of materials obtained by collection of the volatiles derived from peanuts and kibbled carob as identified from the previous Defra-funded project. This mixture was used to develop a simplified mixture containing only a few compounds which were easy to obtain. Activity of the mixture was demonstrated in the laboratory and in representative commercial premises. The intended outcome of this project was the development of an optimised monitoring system which would form an essential tool to guide the integrated management of storage pests.

MILESTONE 1. Identify chemical structure of those components which will comprise the simplified attractant mixture

Introduction

Previous studies examining the potential of volatiles from various foodstuffs to act as attractants for stored product beetles have shown that a mixture of volatiles derived from kibbled carob and from peanuts showed the greatest effectiveness. These volatiles were obtained by aeration of the foodstuffs on to a porous polymer followed by solvent extraction. This is a time consuming process and as natural materials are used the product can show considerable variation between batches. This carob-peanut extract contains a great number of volatiles only a few of which are likely to act as attractants. Rapid screening of mixtures of compounds can be achieved using gas chromatography-electroantennography (GC-EAG). Electroantennography is a technique that measures the change in potential across the antenna generated by the interaction of volatile compounds and their corresponding chemoreceptor cells. By splitting the effluent from a gas chromatograph so that half passes over the insect antenna and half goes to the detector it is possible to determine which compounds cause an electrophysiological response. Those compounds that produce this response can then be identified using gas chromatography- mass spectroscopy (GC-MS).

Materials and methods

Collection of carob and peanut volatiles

Volatiles were obtained by separate aerations of kibbled carob pods and kibbled organic peanuts onto Porapak Q and extraction of the volatiles into pentane (Collins *et al*, in press). The concentrations of the samples obtained were determined using gas chromatography using an external standard of methyl laurate (200 ng/µl). The extracts were diluted and mixed at a ratio of 36:1 carob:peanut to give a total concentration of 7.9 µg/µl in pentane.

Gas Chromatography (GC)

Aliquots (1 µl) of the carob/peanut extract were analysed on a Hewlett Packard 5890 series II gas chromatograph. The extract was injected at 250°C in splitless injection mode (purge on after 1.0 min), onto a Chrompack CPSil-5CB column (100% polydimethylsiloxane, 50m x 0.32mm internal diameter, 1.2 µm film thickness). Helium was used as the carrier gas, and the GC operated in constant pressure mode (15 psi, 31.9

cm/s at 40°C GC oven temperature, split vent flow 50.0 ml/min) with flame ionisation detector at 280°C. The GC oven temperature was initially 40°C held for one minute, rising at 10°C/min to 120°C and then at 3°C/min to 220°C, held at this temperature for 10 min, increased at 10°C/min to 250°C and then held there for 10 min. The FID signal was analysed by PC using HP 3365 Series II Chemstation software version A.03.34.

Insects

Adult insects of the following species and strains were used: *O. surinamensis* susceptible; *S. granarius* Windsor; and *C. ferrugineus* C124. Zero to two-week-old adults of each of the above species were used. *O. surinamensis* and *S. granarius* were reared in the dark at 25°C and 70% r.h. *C. ferrugineus* was reared in the dark at 30°C and 70% r.h. *O. surinamensis* and *C. ferrugineus* were fed wheatfeed, rolled oats and dried de-bittered brewer's yeast mixed 5:5:1 by weight. *S. granarius* was fed whole wheat. All of the strains used were insecticide susceptible. The culture population density for all three species was approximately 3,000 insects per 0.75 l jar half filled with food. Insects were removed from culture and held in clean glass tubes (75 mm x 25mm diameter) without food at the test conditions for 24h prior to testing. A maximum of 20 insects was held in each tube. The top 20 mm of the tube was coated with Fluon™ to prevent insect escape. The sex of the adult beetles was determined using external features as described by Halstead (1963).

Electroantennography (EAG)

Test materials were carob-peanut extract (7.9 µg/µl) and a pentane control. Recordings were made by placing the live insect into an electrical circuit, passing an olfactory stimulus over the antenna and recording the response as described in Collins *et al* (in press). Each electrode consisted of a piece of chloridised silver wire inserted into a glass capillary tube filled with saline (Roelofs, 1984). Signals from the recording electrode were interfaced to a PC *via* a Syntech amplifier and Syntech Autospike Interface Box. The data were analysed using Syntech EAG software version 2.6d. The odour delivery system was as described by White and Birch (1987). All materials were presented in a 10 µl volume. The amount of material presented is expressed in terms of the amount that was applied to the filter paper prior to insertion in the test cartridge. Solvent was allowed to evaporate before placing the filter paper strip in the test cartridge. The test material was released into a continuous flow of humidified air that was passed over the antenna (1000 ml/min) with a pulse duration of 1s (500 ml/min plus 500 ml/min continuous flow to give a total of 1000 ml/min), regulated by a Syntech Stimulus Controller. Intervals of 120 s were left between presentation of each test substance. Pentane was used as the control to take into account any response caused by the solvent or the mechanical disruption of the airflow. Response was measured as the maximum amplitude of depolarisation elicited by a stimulus. The response to the control was subtracted from the response to the test materials. EAG recordings were made before and after each coupled GC-EAG test to ensure that the insect was responding before the GC programme was started and to test for fatigue at the end of the programme.

GC-EAG

The coupled GC-EAG system used was based on that described by White and Birch (1987). The column effluent was split 1:1 between two identical lengths of 0.32mm fused silica capillary column using a universal quick seal splitter (Chrompack). One length led to the FID detector while the other ran, via a copper tube surrounded by a heating tape maintained at 230°C, to a glass stimulus delivery tube. The effluent (0.9 ml/min) was diluted with humidified air to give a total airflow of ca. 1000 ml/min and passed over the antenna of the test insect. Insects were prepared as described above. Signals from the recording electrode were interfaced to a PC *via* a Syntech amplifier and the GC. The data were analysed using HP 3365 Series II Chemstation software version A.03.34. Five male and five female insects of each species were tested and peaks in the carob peanut mixture that gave rise to EAG responses were determined.

Source of test compounds

All compounds used for this work were sourced from Sigma-Aldrich (Gillingham, UK). Nonyl isobutyrate was not available and was therefore synthesised from nonan-1-ol and isobutyric acid.

GC-MS identification of EAG active components

Aliquots (1µl) of carob/peanut extract, at the same concentration as used for the GC-EAG analysis, were analysed on a Hewlett Packard 5890 series II gas chromatograph coupled to a VG Trio-1 mass spectrometer. The extract was injected at 250°C in splitless injection mode (purge on after 1.0 minute), onto a Chrompack CPSil-5CB column (100% polydimethylsiloxane, 50 m x 0.32 mm internal diameter, 1.2 µm film thickness). The oven temperature was initially 40°C for 1 min rising at 10°C per minute to 120°C and then at 3°C/min to 220°C and held at this temperature for 5 minutes. Helium was used as the carrier gas, and the GC operated in constant pressure mode (2 psi, 27.1cm/sec at 40°C GC oven temperature, split vent flow 50.0 ml/min). The mass spectrometer source and interface temperatures were 200°C, and 275°C respectively. The mass spectrometer was operated in electron impact mode (EI+) at 70 eV, and scanned from 33-350 amu once every second. Mass spectrometer acquisition commenced after 6 minutes (solvent delay time).

The GC-MS conditions were matched as closely as possible with those used for GC-EAG analysis of carob and peanut volatiles. However, the vacuum from the mass spectrometer causes differences between the GC-MS and GC-EAG carrier gas flow rates (for the same inlet pressure) and therefore in the retention times. The carrier gas inlet pressure of the GC-MS analysis was set at a lower value than with the GC-EAG to keep the linear gas velocity as similar as possible.

Kovats retention index (KRI) was used to aid identification of some of the unknowns, and authentic standards, where available, were injected for confirmation. The n-alkanes C_{12} to C_{16}, which were present in the carob/peanut extract, were used to calculate the KRI's of compounds of interest for comparison with

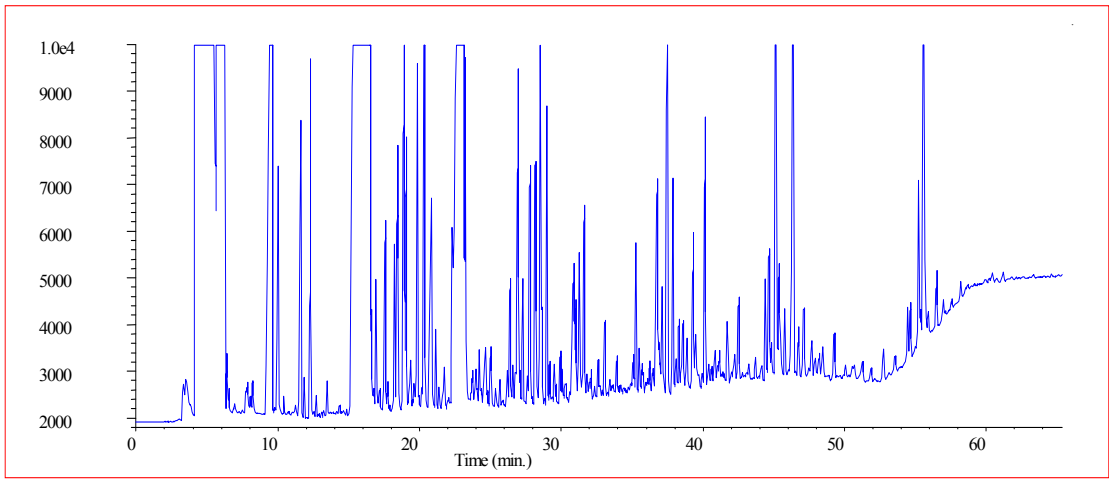
literature KRI values of volatile organic compounds (VOCs). The amounts of compounds represented by the peaks were estimated by comparison with a standard of 200 ng methyl laurate.

Results

GC

There were over 180 peaks on the chromatogram of the carob-peanut mixture and, although GC conditions were optimised for separation of the components, there were still overlapping and hidden peaks and some peaks which were very close together (Figure 1).

Figure 1. Gas chromatogram showing best possible separation of components



EAG

EAG responses were obtained from all of the test insects both before and after each GC-EAG run. Comparisons of the EAG responses obtained before each GC-EAG run showed that there were no statistically significant differences between the sizes of the responses of males and females within each species (t-tests, n = 5 in each case). Neither was there any significant difference between the sizes of responses of different species (one-way Anova, n = 10 in each case) (Table 1)

Table 1. Mean responses (mV) to the carob-peanut mixture before and after GC-EAG

	O. surinamensis		***S. granarius***		***C. ferrugineus***	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Before GC-EAG	0.366 ± 0.149	0.337 ± 0.116	0.700 ± 0.131	0.442 ± 0.082	0.585 ± 0.159	0.427 ± 0.126
After GC-EAG	0.279 ± 0.125	0.329 ± 0.116	0.588 ± 0.129	0.181 ± 0.096	0.387 ± 0.133	0.488 ± 0.229

GC-EAG

The total number of compounds on the mixture chromatogram which elicited a response from any of the males and females of each species are shown in Table 2. Female *S. granarius* responded to a significantly

larger number of compounds than male *S. granarius* (t-test, $P < 0.05$). There were no significant differences between the numbers of compounds responded to by females and males of *O. surinamensis* or *C. ferrugineus.* The total numbers of compounds responded to by both males and females varied between species: both sexes of *O. surinamensis* responded to 12 peaks, *S. granarius* to 43 peaks and *C. ferrugineus* to 32 peaks. The list of peaks to which all 10 of the replicates of a species gave an EAG response is shown in Table 3. The total number of peaks to which all *O. surinamensis*, *S. granarius* and *C. ferrugineus* responded was 4, 10 and 12 respectively.

Table 2. Numbers of peaks on the mixture chromatogram which elicited a response by males (n = 5) or females (n = 5) of each species

	O. surinamensis	***S. granarius***	***C. ferrugineus***
Females	12	85	65
Males	17	65	71

GC-MS

The structures of 18 of the compounds that all 10 replicate insects of a species responded to were tentatively identified (Table 3). Authentic standards were available for 14 of these but not for 4-methylbutyrolactone, 3-ethylacetophenone, nonyl isobutyrate and 2-tridecen-4-one.

Of the seven compounds whose structure could not be determined, one compound produced peaks at 18.354 and 18.547 min and is likely to be a chiral compound. Another compound with a retention time of 31.039 minutes has been tentatively identified as a 14-carbon branched alkane, with a likely molecular weight of 198. However, branched alkanes of this molecular weight and structure are not available commercially for use either in confirmation by GC-MS or for testing of behavioural activity.

Sixteen other compounds that elicited EAG responses from some, but not all of the 10 replicate adults were also identified (Table 4). All of these compounds are available commercially. Of these compounds, hexanol, hexanoic acid, and hexyl isobutyrate appeared to be the most promising as they elicited EAG responses from some of the individuals of all three species.

Table 3. GC-MS identification of compounds that elicited EAG response from all 10 adults tested

Retention time (min)	Conc.(ng)	Species responding	Identification by GC-MS
10.071	25.6	Sg	butyric acid
14.470	0.8	Sg	4-methylbutyrolactone
17.689	21.2	Cf	2-ethylhexanol
17.871	3.1	Cf	3-octen-2-one
*18.354	17.3	Cf	ethyl isobutyrate*
*18.547	39.4	Cf	ethyl isobutyrate*
18.451	~5.5	Cf	2-acetylpyrrole
20.861	24.7	Os	2-phenylethanol
22.692	140.6	Sg	E-2-nonenal
23.760	6.3	Sg	4-ethylbenzaldehyde
27.301	15.2	Sg	3-ethylacetophenone
30.895	19.0	Os, Sg	octyl isobutyrate
30.895	9.5	Os, Sg	gamma-nonanoic lactone
31.039	14.1	Sg	Branched alkane (mol wt. 198)
32.638	5.1	Os	hexyl hexanoate
33.955	4.4	Cf	tetradecane
35.284	16.8	Cf	nonyl isobutyrate
36.496	4.7	Sg	Unknown 1
36.785	34.3	Sg	tridecan-4-one & Unknown 2
38.321	16.8	Cf	pentadecane
38.897	9.4	Cf	2-tridecen-4-one
39.328	21.1	Cf	Unknown 3
45.802	11.6	Cf	Unknown 4
46.377	165.7	Cf	pentadecan-2-one

* formed by chemical breakdown of inside GC-MS giving a split “double” peak at 18.354 and 18.547 minutes

Table 4. GC-MS identification of compounds that elicited EAG responses from some of the 10 replicate adults tested

	Retention time (minutes)	Species responding	Identification by GC-MS	Confirmed by GC-MS?
1	8.259	Cf, Sg	2-pentanol	yes
2	9.650	Sg	isobutyric acid	
3	11.756	Cf	2-methylbutyric acid	
4	10.533	Sg	hexanal	
5	12.418	Os, Cf, Sg	hexanol	
6	16.627	Os, Cf, Sg	hexanoic acid	yes
7	19.956	Os, (Aa)	2-nonanone	yes
8	20.449	Os,	nonanal	
9	21.408	Os	3-nonen-2-one	
10	22.323	Os, Cf, Sg	hexyl isobutyrate	yes
11	23.156	Sg	octanoic acid	
12	28.561	Os, Sg, (Aa)	2-undecanone	yes
13	29.022	Cf	2-undecanol	yes
14	29.526	Sg	tridecane	
15	37.508	Os	2-tridecanone	
16	42.539	Cf	hexadecane	

Discussion and conclusions

The results from the GC-EAG study revealed that the carob-peanut mixture contains several compounds to which all individuals tested for all three species responded. The variability in the number of compounds to which individual insects within a species responded are probably due to differences in the physiological status of the insects and in the experimental insect preparation. It is unlikely that the statistically significant difference in numbers of peaks responded to by male and female *S. granarius* can be attributed to these sources of variation. However, it is possible that the females simply responded to smaller amounts of these components than the males. This difference between *S. granarius* and the other two species tested may be related to a corresponding difference in life history. The larvae of *O. surinamensis* and *C. ferrugineus* are external grain feeders and are capable of moving around to find food whereas the larvae of *S. granarius* are restricted to the grain in which their eggs were oviposited by the adult female. Thus there would be great selection pressure for the adult female to choose the best available grain in which to oviposit.

The chemical structure was tentatively identified for the majority of those compounds that elicited an EAG response in all 10 replicate beetles tested. The structure of most of these compounds was confirmed by GC analysis of authentic standards. A further 16 compounds which elicited an EAG response in some of the

beetles tested were also tentatively identified. Six of these were confirmed. The majority of chemicals identified are readily available and formed a good basis for investigation of their behavioural effects on the key beetle pest species.

MILESTONE 2. Agree which additional species of beetle should be tested and identify premises at which trials should be undertaken

The laboratory studies within this project focus on the three principal beetle pests of grain storage premises in the UK, *O. surinamensis, S. granarius* and *C. ferrugineus*. It is important that a lure in a monitoring device will attract as many different species as possible as the species present will not be known. Key industry representatives were therefore consulted to determine the species considered to be of greatest importance and where the lure would ideally augment early detection. In addition it was important to identify the type of premises that could be used in the latter stages of the project for validation of the lure.

A letter was sent to four industrial bodies with a list of eleven different insect species. It was requested that these were ranked in terms of importance from high to none. Using this information additional species for testing were chosen (Table 5). It was also decided that in addition to testing of insect strains that had been reared in the laboratory for a number of years where possible recently collected field strains would be tested to ensure that behavioural traits were similar (Table 5).

In consultation with industrial partners it was agreed that to represent different industry sectors the following types of premises would ideally be used in the latter stages of the project for validation of the lure and development of a protocol: maltings, grain store (floor storage), grain store (bins), flour mill, animal feed mill and port.

Table 5. Additional species chosen after ranking of importance by industry and strains tested

Species	Lab Strain	Field Strain
O. surinamensis		Huntingdon Daventry Norfolk
C. ferrugineus		Daventry
S. granarius		Dorset
O. mercator	lab ref. S. stock	
T. castaneum	FSS II S. Stock	Norfolk
T. confusum	Lab. Susc.	
R. dominica	Salisbury ex. S. stock	
S. oryzae	Droxford S. stock	
S. zeamais	ex. s. stock	
A. advena	Susc. S. stock	Staplehurst
S. paniceum	Lab strain	Scotland
T. stercorea	Datchet	
P. tectus	PICL	
L. bostrychophila	Lab strain	

MILESTONE 3. Complete behavioural assessment of components to include in simplified attractant mixture and obtain in sufficient quantity for trials

Introduction

The studies undertaken for milestone 1 identified the compounds that produced an electrophysiological response in each of the three species tested. Although this indicates those compounds that the insect is able to detect the method does not provide any information on the behavioural effect, if any, that may be elicited in response to the compound. Behavioural bioassays were therefore necessary to determine whether any of the compounds identified would act as attractants. It is also important to establish the threshold concentrations required to produce a behavioural response, as this information will guide the amounts to be incorporated in prototype lures. Although potential key compounds to act as attractants for the three main species had been identified it was important that no potential attractant was excluded at an early stage. Therefore a literature search was undertaken and compounds that had previously been identified as attractants were included in the screen.

For a lure to be commercially viable it is important to ensure that the constituents are readily available in large quantities, relatively inexpensive, and non-toxic. The compounds identified as potential attractants were reviewed against these criteria to ensure suitability for use in the prototype lure.

Materials and Methods

The insect species and strains used and the culture methods were as described for milestone 1. A single-choice pitfall bioassay was used (Morgan and Healey, 1993) as this had previously been found to be the most appropriate bioassay method for this type of study (Collins *et al.*, 2004). For the tests on *S. granarius*, pitfalls with the further refinement of a 5mm lip, i.e. the glass tube projected 5mm above the level of the filter paper in the arena, were used to deter beetles from falling in by chance, as recommended by Morgan *et al*. (1998). Pitfall bioassays were carried out using 200 mm diameter arenas for all three species. One aliquot (5 µl) of the test solution was applied to each 20 mm diameter filter paper, which was then placed at the bottom of the pitfall tube. Twenty insects were tested in each arena. Tests were conducted in darkness. The number of responders was recorded after 1 h. Tests were conducted in controlled environment rooms at 20 ± 1°C, 50 ± 3% r.h. The following tests were undertaken:

1. Identification of the optimum amount of carob-peanut extract to use as a positive control in subsequent tests with individual compounds. Ten replicates were carried out of each of: pentane control and five amounts (1 ng, 10 ng, 100 ng, 1 µg, 10 µg) of the carob-peanut extract.
2. Initial screening of individual compounds for a behavioural response. Compounds were chosen for testing for one of the following reasons: (a) compound was present in the carob-peanut extract and elicited an EAG response; (b) compound was present in the carob-peanut extract but not all replicate insects gave an EAG response; (c) structural isomers of compounds present in carob-peanut extract which elicited an EAG

response but which were not available for purchase and not easily synthesised; (d) compounds reported in the literature to elicit a behavioural response from the species of interest. The compounds tested are listed in Tables 6a -6c. All of the compounds were tested at two concentrations in 5 µl of pentane. The amounts chosen were equivalent to the concentration in the carob-peanut extract used for GC-EAG and equivalent to the total amount of volatiles in the carob-peanut extract necessary to elicit a behavioural response as determined in test 1. For compounds in category c, the amounts tested were based on the amounts of their structural isomers present in the carob-peanut extract. For compounds in category d, the amounts tested were based on the amounts reported in the literature as being necessary to elicit a behavioural response. The results were compared with those from controls: solvent (pentane) and positive (carob-peanut extract at the optimum amount identified in previous tests). Ten replicates of each concentration and control were carried out.

3. Further pitfall bioassays were carried out with a number of the compounds that gave a positive response in the initial screening pitfall bioassays to establish the response thresholds with the three species. The criteria used to prioritise the testing of these compounds were toxicity, efficacy, availability and the extent to which the compounds occur naturally in the commodities in which traps are used. None of the compounds that gave a response in the initial screening tests is toxic at the amounts that would be present in a lure. They are all are readily available and present in foodstuffs in small amounts. At least one of the compounds is approved as a food additive. Therefore the remaining criterion used for prioritisation was efficacy of the compounds in screening tests. The compounds chosen for testing were: hexanoic acid, 3-methylbutanol, 4-ethylbenzaldehyde, 4-ethylacetophenone, 2-phenylethanol, E-3-octen-2-one, hexanal, nonanal, 3-methylbutyrolactone, E-2-nonenal and γ-nonanoic lactone. The amounts of each compound tested in 5 µl of pentane were: 1 ng, 10 ng, 100 ng, 1 µg and 10 µg, except hexanoic acid which was tested at higher amount of 100 µg instead of 1ng because there was a possibility that it may be used as a carrier in the lure.

Statistical analysis

For all tests the numbers of insects that responded were square root arcsine transformed and the differences between the responses to the pentane control and the test compound compared using one-way ANOVA. For tests 1 and 2 this was followed by Tukey's pairwise comparisons and by Dunnett’s test for comparisons with a control for test 3.

Table 6. Compounds tested with *S. granarius, O. surinamensis* and *C. ferrugineus*

Compound	Reason for testing*	Amounts tested
S. granarius		
Butyric acid	a	256 ng, 1 µg
E-2-Nonenal	a	100 ng, 1.4 µg
4-Ethylbenzaldehyde	a	63 ng, 1 µg
E-2-Decenal	a	153 ng, 1 µg
Octyl isobutyrate	a	190 ng, 1 µg
γ-Nonanoic lactone	a	95 ng, 1 µg
5-Methylbutyrolactone (isomer of 4-methyl butyrolactone)	c	100 ng, 1 µg
3-Methylbutyrolactone (isomer of 4-methyl butyrolactone)	c	200 ng, 1 µg
4-Ethylacetophenone	c	152 ng, 1 µg
Nonanoic acid	d	1 µg, 10 µg
Hexanal	b	100 ng, 1 µg
Nonanal	d	100 ng, 1 µg
Tridecane	b	1 µg, 10 µg
O. surinamensis		
2-phenylethanol	a	247 ng, 1 µg
Octyl isobutyrate	a	190 ng, 1 µg
γ-nonanoic lactone	a	95 ng, 1 µg
Hexyl hexanoate	a	51 ng, 1 µg
3-Methylbutanol	d	100 ng, 500 ng
Hexanoic acid	d	100 ng, 1 µg
E-3-octen-2-one	b	31 ng, 1 µg
Nonanoic acid	d	1 µg, 10 µg
Hexanal	d	100 ng, 1 µg
Nonanal	d	100 ng, 1 µg
C. ferrugineus		
2-Ethylhexanol	a	212 ng, 1 µg
E-3-Octen-2-one	a	31 ng, 1 µg
2-acetylpyrrole	a	55 ng, 1 µg
Ethyl isobutyrate	a	567 ng, 1 µg
Tetradecane	a	44 ng, 1 µg
Nonyl isobutyrate	a	168 ng, 1 µg
Pentadecane	a	168 ng, 1 µg
Pentadecan-2-one	a	1 µg, 1.657 µg
Octan-3-one	d	1 µg, 10 µg
3-Methylbutanol	d	500 ng, 1 µg

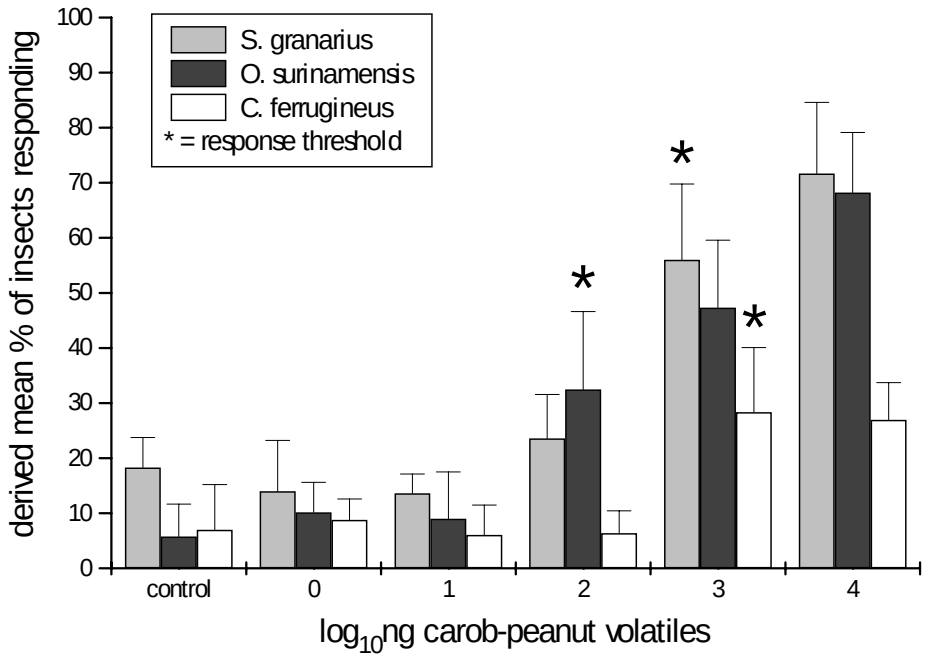
* description under section 2 above

Results

Bioassay test 1.

The response thresholds for varying amounts of carob-peanut extract compared with a pentane control were 100 ng for *O. surinamensis* and 1 µg for *S. granarius* and *C. ferrugineus* ($P < 0.05$) (Figure 2). These amounts were used as positive controls in subsequent tests with individual compounds.

Figure 2. Response thresholds of *S. granarius, O. surinamensis* and *C. ferrugineus* to varying amounts of carob-peanut extract in pentane compared with a pentane control ($P < 0.05$)



Bioassay test 2**.**

Five of the compounds tested elicited a positive response from *S. granarius* in the pitfall bioassays ($P < 0.05$) (Table 7a). Seven of the compounds tested elicited a positive response from *O. surinamensis* ($P < 0.05$) (Table 7b). Five of the compounds tested elicited a positive response from *C. ferrugineus* (Table 7c).

Table 7a. Summary of initial pitfall bioassay results with *S. granarius*

Compound*	**Amount required for response**
E-2-Nonenal[a]	1.406 µg
4-Ethylbenzaldehyde[a]	1 µg
Octyl isobutyrate[a]	1 µg
3-Methylbutyrolactone[c]	1 µg
4-Ethylacetophenone[c]	152 ng

Table 7b. Summary of initial pitfall bioassay results with *O. surinamensis*

Compound*	**Amount required for response**
2-Phenylethanol[a]	1 μg
γ -Nonanoic lactone[a]	1 μg
3-Methylbutanol[d]	100 ng
Hexanoic acid[d]	100 ng
3-Octen-2-one[b]	1 μg
Hexanal[d]	100 ng
Nonanal[d]	100 ng

Table 7c. Summary of initial pitfall bioassay results with *C. ferrugineus*

Compound*	**Amount required for response**
Ethyl isobutyrate[a]	1 μg
Nonyl isobutyrate[a]	1 μg
Pentadecan-2-one[a]	1.657 μg
Octan-3-one[d]	10 μg
3-Methylbutanol[d]	500 ng

* letter refers to reason for testing

Bioassay test 3.

A summary of the response thresholds ($P < 0.05$) for each of the compounds with each of the three species tested is shown in Table 8. Two of the compounds, 4-ethylacetophenone and E-2-nonenal, elicited positive responses from all three species tested. Some of the compounds elicited positive responses in the initial screening bioassays and then did not elicit a response in these subsequent tests or required a higher amount of the compound in subsequent tests.

Table 8. Summary of response thresholds for each of the compounds with each of the three species tested

	S. granarius					*O. surinamensis*					*C. ferrugineus*				
Amount of compound	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Hexanoic acid	-	-	-	-	-	-	-	*	*	-	-	-	-	-	*
3-Methylbutanol	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-
4-Ethylbenzaldehyde	-	-	-	*	*	-	-	-	-	-	-	-	-	-	-
4-Ethylacetophenone	-	*	*	*	-	-	-	-	*	*	-	-	-	-	*
2-Phenylethanol	-	-	-	-	*	-	-	*	*	-	-	-	-	-	-
3-Octen-2-one	-	-	-	-	-	-	*	-	-	*	-	-	*	*	*
Hexanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nonanal	nt	nt	nt	nt	nt	-	-	-	-	-	-	-	-	-	*
Ethyl isobutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E-2-Nonenal	-	-	-	-	*	-	-	-	-	*	-	-	*	*	*
3-Methylbutyrolactone	-	-	-	*	*	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
γ -Nonanoic lactone	nt	nt	nt	nt	nt	-	-	-	*	*	nt	nt	nt	nt	nt

Amount of compound 1 - 5 = 1 ng, 10 ng, 100 ng, 1 µg and 10 µg, except hexanoic acid: 10 ng, 100 ng, 1 µg, 10 µg and 100 µg.

nt = not tested

- = response not significantly different from pentane control

* = response significantly different form pentane control ($P < 0.05$)

Discussion

Reasons for testing each of the additional compounds are given below:

- Octan-3-one has been shown to elicit a behavioural response from *C. ferrugineus* at 10 ng with the optimum response at 10 µg (Pierce *et al.*, 1991).
- 3-Methylbutanol has been shown to elicit a behavioural response from *O. surinamensis* at 1 ng with the optimum response at 100 ng (Pierce *et al.*, 1991). This compound has also been shown to elicit a behavioural response from *C. ferrugineus* at 10 ng with the optimum response at 100 µg (Pierce *et al.*, 1991).
- Hexanoic acid is known to be attractive to *O. surinamensis* at 1.2 µg (Stubbs *et al.*, 1985) and at 1 µl (equivalent to 927 ng) (O'Donnell *et al.,* 1983).
- γ-Valerolactone (5-methylbutyrolactone) and α-methyl-γ-butyrolactone (3-methylbutyrolactone) are isomers of 4-methylbutyrolactone which elicited an EAG response from *S. granarius* but

which is unavailable. It is not feasible to produce this compound for testing and lure production because there is no simple synthetic route.

- 4-Ethylacetophenone was tested as a substitute for 3-ethylacetophenone, which elicited an EAG response from *S. granarius,* but which is unavailable. 3-Ethylacetophenone can be found as an impurity in commercially available 4-ethylacetophenone.
- 3-Octen-2-one was tested with *O. surinamensis* because it elicited an EAG response from some of the *O. surinamensis* tested and from all of the *C. ferrugineus* tested.
- Nonanoic acid is known to elicit a behavioural response from *O. surinamensis* at 1 µg with the optimum response at 1000 µg (Pierce *et al*., 1990). 500 µg of this compound was found to be highly attractive to *O. surinamensis* when placed on a wick above a bioassay arena (O'Donnell *et al.,* 1983). Nonanoic acid was tested with *S. granarius* because it is a main component in two mixtures of compounds one of which is known to be attractive to *S. granarius* (Collins and Chambers, 2003) and the other of which is known to be attractive to *S. zeamais* (Hodges *et al.,* 1998).
- Hexanal elicited an EAG response from some *S. granarius*. This compound has also been found to elicit a behavioural response from *O. surinamensis* at 100 ng with the optimum response at 1 µg (Pierce *et al*., 1990). Mikolajczak *et al*. (1984) found that hexanal elicited a behavioural response from *O. surinamensis* at 10 µg and 100 µg with the optimum response at 10 µg. Hexanal is a main component in the peanut extract.
- Nonanal elicited an EAG response from some of the *O. surinamensis* tested. It has been found to elicit a behavioural response from *O. surinamensis* in a two-choice pitfall bioassay at 100 ng and 1 µg (optimum amount) (Pierce *et al.,* 1990). Nonanal was tested with *S. granarius* because it is a component in two mixtures of compounds one of which is known to be attractive to *S. granarius* (Collins and Chambers, 2003) and the other of which is known to be attractive to *S. zeamais* (Hodges *et al.,* 1998).
- Tridecane elicited an EAG response from some *S. granarius*. It was identified as a main component in several aeration extracts: wheat, oats, maize and barley (data from Defra contract CE0310).
- 1-Decene may be attractive to *S. granarius* because compounds of this type (1-alkenes) are present in the kairomone lure for the Trécé Inc. Storgard® Dome™ Trap which is highly attractive to this species (Collins & Chambers, 2003). The kairomone lure consists of mixture of oils from solvent extraction of vegetable material.

There are no records in the literature of any of the compounds tested eliciting a behavioural response from *S. granarius*. However, there is evidence of a behavioural response to γ-lactones in that some γ-lactones are feeding deterrents to *S. granarius* and a number of other stored product insects, although this is not reported for *O. surinamensis* or *C. ferrugineus* (Ciunik *et al.,* 2000; Daniewski *et al.,* 1993; Nawrot, *et al.,* 1983 &

1985). Therefore, careful consideration should be given to the inclusion in the lure of 3-methylbutyrolactone and γ-nonanoic lactone. There is also evidence that C5 - C19 fatty acids, including nonanoic acid, are repellent to *Sitophilus oryzae* when applied to wheat in large amounts (Shaaya *et al.,* 1976). We now know that the following compounds increase the response of *S. granarius* in pitfall bioassays: E-2-nonenal, 4-ethylbenzaldehyde, 4-ethylacteophenone, 2-phenylethanol and 3-methylbutyrolactone. Although 3-methylbutyrolactone is a γ-lactone, this particular γ-lactone may be an attractant or it may increase the response in pitfall bioassays when high amounts are present by increasing the general activity of the insects.

Three of the compounds tested with *O. surinamensis* had not previously been recorded in the literature as eliciting a behavioural response: 4-ethylacetophenone, γ-nonanoic lactone and 3-octen-2-one. Three of the compounds, which increased the response in bioassays during the present study, have also been shown to elicit behavioural responses by other authors: E-2-nonenal, 2-phenylethanol and hexanoic acid. During the present study, E-2-nonenal elicited a response at 10 µg. Using older insects (4-6 weeks old) and a two-choice pitfall bioassay Mikolajczak *et al.* (1984) found that this compound elicited a response at 100 ng, with the optimum response at 10 µg and a strong repellent effect at 100 µg. During the present study, 2-phenylethanol elicited a response at 100 ng and 1 µg. Using a two-choice pitfall bioassay with a smaller arena, a longer bioassay time (2 h) and older insects (5-12 weeks old), Pierce *et al.* (1991) found that this compound elicited a response at 10 ng and 1 µg with the optimum response at 100 ng and a strong repellent effect at 100 µg. Hexanoic acid elicited a response during the present study at 100 ng and 1 µg. Using a different type of bioassay, O'Donnell *et al.* (1983) and Stubbs *et al*. (1985) found that hexanoic acid elicited a response at 1 µl (equivalent to 927 ng) and 1.2 µg, respectively. The results of Stubbs *et al.* (1985) show that there may be a repellent effect at 120 µg.

Three of the compounds tested with *O. surinamensis* elicited a response in initial bioassays (with the carob-peanut extract in the same room) but not in dose response bioassays: 3-methylbutanol, hexanal and nonanal. Using their pitfall bioassay method, Pierce *et al.* (1991) found that 3-methylbutanol elicited a response from 1 ng to 10 µg with the optimum response at 100 ng. Hexanal elicited a response at 100 ng, with the optimum response at 1 µg and becoming repellent at 100 µg (Pierce *et al*., 1990) with 5-12 week old insects. Mikolajczak *et al*. (1984) found that hexanal elicited a behavioural response from *O. surinamensis* at 10 µg and 100 µg with 4-6 week old insects. Pierce *et al.* (1990) found that nonanal elicited a behavioural response from *O. surinamensis* in their two-choice pitfall bioassay between 100 ng and 10 µg (optimum amount: 1 µg) with 5-12 week old insects. However, using 4-6 week old insects, Mikolajczak *et al.* (1984) found that there was no significant behavioural response to nonanal. Hexanal and nonanal, together with octanal in a 1:1:1 mixture elicited a response from *O. mercator* in pitfall bioassays at amounts ranging from 10 ng and 100 µg. This mixture also increased the response of *O. mercator* to cucujolide aggregation pheromones (Pierce *et al.,* 1990). 3-Methylbutanol increased the responses of both *O. surinamensis* and *O. mercator* to their respective cucujolide aggregation pheromones (Pierce *et al.,* 1991). It is, therefore, possible that the addition of 3-

methylbutanol, nonanal and hexanal to the multi-species lure in low amounts may increase the overall behavioural response, even if they are added in amounts which do not elicit behavioural responses from the individual compounds. This may also explain why responses were obtained in this study when the carob-peanut extract was present and not when it was absent. It is possible that these compounds augment responses to food attractants as well as to pheromones.

One of the compounds tested with *O. surinamensis*, nonanoic acid, did not give any response at either 1 µg or 10 µg. However, this compound was found to increase the response at 1 µg with the optimum response at 1000 µg by Pierce *et al*. (1990) and 500 µg was found to be highly attractive to *O. surinamensis* when placed on a wick above a bioassay arena (O'Donnell *et al.,* 1983). The bioassays used by these authors are different to that used during this study in a number of parameters: they used older insects (5 - 12 weeks old and 0-3 weeks old, respectively), smaller bioassay arenas were used, the duration of the bioassays differed, for the former bioassay the insects were held at low population density for one week prior to testing and the insect culture media were different (rolled oats and yeast only with no wheatfeed and wheat flour rather than wheatfeed with rolled oats and yeast, respectively). Both Pierce *et al.* (1990) and O'Donnell *et al.* (1983) found that large amounts of nonanoic acid were required to elicit a behavioural response. It would, therefore, not be practical to include this compound in a multi-species lure.

The compounds found to or confirmed to increase the response of *O. surinamensis* in pitfall bioassays during this study are: 3-methylbutanol, nonanal, hexanal (prior three compounds in the presence of carob-peanut volatiles only), 2-phenylethanol, 4-ethylacetophenone, γ-nonanoic lactone, 3-octen-2-one, hexanoic acid and E-2-nonenal.

Many of the compounds tested with *C. ferrugineus* have not previously been recorded in the literature as eliciting a behavioural response. Such compounds which were found increase the response of *C. ferrugineus* in pitfall bioassays are: hexanoic acid, 4-ethylacetophenone, ethyl isobutyrate (only with carob-peanut volatiles present), nonyl isobutyrate, E-2-nonenal, 3-octen-2-one and pentadecan-2-one.

Some of the compounds tested with *C. ferrugineus* had been tested during other studies and a comparison of the results follows. The amount (10 µg) of octan-3-one required to elicit a behavioural response was greater than that reported by Pierce *et al.* (1991). They recorded a response at 10 ng, optimum response at 10 µg, decreasing at 100 µg and becoming repellent at 1000 µg. A greater amount of 3-methylbutanol was also required during this study than in that of Pierce *et al.* (1991). The amount of this compound required to elicit a response during this study was 1 µg whereas Pierce *et al.* (1991) recorded a response at 10 ng with the optimum response at 100 µg. *C. ferrugineus* did not respond to 2-phenylethanol during this study however Pierce *et al.* (1991) recorded a response at 10 µg becoming repellent at 100 µg. The explanation for the discrepancies in response thresholds may be due to the bioassays used during the present study and that of

Pierce *et al.* (1991) not being directly comparable: Pierce *et al.* (1991) used a two-choice pitfall bioassay with a smaller arena, a longer bioassay time (2 h), they used older insects (5-12 weeks old) and the insect culture media were different (no wheatfeed; rolled oats and yeast only). Any of these factors could account for the differences in response thresholds or differences in whether or not a response was recorded. The response threshold for nonanal was found to be 10 µg during this study, which used mixed sex insects. Using cardboard traps, Mushobozy *et al.* (1993) recorded responses to nonanal of 100 ng and 10 µg from male and female *C. ferrugineus,* respectively. They also noted that the increase in the number of insects caught over the control traps was very low, that this is not a good attractant for *C. ferrugineus* and that if either nonanal or 1-octen-3-ol is added to multispecies baits to increase their efficacy for other species, there should be little adverse effect on the response of *O. mercator, C. ferrugineus* or *C. pusillus.* The compounds found to or confirmed to increase the response of *C. ferrugineus* in pitfall bioassays during the present study are: ethyl isobutyrate (only with carob-peanut volatiles present), nonyl isobutyrate, pentadecan-2-one, octan-3-one, 3-methylbutanol, hexanoic acid, 4-ethylacetophenone, 3-octen-2-one, E-2-nonenal and nonanal.

A summary of all of the compounds which increased the response over the pentane control in the initial and dose response pitfall bioassay, together with the range of amounts over which they elicited a response, is shown in Table 9.

Two of the compounds tested elicited a response from all three species: E-2-nonenal and 4-ethylacetphenone. Five of the compounds tested elicited a response from two of the species tested: 2-phenylethanol, 3-methylbutanol, hexanoic acid, 3-octen-2-one and nonanal. These compounds should form the basis for the formulation of a mixture of components for testing as a multi-species lure. Given the role of hexanal in increasing the response of *Oryzaephilus* species to aggregation pheromones, consideration should also be given to the inclusion of this compound in the lure mix. The required release rates will be based on the pitfall bioassay dose response results but the amount of the compound on the filter paper does not necessarily give a good indication of the amount of the compound detected by the insects in the air inside a pitfall arena. In general, the amount of the compound in the air will increase with the amount of the compound on the filter paper but this is not necessarily a linear relationship and the relationship will be different for each compound depending on its physical properties (Cometto-Muniz *et al.,* 2003). The amount of the compounds in the air in the pitfall arenas is currently being investigated by spme-gc-ms. Comparisons are being drawn between these measurements and release rates at realistically low temperatures in a grain silo in order to establish realistic required release rates from a lure.

Conclusions

- Five compounds increased the response of *S. granarius*: E-2-nonenal, 4-ethylbenzaldehyde, 4-ethylacteophenone, 2-phenylethanol and 3-methylbutyrolactone.

- Nine compounds increased the response of *O. surinamensis*: 3-methylbutanol, nonanal, hexanal (prior three compounds in the presence of carob-peanut volatiles only), 2-phenylethanol, 4-ethylacetophenone, γ-nonanoic lactone, 3-octen-2-one, hexanoic acid and E-2-nonenal.
- Ten compounds increased the response of *C. ferrugineus*: ethyl isobutyrate (only with carob-peanut volatiles present), nonyl isobutyrate, pentadecan-2-one, octan-3-one, 3-methylbutanol, hexanoic acid, 4-ethylacetophenone, 3-octen-2-one, E-2-nonenal and nonanal.
- Two compounds elicited a response from all three species: E-2-nonenal and 4-ethylacetphenone.
- Five compounds elicited a response from two of the species tested: 2-phenylethanol, 3-methylbutanol, hexanoic acid, 3-octen-2-one and nonanal.

Table 9. Summary of compounds which increased the response over the pentane control in the initial and dose response (DR) pitfall bioassays. Their ranges of effective amounts on the filter paper are shown

	S. granarius		***O. surinamensis***		***C. ferrugineus***	
	Initial	**DR**	**Initial**	**DR**	**Initial**	**DR**
E-2-nonenal	1.4 µg	10 µg	nt	10 µg	nt	100 ng - 10 µg
4-ethylbenzaldehyde	1 µg	1 µg - 10 µg	nt	-	nt	-
3-methylbutyrolactone	1 µg	1 µg - 10 µg	nt	nt	nt	nt
4-ethylacetophenone	152 ng	10 ng - 1 µg	nt	1 µg - 10 µg	nt	10 µg
2-phenylethanol	nt	10 µg	1 µg	100 ng - 1 µg	nt	-
γ-nonanoic lactone	-	nt	1 µg	1 µg - 10 µg	nt	nt
3-methylbutanol	nt	-	100 ng	-	500 ng	1 µg
Hexanoic acid	nt	-	100 ng	100 ng - 1 µg	nt	10 µg
3-octen-2-one	nt	-	1 µg	10 ng & 10 µg	-	100 ng - 10 µg
Hexanal	-	-	100 ng	-	nt	-
Nonanal	-	nt	100 ng	-	nt	10 µg
Ethyl isobutyrate	nt	-	nt	-	1 µg	-
Nonyl isobutyrate	nt	nt	nt	nt	1 µg	nt
Pentadecan-2-one	nt	nt	nt	nt	1.66 µg	nt
Octan-3-one	nt	nt	nt	nt	10 µg	nt

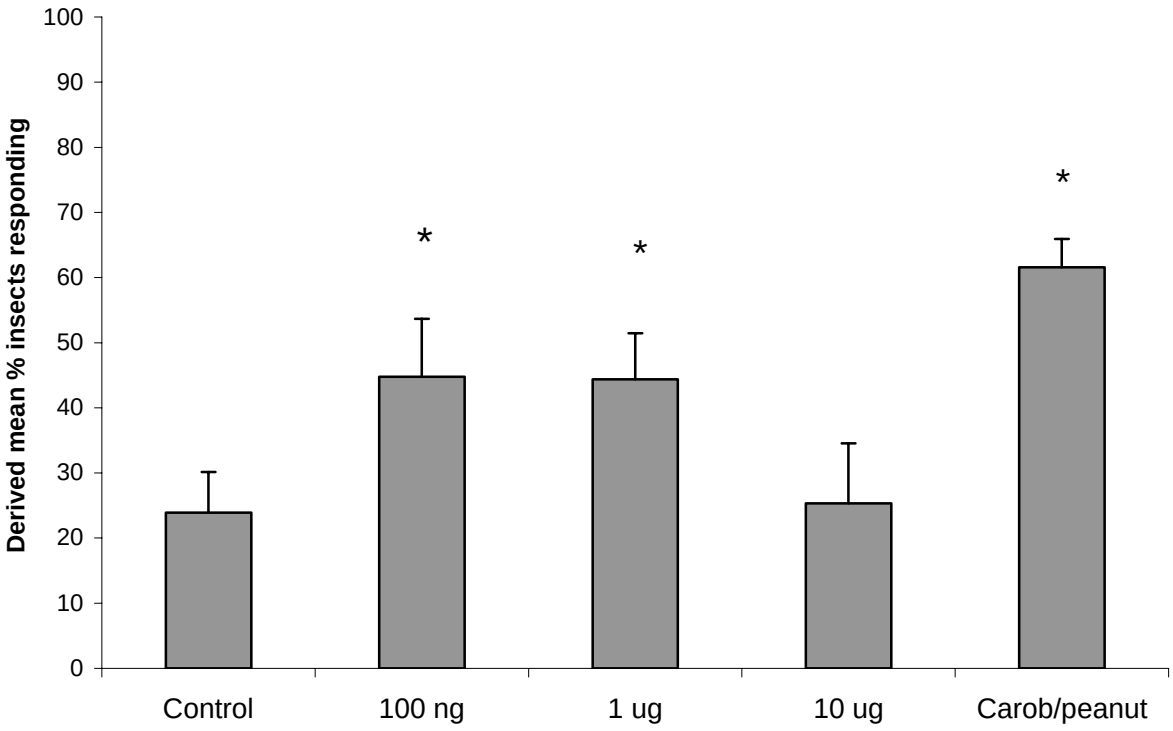
Testing of mixtures

Introduction

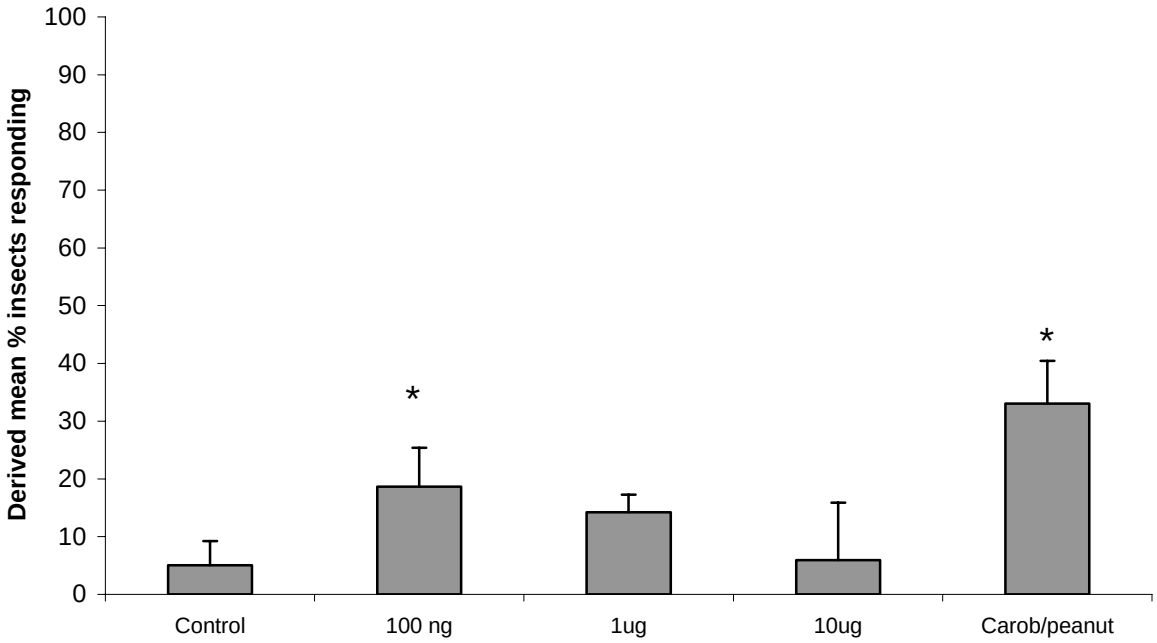
The work described above identified the compounds and the concentrations required to attract the individual species. In order to attract all three species it was necessary to mix the individual compounds and examine the effect of the mixtures on the three key test species. This work commenced with 10 compounds - hexanoic acid, 3-methylbutanol, 4-ethylbenzaldehyde, 4-ethylacetophenone, 2-phenyl ethanol, 3-octen-2-one, hexanal, nonanal, E-2-nonenal and γ-nonanoic lactone. A series of trials were undertaken firstly with a mixture of the 10 compounds and then trying different mixtures. In general, the compounds were mixed together in a 1:1 ratio but some tests also examined the effect of different ratios of the compounds. All tests were undertaken in the laboratory using the pitfall bioassay method as previously described. The mixture that was shown to have the greatest effect against all three species was a six component mixture of hexanoic acid, 3-methylbutanol, 4-ethylacetophenone, 3-octen-2-one, nonanal and E-2-nonenal. There was a significant increase in response for *S. granarius* using 100 ng and 1 μg of each compound, for *O. surinamensis* using 100 ng of each compound and for *C. ferrugineus* using 1 μg and 10 μg of each compound (Figure 3 a-c). This mixture was chosen for formulation in a lure dispenser for further laboratory and field scale evaluation.

Figure 3. Response of *S. granarius, O. surinamensis* and *C. ferrugineus* to a six-component mixture in pitfall bioassays. The amounts are the amount of each individual compound in the six-component mixture

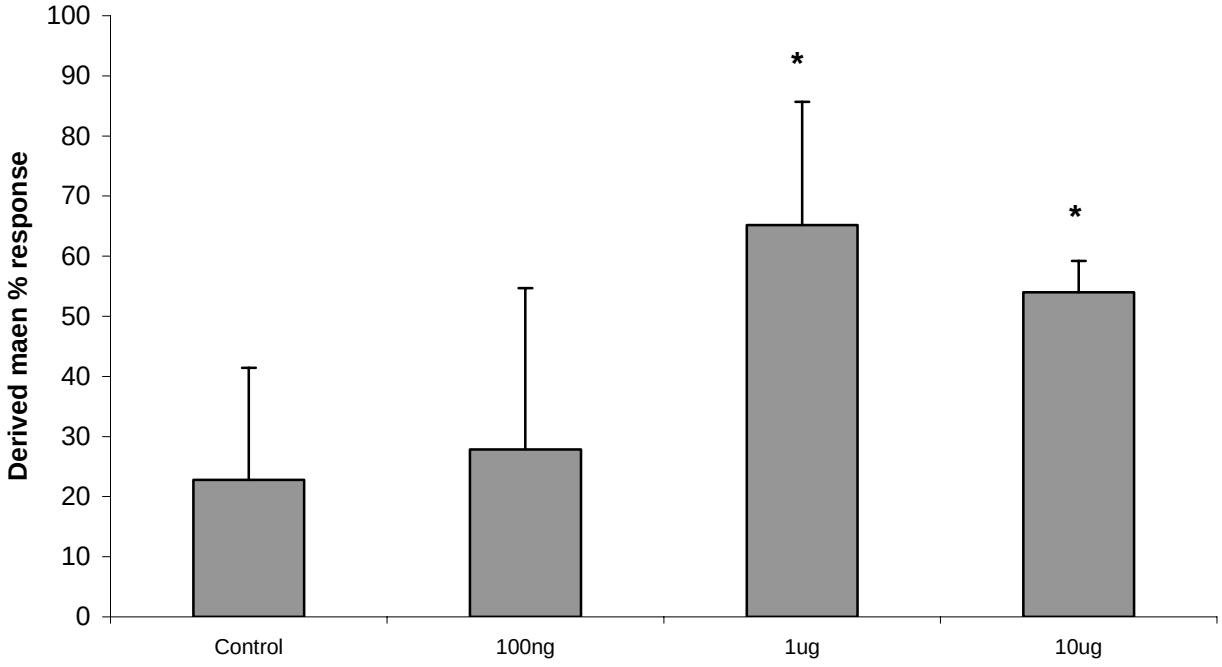
(a) *S. granarius*



(b) *O. surinamensis*



(c) *C. ferrugineus*



MILESTONE 4. Develop and conduct laboratory tests of formulation to ensure that lure will work well in empty premises and in presence of grain

Introduction

Once the ability of the compound to attract the different insect species, the threshold concentrations required for response and the effect of a mixture of the compounds had been established it was necessary to formulate the compounds in an appropriate lure dispenser and establish whether this would attract insects both in the laboratory and on a practical scale basis. This was conducted in three parts:

1. Experiments were undertaken to identify whether the lure would remain effective in the presence of grain
2. The compounds identified were formulated into different types of dispenser and the effectiveness assessed in laboratory bioassays
3. Trials of the prototype lure were undertaken in a grain bulk to assess effectiveness over a six week period.

1. Identification of the ability of prototype lures to attract insects in the presence of grain using headspace Solid Phase Micro-extraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS)

Introduction

In a previous project lures constructed of high density polythene loaded with volatiles obtained from kibbled carob and peanuts were found to be highly attractive to three species of grain pests *O. surinamensis*, *C. ferrugineus* and *S. granarius* in laboratory bioassays. However, when these lures were tested in grain bins (30 tonnes) the performance of these lures was dramatically reduced. The aim of this work was to determine why the performance of lures placed in PC™ traps placed in grain bins was inferior to their performance in pitfall bioassays. It was suspected from previous SPME analysis of lures in the laboratory that the polythene lures may not have released enough material at low temperatures and in an open area (such as a grain store) to attract the insects. The two other main possibilities were that:

(a) The volatiles being released by the lures were absorbed by the grain
(b) The lures were not as attractive to the insects in the presence of grain

These possibilities were examined in tests in both the laboratory and the Storage Research Unit at CSL by analysing the volatiles released in the headspace using solid phase microextraction (SPME) followed by GC-MS.

Materials and methods

The polythene tube lures consisted of 50 mm lengths of rigid, high-density polythene tubing (922 kg/m^3 nominal density) with an internal diameter of 1.6 mm and wall thickness of 0.8 mm which were heat sealed at both ends to contain the volatiles. Each lure contained 80 µl of a synthetic mixture of volatiles.

SPME sampling of lures in sealed and open beakers

Single polythene lures each containing 80 µl of synthetic mixture were placed into 600 ml Pyrex beakers (previously muffled at 400°C for 2 hours to remove volatiles). Aluminium foil was then stretched over the top of the beaker to create a seal. The lures were left in the beaker for 30 minutes for the volatiles to equilibrate and then sampled by SPME (portable SPME field samplers (carboxen / polydimethylsiloxane 75 µm film thickness, 1 cm fibre length (Supelco) for 30 minutes.

The headspace above polythene lures was sampled at 20°C and 7°C in sealed beakers and at 7°C in open beakers. A surrounding temperature of 7°C was achieved by placing the beakers in an empty fridge. This temperature was chosen to be similar to the daytime temperatures recorded in the previous study when lures were tested in a grain store. The experiments were conducted in the laboratory in glass beakers, as this removed the effect of the presence/absence of grain and solely focused on (a) the effect of temperature on concentration of volatiles in the headspace and (b) whether the lure released volatiles into a sealed or open container.

SPME sampling of volatiles from inside a PC™ trap (containing a lure) placed on the surface of grain in a 30 tonne grain bin

A single polythene tube lure was placed inside the lid of a PC™ trap that was then placed on the surface of the grain in a grain bin. The volatiles inside the PC™ trap were sampled after 1 week by pushing the SPME needle (portable SPME field samplers (carboxen / polydimethylsiloxane 75 µm film thickness, 1 cm fibre length (Supelco)) through one of the holes in the lid of the PC™ trap. The volatiles were sampled for 30 minutes and then analysed by GC-MS.

GC-MS analysis

The volatiles were analysed on a Hewlett Packard 5890 series II gas chromatograph coupled to a VG Trio-1 mass spectrometer. The SPME fibres were desorbed at 270^0C for 1 minute in splitless injection mode (purge on after 1.0 minute), onto a Chrompack CPSil-5CB column (100% polydimethylsiloxane, 50 m, 0.32 mm internal diameter, 1.2 µm film thickness, no. 9019903). The oven temperature was initially 40°C for 1 min, rising at 10°C per minute to 120°C, then at 3°C/min to 220°C, and then at 10°C/min to 270°C and held at this temperature for 15 minutes. Helium was used as the carrier gas, and the GC operated in constant pressure mode ((4 psi, 30.4 cm/sec (at 40°C GC oven temperature), split vent flow 50.0 ml/min). The mass spectrometer source, and interface temperatures were 200°C, and 275°C respectively. The mass spectrometer was operated in electron impact mode (EI+) at 70 eV, and scanned from 33-350 amu once every second. Mass spectrometer acquisition commenced after 0 minutes.

Results

SPME Sampling of lures in sealed and open beakers

The concentration of volatiles released from lures in sealed beakers was markedly lower at 7°C compared to 20°C (Table 10). For the most volatile component, ethyl isobutyrate, the concentration in the headspace was approximately 3-fold lower at 7°C than at 20°C. For the less volatile components, such as 4-ethylacetophenone the drop in concentration was far greater (130 fold).

When lures were sampled at 7°C in an open beaker the concentrations were reduced still further, with the 5 least volatile components not being detected at all and the 5 most volatile components detected in only trace amounts. This is due to volatiles being able to diffuse out of top of the open beaker stopping their build up. The open beaker mimics a PC™ trap which is also open at the top. For ethyl isobutyrate the concentration at 7°C in an open beaker was approximately a 1/700th of the concentration compared to that found in a sealed beaker at 20°C.

SPME sampling of volatiles from within a PC™ trap placed on the surface of grain in a 30 tonne bin

The concentrations of volatiles released from polythene lures in a PC™ trap at 8°C and from a lure in a sealed 600 ml beaker in the laboratory at 20°C are compared in Table 11. For the most volatile component, ethyl isobutyrate the concentration inside the PC™ trap was approximately 1/200th of that in a sealed beaker at 20°C. For a less volatile component, 4-ethylacetophenone the concentration in the PC™ trap was approximately 1/4600th of that in a sealed beaker at 20°C.

Table 10. SPME headspace sampling of sealed polythene tube lures loaded with 80 µl of a synthetic mixture (results are given as GC-MS area counts)

	1 ethyl isobutyrate	2 3-methyl butanol	3 hexanal	4 3-octen-2-one	5 hexanoic acid	6 nonanal	7 E-2-nonenal	8 2-phenyl ethanol	9 4-ethyl aceto-phenone	10 γ-nonanoic lactone
Loading (mg)	1.4 mg	1.4 mg	1.4 mg	1.4 mg	41 mg	1.4 mg	1.4 mg	3.4 mg	3.4 mg	3.4 mg
Sealed beaker @ 20°C	54 871 188	6 749 906	19 600 096	11 211 951	25 111 982	2 110 013	2 090 737	1 895 394	5 011 988	114 700
Sealed beaker @ 7°C	15 803 180	581 814	7 142 993	790 993	984 382	176 103	85 808	18 093	36 403	n/d
Open beaker @ 7°C	74 404	4 680	28 498	2 380	48 567	n/d	n/d	n/d	n/d	n/d

Table 11. SPME headspace sampling of polythene tube lures loaded with 80 µl of synthetic mixture placed in a sealed beaker in the laboratory at 20^{0}C and inside a surface PC™ trap in a grain bin at 8^{0}C (results are given as GC-MS area counts)

	1 ethyl isobutyrate	2 3-methyl butanol	3 hexanal	4 3-octen-2-one	5 hexanoic acid	6 nonanal	7 E-2-nonenal	8 2-phenyl ethanol	9 4-ethyl aceto-phenone	10 γ-nonanoic lactone
Loading (mg)	1.4 mg	1.4 mg	1.4 mg	1.4 mg	41 mg	1.4 mg	1.4 mg	3.4 mg	3.4 mg	3.4 mg
(I) Inside Sealed beaker @ 20°C	80 203 560	4 871363	9 148670	26 541 218	57 247 340	1 743 783	11 870 662	4 439 722	23 760 760	646 700
(II) Inside PC™ trap grain silo @ 8°C	412 005	32 831	87 226	32 258	947 367	6 974	4 624	4 064	5 125	n/d

Discussion

The solubility of volatiles in the non-polar polythene tubing declines with decreasing temperature, reducing the rate of diffusion of volatiles through the walls of the polythene lure. The vapour pressure of the compounds in the air also declines with decreasing temperature. When lures are tested in pitfall bioassays there is only a low volume of headspace (approximate volume 3.5 litres) which allows the volatiles to build up in the air. In grain stores the volume of headspace above the traps is very much larger which prevents the volatiles building up to the same levels as in a bioassay arena. Air currents above the grain and through the grain (if ventilated) will also sweep volatiles away from the trap preventing their build up. All these factors will markedly reduce the concentrations of volatiles in the air above PC™ traps in the grain store at low temperatures compared to laboratory bioassays conducted at 20°C.

The possibility that insects do not respond well to lure volatiles when already on a commodity is not provable by this work. However, the fact that the lures released very low levels of volatiles leads to the conclusion that lack of sufficient volatiles released by the lures reduced their attractiveness in the grain bins. It is unlikely that chemical breakdown was a major factor due the relatively low temperatures in the grain store. Most of the chemicals identified as attractive to the three species of insect are stable at 0 to +5°C, or at room temperature.

2. Formulation and testing of lure components in laboratory studies.

Introduction

Identification of the attractive components of the carob-peanut mixture is only part of developing an effective lure. It is necessary that these components are formulated and incorporated in a dispenser that is able to provide the correct release rate of each of the components, is able to remain effective for at least six weeks, is easy to use and is cheap to produce. Initial testing of a prototype lure in the CSL Grain Storage Research Unit had used the same lure dispenser as is currently supplied for use in the PC™ Floor trap. However, it was shown that the volatiles were lost rapidly and the lures were not effective over a six week period (see 'Testing of prototype lures in the CSL Grain Storage Research Unit' below). Various different types of lure dispenser were therefore assessed in laboratory bioassays prior to testing the lure under more realistic field conditions. The production of the lures and the results of laboratory tests with the three key beetle pest species *O. surinamensis*, *S. granarius* and *C. ferrugineus* is described.

Materials and Methods

The six compounds, hexanoic acid (9.27 mg), 3-methylbutanol (8.09 mg), 4-ethylacetophenone (9.93 mg), 3-octen-2-one (8.57 mg), nonanal (8.27 mg) and E-2-nonenal (8.46 mg), were formulated into different types of lure dispenser by Russell IPM. The compounds were mixed with a matrix that was present to stabilise the release rate at different temperatures and butylated hydroxytoluene (BHT, 10 mg) to prevent breakdown of the compounds.

Four different lure dispensers were provided by Russell IPM:

1. The ring dispenser. This consisted of a cotton filter 10 mm in diameter and 5 mm thick loaded with the six components, BHT and the matrix and was encased in a rubber ring with an inner diameter of 10 mm and an outer diameter of 13 mm. The matrix used was solid at room temperature and was heated gently to a liquid form in order to add the attractant compounds and BHT.
2. Light bag - the ring dispenser containing the attractants, matrix and BHT was placed in a thin polyethylene sachet (Transatlantic Plastics Limited), 500 gauge, and 125 micron and sealed at both ends.
3. Heavy bag - the ring dispenser containing the attractants, matrix and BHT was placed in a thicker gauge polyethylene sachet (Transatlantic Plastics Limited), 1000 gauge, 125 micron and sealed at both ends in order to further slow the release of the attractants.
4. Vial - a polyethylene vial containing a smaller cotton filter (6mm diameter, 15 mm in length) on to which was loaded the attractants, matrix and BHT. The vial was 30mm long and had an internal diameter of 8mm and wall thickness of 1mm.

A fifth type of dispenser was produced at CSL. This consisted of a 50 mm length of rigid, high density polythene tubing (922 kg/m^3 nominal density) with an internal diameter of 1.6 mm and wall thickness of 0.8 mm. The compounds and BHT were added to the tube which was heat sealed at both ends to retain the mixture. This lure did not contain the matrix

All lures were kept in a freezer at -18°C prior to use and were sealed in aluminium foil envelopes. Lures were tested in pitfall bioassays at 20°C, 50% rh. A one hour test period was used. Lures were removed from the freezer 24 hours prior to testing and left in a laboratory at approx. 20°C and ambient rh. The lures were then tested and this was defined as Week 0. Immediately after testing the lures were returned to the laboratory and left at ambient conditions for the specified time periods (Week 1, Week 3 and Week 6). A single set of lures was used for the same species throughout the test period. Ten replicate lures were tested in total.

Results

O. surinamensis

A significant increase in response ($P < 0.05$) compared to the control was found at Week 1 for the vial, at Week 3 for the tube and the vial and at Week 6 for the tube and the vial (Figure 4a). The ring, light and heavy bag lures did not increase the response at any of the time periods tested.

S. granarius

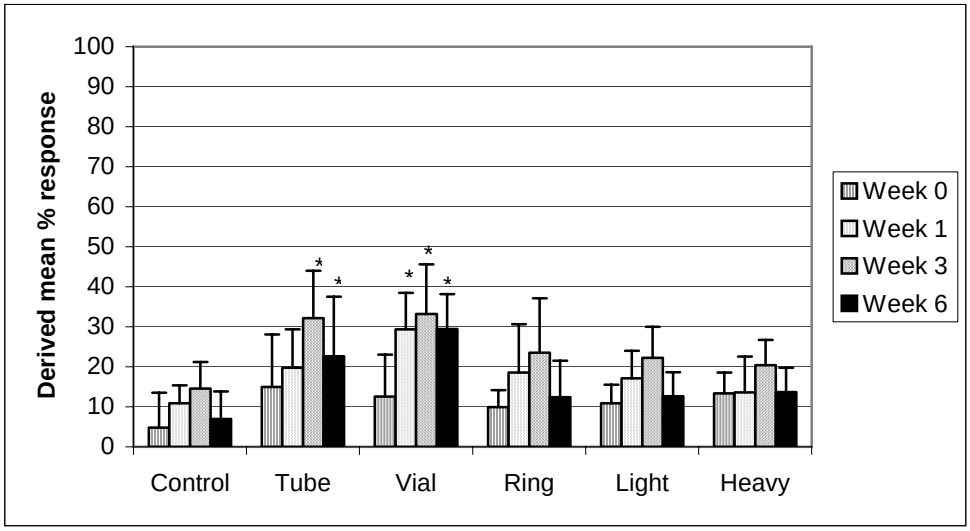
A significant increase in response ($P < 0.05$) compared to the control was found at Week 0 for the light and heavy bag lures, Week 1 for the tube, light and heavy bag lures, at Week 3 for the tube lure only and at Week 6 for the tube, vial and heavy bag lures (Figure 4b). Generally the greatest response to the lures was observed for one-week-old lures.

C. ferrugineus

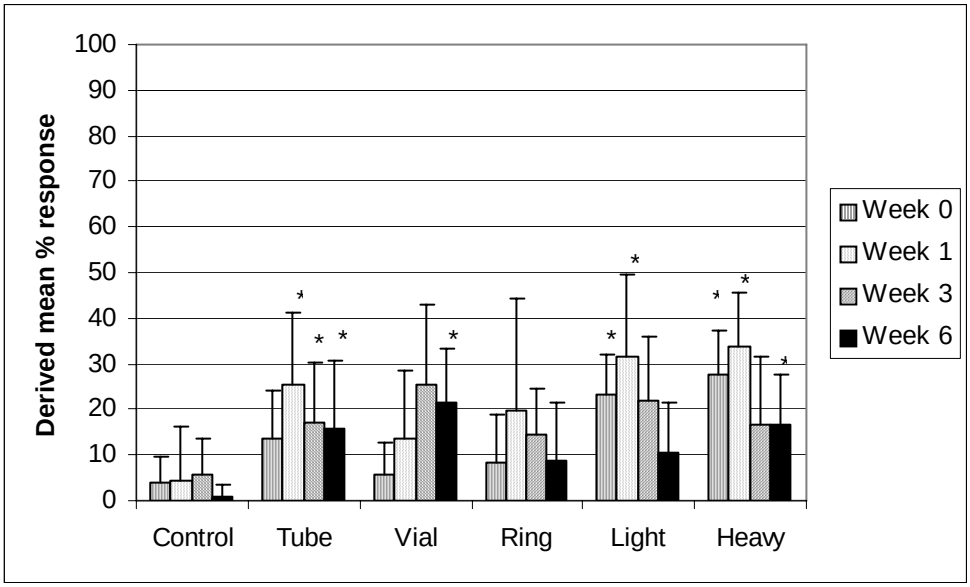
A significant increase in response ($P < 0.05$) compared to the control was found at Week 0 for the tube, vial and heavy bag lures, at Week 1 for all dispenser types excluding the heavy bag lure and at Week 6 for the vial lure. A significant decrease in response compared to the control was found at Week 3 for the light and heavy bag lures (Figure 4c) although the high control response observed at Week 3 is the most likely cause for this observation. .

Figure 4. Response of *O. surinamensis* (a), *S. granarius* (b) and *C. ferrugineus* (c) to volatiles released from different lure dispensers in pitfall bioassays

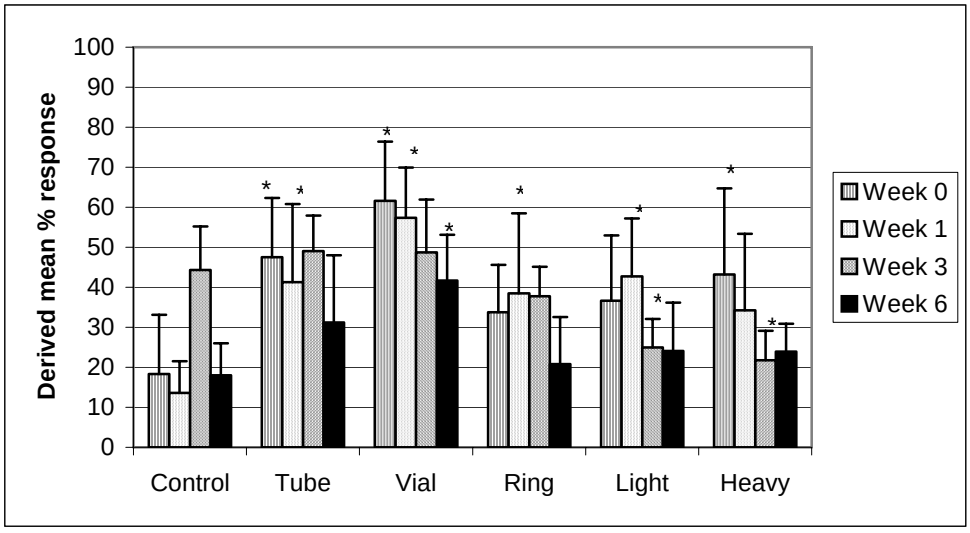
(a) *O. surinamensis*



(b) *S. granarius*



(c) *C. ferrugineus*



Columns marked by * indicate that the response was significantly greater than the response to the control ($P < 0.05$)

Conclusions

The responses by *O. surinamensis* and *S. granarius*, although significant in some cases, were generally low. The response of *C. ferrugineus* was much greater than that for the other two species. From these results, the tube and vial lure dispensers appear to be the best option for all three species. As the polythene vial lure is easier to produce by the industrial partner it was concluded that this should be used in further trials in the CSL Grain Storage Research Unit.

3. Testing of prototype lures in the CSL Grain Storage Research Unit

Introduction

The aim of this step was to test the formulated lure under conditions as close as possible to those under which it will be used. The three species chosen for testing the lure in the grain bulk were *O. surinamensis, S. granarius* and *C. ferrugineus.* Tests of the lure in the PC™ Floor Traps relied on free-roaming populations of insects present in the Storage Research Unit. Separate trials were carried out with three different lure formulations:

1. The type of lure dispenser currently supplied by Russell IPM for use in the PC™ Floor Trap (rubber ring type) A gel matrix was added for this trial to counteract the affect of temperature on volatile release rate. This lure dispenser was also tested without added attractant components to ascertain whether the gel matrix had an effect on trap catch.
2. A vial type lure containing attractants and no gel.
3. A vial type lure as above containing lower amounts of attractants and no gel.

The grain was fumigated with methyl bromide between trials of lure types 1 and 2 (trials 2 and 3) and fresh insects were introduced to the grain bulk.

Materials and Methods

Wheat

The grain bulk was made up of four loads of feed wheat with a total weight of 116 tonnes. A 2kg sample from each load was sieved and no insects were found. The average moisture content of the four loads (determined by the oven method ISO 712) was 13.1%. The grain bulk was 9.8 m wide and 9.7 m long, with a plateau extending 2.5 m from the back wall at a depth of 2.3 m, and the slope extending from this area to the front.

Insects

Adult insects of the following species and strains were used: *O. surinamensis* (Lab. Susc.), *S. granarius* (Windsor) and *C. ferrugineus* C124 and reared as previously described. Mixed age adults of each species were used. The culture population density for all three species was approximately 10,000 insects per tank (26 x 22 x 37 cm or 27 x 30 x 41 cm).

Batching, acclimation and introduction of insects into grain bulk

Initial populations of one adult insect per 1 kg of grain of each of *O. surinamensis* and *S. granarius* and one adult insect per 2 kg of grain of *C. ferrugineus* were established as follows: *O. surinamensis* and *S. granarius* were measured, by volume, into batches of 710 insects for the plateau of the bulk, 734 insects for the slope of the bulk and 694 for the bins. *C. ferrugineus* were measured, by volume, into batches of 355 insects for the plateau of the bulk and 367 insects for the slope of the bulk. The three species were mixed and held in 115 x 150 ml round deli pots containing 50g kibbled wheat. The insects were acclimated to test conditions in a CE room (15ºC, 60% rh) for four days. The grain under the plateau of the bulk constituted 36.7% of the bulk and the grain under the slope the remaining 63.3%. This was taken into account by introducing more insects at the insertion points on the slope than on the plateau. Therefore, two batches of insects were introduced at each insertion point on the slope of the bulk. They were inserted via a plastic tube pushed into the grain from which the grain had been removed by suction. The insects were introduced at four depths at each insertion point on the plateau (60 insertion points): 0.5m, 1m, 1.5m and 2m. On the slope, insects were inserted at 0.5m, 1m and 1.5 m in rows 1 and 2, 0.5m and 1m in row 3, and 0.5m only in rows 4 and 5 (50 insertion points). Insects were inserted into the grain in the bins at nine points and at the same four depths as for the plateau of the grain bulk (36 insertion points per bin). The insects were left to disperse through the grain for one week before the start of the trial.

Traps

Within each trial the same lure dispenser was used in PC™ Trap (Cogan and Pinniger, 1989) and in PC™ Floor Traps (Collins and Chambers, 2003; Collins et al., 2003). The PC™ Pitfall Traps used were as supplied for sale by Igrox Limited, White Hall, Worlingworth, Woodbridge, Suffolk, IP13 7HW, UK. The lures were attached to the inside of the PC™ Pitfall Trap lids with paper clips. The PC™ Floor Traps used

were also as supplied by Igrox Ltd except that the lure used was the experimental lure rather than the supplied lure.

Lures

1. Type 1. The type of lure dispenser currently supplied by Russell IPM for use in the PC™ Floor Trap: rubber ring type with a cellulose acetate filler, a preservative (butylatedhydroxytoluene (BHT)) and a gel matrix (formulation commercial in confidence) to counteract the affect of temperature on volatile release rate (Figure 5).
2. Type 2. A polyethylene vial dispenser containing cellulose acetate filler, attractants and no gel or BHT (Figure 6).

The amount of volatile components added to each type of lure is shown in Table 12.

Figure 5. Type 1 lure currently supplied by Russell IPM for use in the PC™ Floor Trap

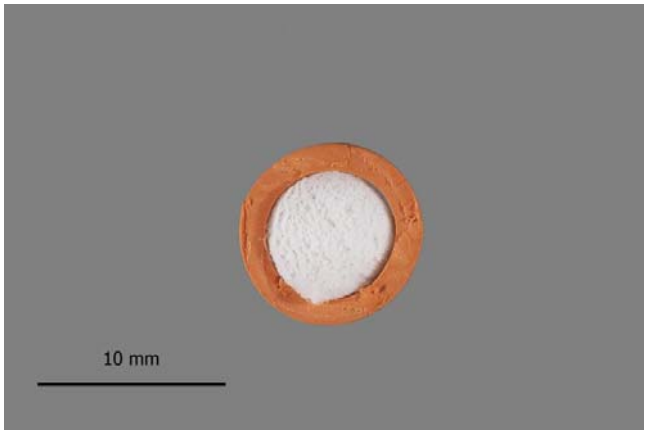


Figure 6. A vial lure containing cellulose acetate filler

Table 12. Amount of volatile components added to each type of lure tested (mg)

	Trial 1		Trial 3		Trial 4	
Compound	**PC**	**Floor**	**PC**	**Floor**	**PC**	**Floor**
hexanoic acid	9.27	9.27	1	10	0.1	1
3-methylbutanol	8.09	8.09	1	10	0.1	1
4-ethylacetophenone	9.93	9.93	0.25	1	0.01	0.10
3-octen-2-one	8.57	8.57	1	10	0.1	1
nonanal	8.27	8.27	1	10	0.1	1
E-2-nonenal	8.46	8.46	1	10	0.1	1
Total	**52.59**	**52.59**	**5.25**	**51**	**0.51**	**5.1**

Insect monitoring

In the grain bulk twenty pairs of PC™ Pitfall Traps (surface and buried at a depth of 5 cm) were placed in a 2 m grid on the plateau and on the slope (Figure 7). Alternate pairs of traps contained lures. For surface traps with attractants, surface traps without attractants, buried traps with attractants, buried traps without attractants N = 10.

Figure 7. Positions of insect insertion, trapping and environmental monitoring points in grain bulk

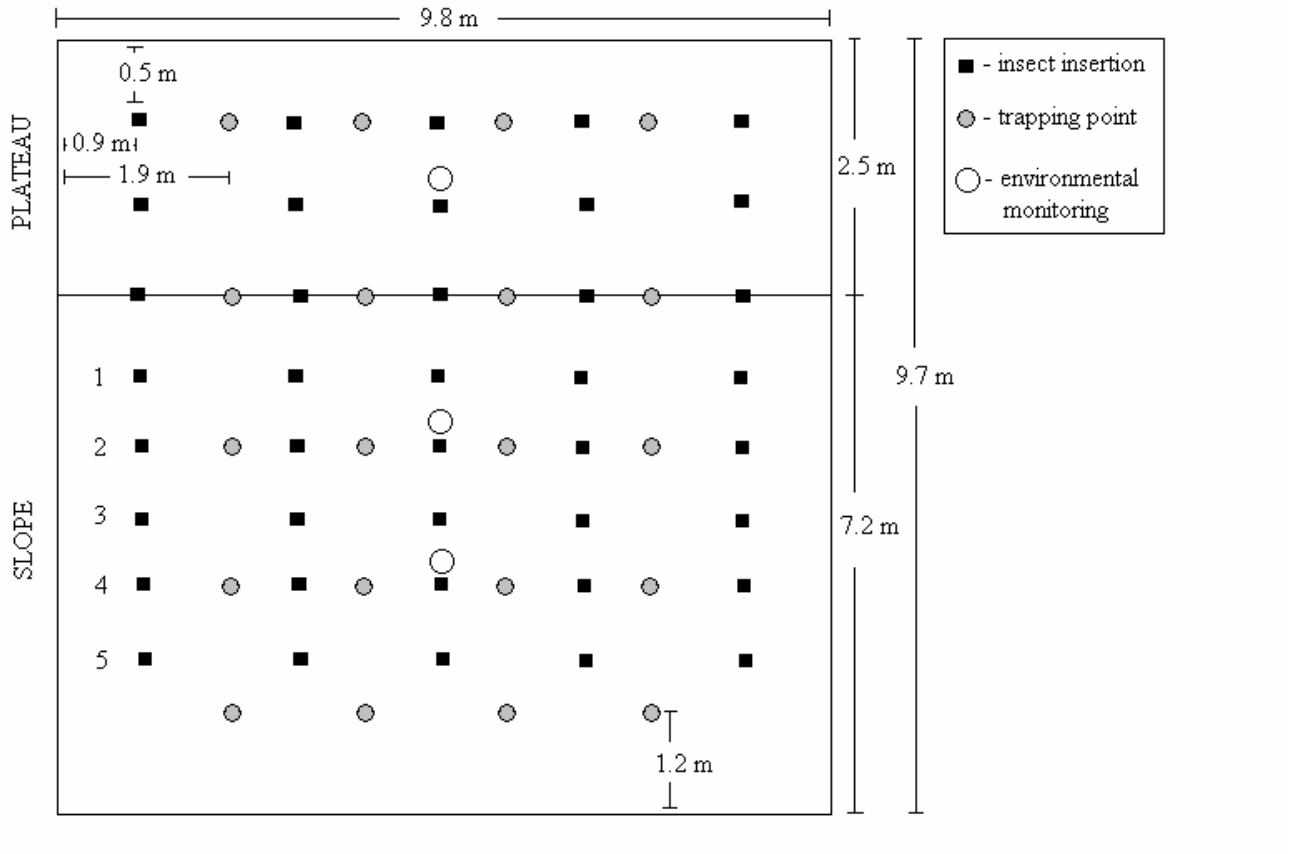
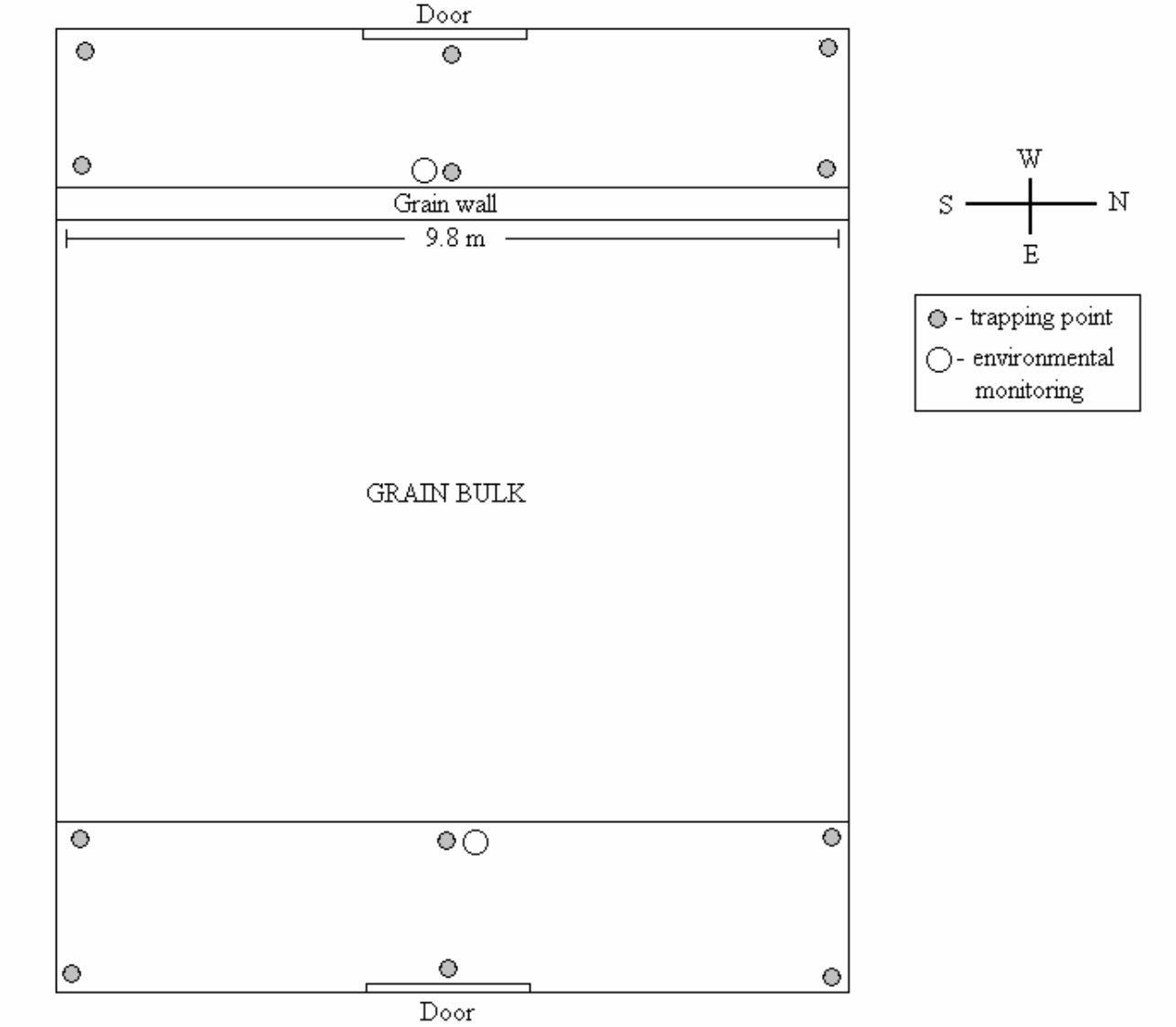


Figure 8. Positions of PC ™ Floor Traps and environmental monitoring points around the grain bulk



The PC™ Floor Traps were placed at 4.9 m intervals along the front edge of the grain bulk, along the back edge of the grain walling, along the back wall and along the front wall in the area containing the grain bulk (Figure 8). Twelve traps were used in total. Alternate traps contained lures. For floor traps with attractants and floor traps without attractants N = 6.

The PC™ Pitfall Traps and PC™ Floor Traps were checked weekly for at least six weeks and their contents identified, counted and disposed of. The same lures were used over the entire monitoring period for each trial.

The same arrangement of traps was used in each area for each trial except that traps containing vial lures without the attractants were used as the controls rather than empty traps in trial 3.

Environmental monitoring

The temperature in the grain bulk was recorded at depths of 10 cm and 1 m at three positions using Tinytalk miniature temperature data loggers (Product TK-0023 Gemini Data Loggers (UK) Ltd., Chichester, U.K.). Samples of grain were taken at weekly intervals from adjacent points, at a depth of 5 cm, for moisture content analysis. The temperature in each bin was recorded at the surface of the grain and at depths of 10 cm and 1 m at a point on the circumference of the circle on which the traps were placed using a combination of thermocouples and Squirrel data loggers. A sensor array with single thermistor sensors at the surface and 1m depths was also used (Cook and Watts, 2003). An ambient recording was made using a sensor 1 m above the top of one of the bins. Samples of grain were taken at weekly intervals from adjacent points, at a depth of 5 cm, for moisture content analysis. The temperature and rh on the floor of the Storage Research Unit were recorded, using Tinytags, (Tinytag Plus TGP-1500. Gemini Data Loggers (UK) Ltd., Chichester, U.K.), at two positions: one behind the grain walling and one in front of the grain bulk.

Trials

1. Type 1 lure with BHT, gel and attractive components in PC™ Pitfall Traps in the floor store and in PC™ Floor Trap. The insects were introduced into the grain bins on 9th August 2004 and into the grain in the floor store on 10th August 2004. The traps were placed in the floor store and on the floor on 16th August 2004. The contents of the traps were identified and counted weekly for seven weeks from 24th August 2004 to 4th October 2004 for the PC™ Pitfall Traps and for six weeks from 24th August 2004 to 28th September 2004 for the PC™ Floor Traps.
2. Type 1 lure with gel only in PC™ Pitfall Traps in the floor store. Insect introduction was as above. The traps were placed in the grain in the floor store on 12th November 2004 and their contents identified and counted after one week on 19th November 2004. The positions of the traps with lures and the empty control traps were exchanged and traps placed in the grain in the floor store on 23rd November 2004. Their contents were identified and counted after one week on 30th November 2004.
3. Type 2 vial lure containing cellulose acetate filler, attractants and no gel in PC™ Pitfall Traps in floor store and in PC™ Floor Traps on the floor. The insects were introduced into the grain in the floor store on 27th May 2005. The traps were placed in the floor store and on the floor on 3rd June 2005. The contents of the traps were identified and counted weekly for six weeks from 10th June 2005 to 15th July 2005. During this trial, the volatiles within the surface traps were sampled (see method below).
4. Type 2 vial lure containing cellulose acetate filler, lower amounts of attractants and no gel in PC™ Pitfall Traps in floor store and in PC™ Floor Traps on the floor. Insect introduction was as above. The traps were placed in the floor store and on the floor on 27th July 2005. The contents of the traps were identified and counted weekly for six weeks from 3rd August 2005 to 7th September 2005.

Sampling of headspace inside PC™ Pitfall Traps containing Russell vial lures by Static Headspace Solid-Phase Microextraction (SHS-SPME) during Trials 1, 3, and 4

The headspace inside surface PC™ traps was sampled using Supelco 75 µm Carboxen/Polydimethylsiloxane PDMS portable SPME fibres. The SPME fibres were conditioned in gas chromatograph injection ports at 270-280ºC, using helium as a carrier gas, for 1 h prior to use. The SPME needles were pushed through one of the inner ring of holes in the PC™ trap lids, and the fibres exposed by depressing SPME plungers.

In trial 1, the SPME needles were pushed as far into the traps as possible (61 mm, SPME on bottom notch). In trials 3 and 4 SPME needles were held at 42 mm (SPME on middle notch). The maximum depth of a PC™ trap is 130 mm (as measured from the apex of the trap). The headspace sampled for 60 minutes.

In trial 1 a single PC™ trap containing a lure was sampled 7, 16, 23, and 28 days after traps were placed into the grain. The headspace outside the trap was also sampled at a distance of 50 mm from the outside edge of the trap, just above the grain surface (2 mm) for 1 h. A background headspace sample was obtained by sampling the grain with no traps present, 2 mm above the grain, for 1 h.

In trials 3 and 4 the headspace inside traps was sampled for 1 h at 6, 13, 20, 27, 34 and 41 days after traps were placed in the grain bulk. The grain surface temperature near the traps was also measured at the time of sampling because this would affect the release rate of volatiles from the lure. Three different surface PC™ traps containing an attractant lure, and one empty PC™ trap were sampled each week.

In trial 4, in addition to sampling of surface traps, buried traps were also sampled. Three buried traps containing lures, and one control (empty) trap were sampled each week for 6 weeks. For sampling of the buried traps the SPME samplers were pushed through the grain (approx. 20 mm) until the samplers pushed up against the tops of the traps, near to the central part of the lids. The exact positions of the buried traps were determined by pushing a pen, or fingers through the grain. The outer needles of the SPME samplers were pushed through holes near to the centre of the lids, locked in place on the middle notch, and the plungers then depressed to 50% of maximum, so that the fibres were at a depth of 30 mm inside trap. The plungers were not fully depressed to prevent damage to the SPME fibre caused by the fibres touching the insides of the PC™ traps, which narrow towards the base. The SPME samplers were held in position using clamps and retort stands. The sampling time was 1 h.

Sampling of headspace inside PC™ Floor Traps containing Russell vial lures by Static-Headspace Solid-Phase Microextraction (SHS-SPME) during Trials 3 and 4

Floor traps (positions 1, 2, 4, and 6) were sampled for 1 h each week for 6 weeks in trials 3 and 4. The SPME outer needles were pushed approx. 1 mm through one of the inner ring of holes in the floor trap lids and the plungers then depressed to 50% of maximum exposing the fibres, so that the fibres were at a depth of 12 mm

inside floor traps. The SPME samplers were clamped to keep them in position during the sampling. Three of the four floor traps sampled contained lures, and one was a control (empty) floor trap (position 1).

Storage and analysis of SPME fibres

The SPME samplers were stored in a fridge at 4ºC until GC-MS analysis. Volatiles from the SPME fibres were analysed on a Hewlett Packard 5890 series II gas chromatograph coupled to a VG Trio-1 mass spectrometer. The volatiles were desorbed at 270ºC for 1 min, in splitless injection mode (purge on after 1.0 min), through a 0.75 mm i.d. inlet liner onto a Chrompack CP-Sil 5CB column (50 m x 0.32 mm internal diameter, 1.2 µm film thickness).

In trial 1, the GC oven temperature was initially held at 40ºC for 1 min rising at 6ºC/min to 180ºC, then at 10ºC/min to 280ºC and then held at this temperature for 15 min. For trials 3, and 4 the GC oven temperature was initially 40ºC for 1 min rising at 10ºC/min to 280ºC and then held at this temperature for 15 min. Helium was used as the carrier gas, and the GC operated in constant flow mode (3.5 psi, at 40ºC GC oven temperature). The mass spectrometer source and interface temperatures were 200ºC and 275ºC respectively. The mass spectrometer was operated in Electron Impact mode (EI+) at 70 eV, and scanned from 10-350 amu once every second.

Statistical methods

Two comparisons were made between traps with and without lures: the numbers of insects caught and the numbers of traps that caught insects (positive traps). Analyses of the numbers of insects caught were performed with GenStat 8. The method was GEE (Generalized Estimating Equations) for repeated observations over multiple weeks. This assumed a poisson distribution with a logarithm link. There was an unresolved question about the number of degrees of freedom (df) with GEE when the distribution is poisson therefore the residual df were used to calculate the probability from the t value rather than infinity df. Thus the interpretation is conservative i.e. a slightly bigger difference may be required for it to be statistically significant. The positive trap data were also analysed by GEE but with a binomial rather than a poisson distribution.

For all analyses, all weeks were considered together then weeks 1 - 3 and weeks 4 -7 (trial 1) or weeks 4 - 6 (trial 3). There were too few samples to consider each week separately. The number of traps was small so only large differences were likely to be detected. It was not possible to analyse data from PC™ Floor Traps from Trial 1 because too few insects were caught in the traps.

Results

TRIAL 1

Environmental monitoring

For the majority of the trial, the temperatures recorded in the grain bulk were between 15°C and 20°C. The temperatures recorded at a depth of 10 cm were more variable, following the ambient air temperature more closely, than those recorded at a depth of 1 m. In the grain bulk, the temperature at 10 cm deep fell below 15°C from week four onward, whereas the temperature at 1 m deep remained stable. The moisture contents recorded in the grain bulk were between 14.5% and 15.5% for the majority of the trial and followed ambient relative humidity.

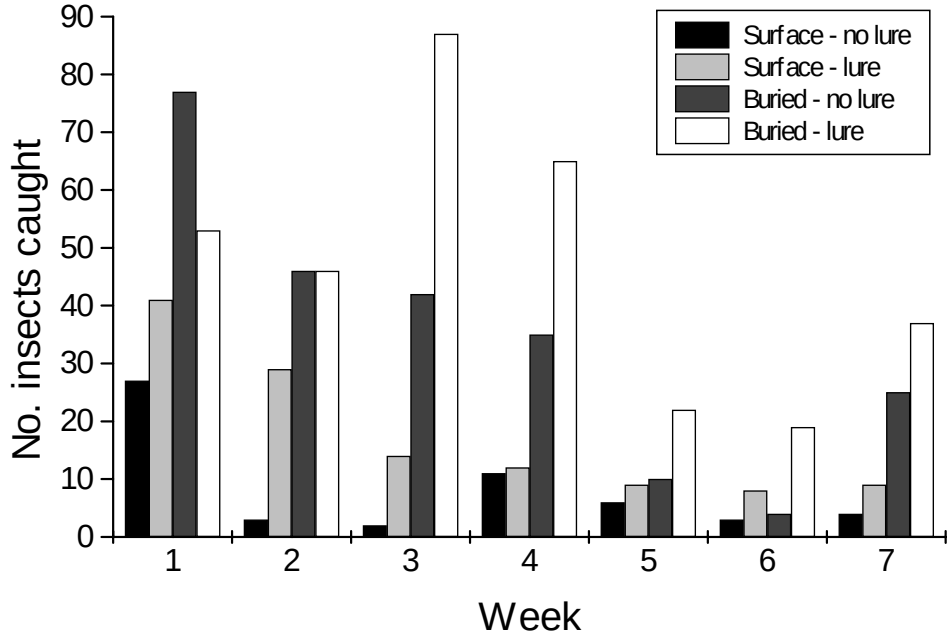
The temperatures recorded on the floor of the Storage Research Unit were between 10°C and 20°C for most of the trial. They decreased during the trial and dipped below 10°C for a short period during the sixth week. The relative humidity recorded on the floor varied between 60 and 100% dipping to 55% during week four of the trial.

Insect monitoring

O. surinamensis - grain bulk

Consistently greater numbers of *O. surinamensis* were caught in buried PC™ Pitfall Traps than in surface traps (Figure 9). The numbers of *O. surinamensis* caught in surface traps decreased over the duration of the trial. This decrease was not evident with buried traps until week five when the temperature dropped below 10°C. More insects were caught in surface traps with lures than without lures ($P = 0.0104$) when data from all weeks were analysed together. There were no significant differences when data from weeks 1 – 3 or 4 – 7 were analysed together, although more insects were caught in traps with lures than without lures during each week from week 3 to the end of the trial. There were no differences in the numbers of insects caught in buried traps. There were no differences in the numbers of positive traps with and without lures in either surface or buried traps (Table 13a and b). Any difference may not have been evident due to the high overall number of insects captured; almost every trap contained *O. surinamensis.*

Figure 9. Total numbers of *Oryzaephilus surinamensis* caught in surface and buried traps in the grain bulk during trial 1



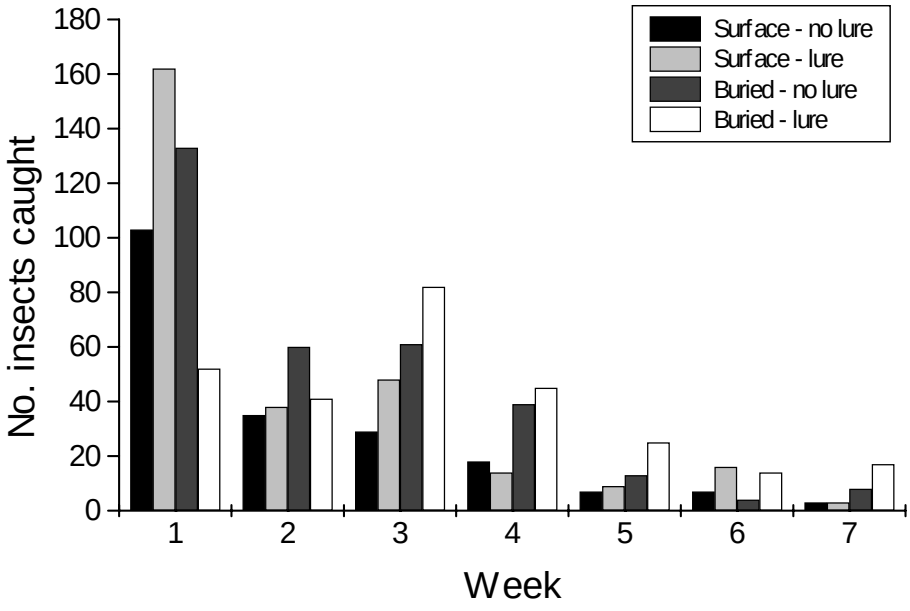
O. surinamensis - PC™ Floor Traps

Over the duration of the trial, there were 11 positive traps with lures and 7 without lures. A total of 16 *O. surinamensis* were caught in traps with lures and 9 in traps without lures.

C. ferrugineus - grain bulk

The numbers of *C. ferrugineus* caught in surface and buried PC™ Pitfall Traps were similar (Figure 10) and in both cases decreased over the duration of the trial. More insects were caught in surface traps with lures than without lures when data from all weeks were analysed together ($P = 0.0112$). There were no differences when weeks 1 –3 or 4 – 7 were analysed together. More insects were caught in buried traps with lures than without lures when data from all weeks were analysed together and for weeks 1 - 3 ($P < 0.0001$ in both cases). There was no difference when weeks 4 – 7 were analysed together. There were no differences in the numbers of positive traps with and without lures in either surface or buried traps (Table 13a and b).

Figure 10. Total numbers of *Cryptolestes ferrugineus* caught in surface and buried traps in the grain bulk in trial 1



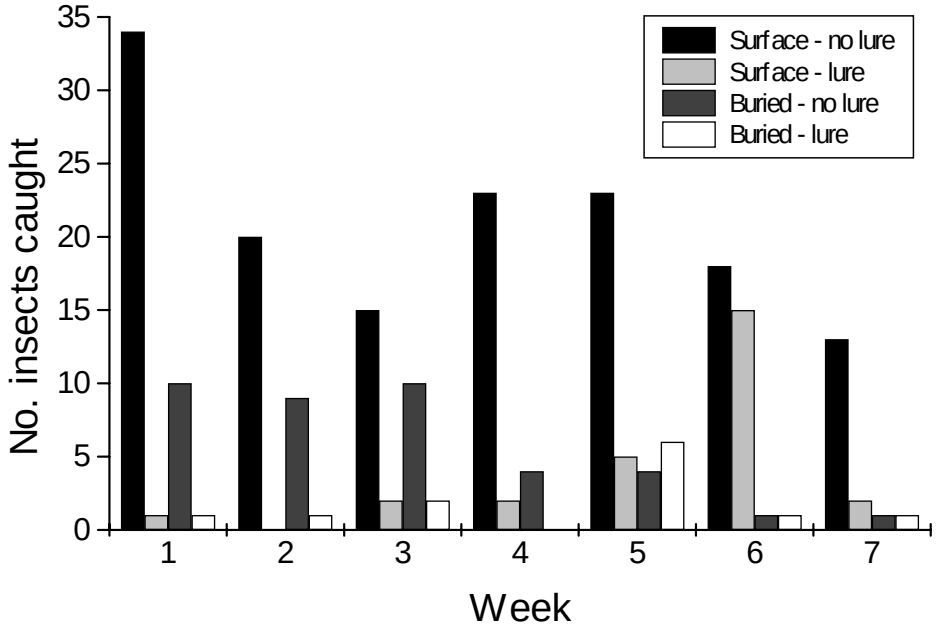
C. ferrugineus - PC™ Floor Traps

Over the duration of the trial, there were 3 positive traps with lures and none without lures. A total of 6 *C. ferrugineus* were caught in traps with lures and none in traps without lures.

S. granarius - grain bulk

Consistently greater numbers of *S. granarius* were caught in surface PC™ Pitfall Traps than in buried traps (Figure 11). More insects were caught in surface traps without lures than with lures when data from all weeks were analysed together and for weeks 1 – 3 ($P < 0.0001$ and $P = 0.0092$, respectively). There was no difference during weeks 4 – 7. More insects were also caught in buried traps without lures than with lures when data from all weeks were analysed together ($P = 0.0003$) but there was no difference when weeks 1 - 3 or 4 – 7 were analysed together. More surface traps without lures than with lures were positive for *S. granarius* when data from all weeks were analysed together and for weeks 1 –3 ($P = 0.0001$ and $P = 0.0183$, respectively). There was no significant difference for weeks 4 – 7 although more insects were caught in traps without lures. More buried traps without lures were positive for *S. granarius* ($P < 0.0006$) but there were no differences when weeks 1 – 3 or 4 – 7 were analysed together (Table 13a and b).

Figure 11. Total numbers of *Sitophilus granarius* caught in surface and buried traps in the grain bulk during trial 1



<u>*S. granarius* - PC™ Floor Traps</u>

Over the duration of the trial, there were 3 positive traps without lures and 1 with a lure. A total of 3 *S. granarius* were caught in traps without lures and 1 in a trap with a lure.

Table 13a. Trial 1. Numbers of surface traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk

	Week	1	2	3	4	5	6	7	1 to 3	4 to 7	1 to 7
O. surinamensis	Lure	9	8	6	6	6	6	7	23	19	48
	No lure	9	2	2	5	4	3	4	13	11	29
S. granarius	Lure	1 a	0	2	2	3	6	2	3 a	11	16 a
	No lure	8	8	6	8	10	7	6	22	23	53
C. ferrugineus	Lure	9	10	9	6	5	8	3	28	16	50
	No lure	10	9	7	6	5	4	3	26	12	44

a = significantly different from traps without lures

Table 13b. **Trial 1. Numbers of buried traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk**

	Week	1	2	3	4	5	6	7	1 to 3	4 to 7	1 to 7
O. surinamensis	Lure	10	9	10	9	7	7	8	29	22	60
	No lure	10	9	10	9	4	4	8	29	16	54
S. granarius	Lure	1 a	1	2	0	3	1	1	4	5	9 a
	No lure	7	4	6	4	1	1	0	17	2	23
C. ferrugineus	Lure	9	10	10	8	8	8	8	29	24	61
	No lure	10	10	10	8	6	4	6	30	16	54

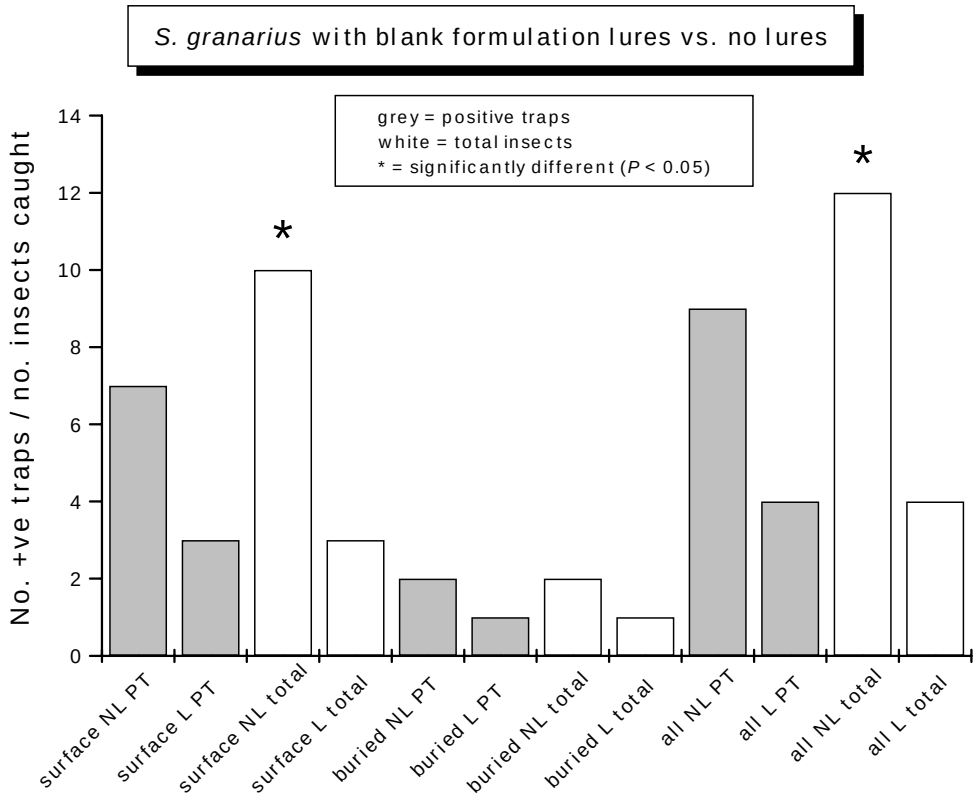
a = significantly different from traps without lures

TRIAL 2

<u>*S. granarius* with type 1 lures with gel only in PC™ Pitfall Traps in the floor store</u>

Greater numbers of *S. granarius* were caught in traps without lures than with lures. More traps were positive for *S. granarius* without lures than with lures (Figure 12). This was also true when the positions of the traps with lures and empty traps were exchanged.

Figure 12. Trial 2 *S. granarius* catches from PC™ Pitfall Traps with the lure with gel and no attractants



NL = no lure, L = lure, PT = positive traps

TRIAL 3

Environmental monitoring

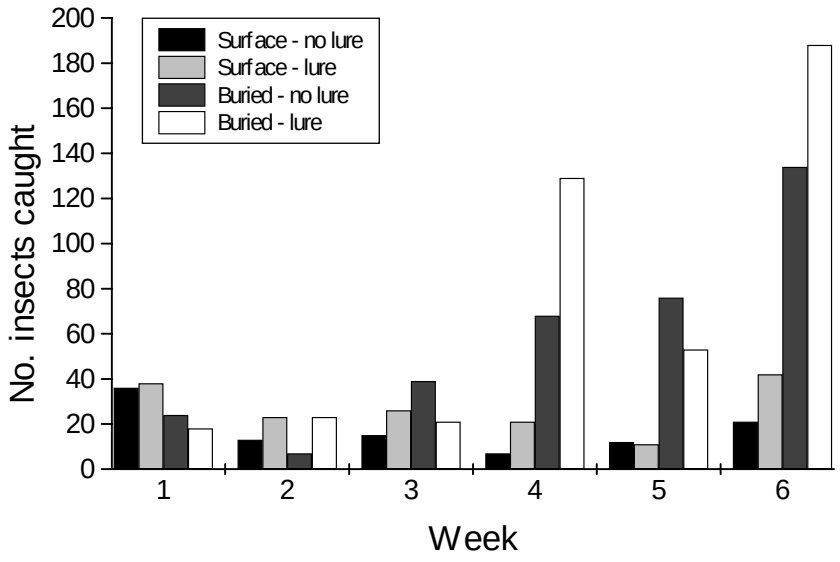
The temperatures recorded in the grain bulk were between 9°C and 22°C. The temperatures recorded at a depth of 10 cm were more variable, following the ambient air temperature more closely, than those recorded at a depth of 1 m. The temperatures at both depths increased over the duration of the trial. At a depth of 1 m this increase was approximately 4°C and at 10cm it was approximately 6°C. The moisture contents recorded in the grain bulk were between 14 % and 16 % and generally decreased during the trial. The temperatures recorded on the floor of the Storage Research Unit were between 10°C and 20°C for most of the trial. They increased by approximately 4°C during the trial, only dipping below 10°C for a short period during the first two weeks. The relative humidity recorded on the floor varied between 55 and 100%.

Insect monitoring

O. surinamensis - grain bulk

Greater numbers of *O. surinamensis* were caught in buried PC™ Pitfall Traps than in surface traps during weeks 4 - 6 (Figure 13). The numbers of *O. surinamensis* caught in buried traps increased over the duration of the trial. This increase was not evident in surface traps. There were no significant differences in the numbers of insects caught in either surface of buried traps during the trial. There were no differences in the numbers of positive traps with and without lures in either surface or buried traps (Table 14a and b). Any difference may not have been evident due to the high overall number of positive traps.

Figure 13. Total numbers of *Oryzaephilus surinamensis* caught in surface and buried traps in the grain bulk during trial 3



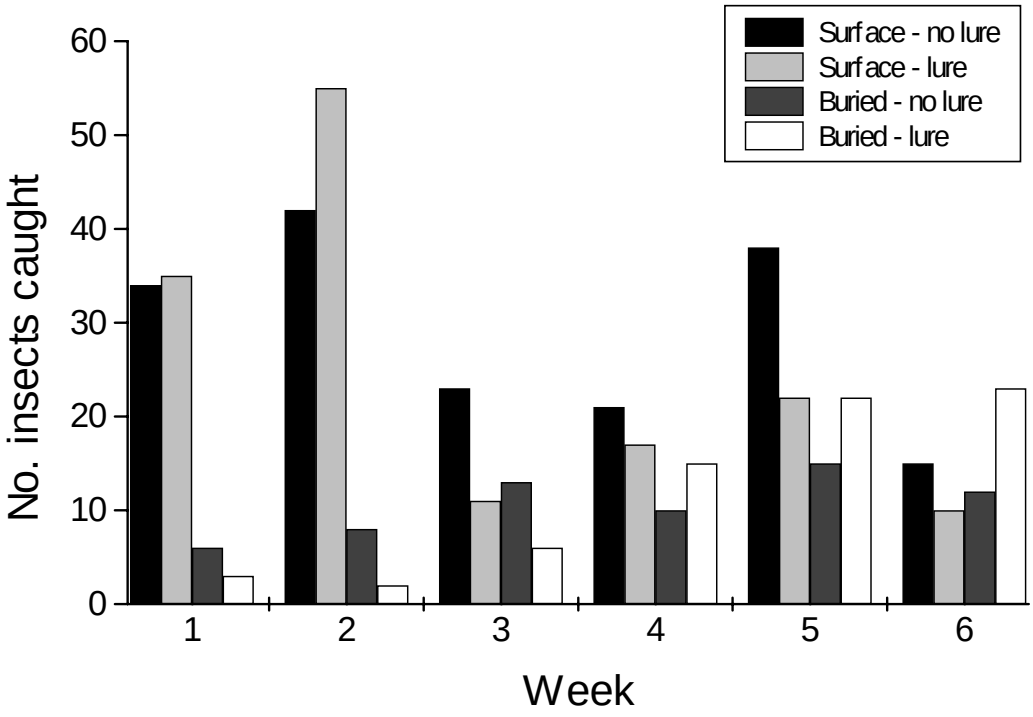
O. surinamensis - PC™ Floor Traps

More *O. surinamensis* were caught in traps with lures than without lures when all of the data were analysed together and during weeks 1 – 3 ($P < 0.0001$ and $P = 0.0006$, respectively). There was no difference when weeks 4 – 6 were analysed together. More traps with lures were positive for *O. surinamensis* when data from weeks 1 – 6 were analysed together ($P = 0.0351$) (Table 14c). A total of 360 *O. surinamensis* were caught in traps with lures and 68 in traps without lures.

S. granarius - grain bulk

Greater numbers of *S. granarius* were caught in surface PC™ Pitfall Traps than in buried traps (Figure 14). The numbers of *S. granarius* caught in buried traps increased throughout the trial. This increase was not evident in surface traps. There were no significant differences in the numbers of insects caught in either surface of buried traps during the trial. There were no differences in the numbers of positive traps with and without lures in either surface or buried traps (Table 14a and b).

Figure 14. Total numbers of *Sitophilus granarius* caught in surface and buried traps in the grain bulk during trial 3



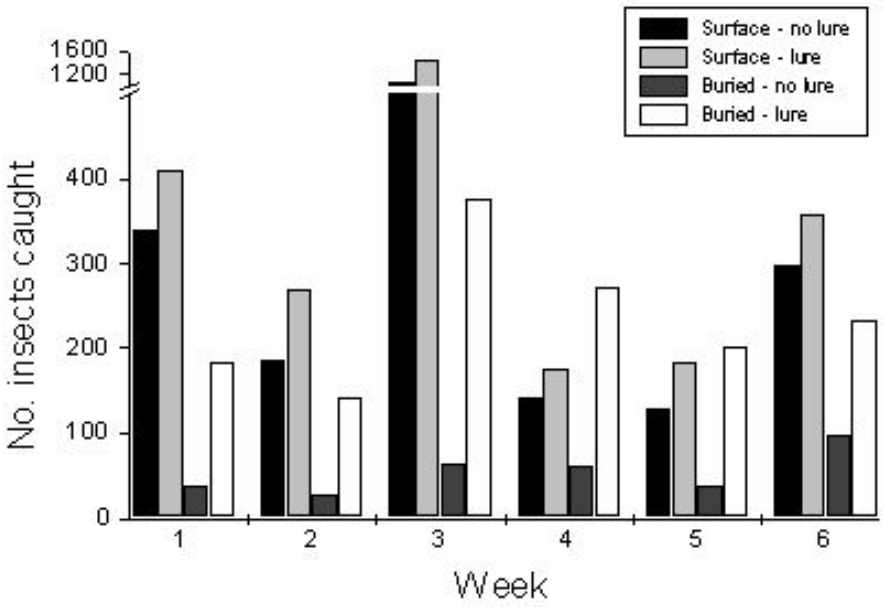
S. granarius - PC™ Floor Traps

There were no significant differences in either the numbers of traps that were positive for S. granarius or the total number of *S. granarius* caught in floor traps with and without lures (Table 14c). The total number of insects caught in traps with lures was 307 and 117 were caught in traps without lures. Insects were caught in only half of the traps with lures and half of the traps without lures. Discounting the other traps, variation was too high and replication too low to detect any differences.

C. ferrugineus - grain bulk

Very large numbers of *C. ferrugineus* were caught in both the surface and buried PC™ Pitfall Traps throughout the trial. Greater numbers of *C. ferrugineus* were caught in surface traps than buried traps during the first three weeks but there was little difference during the last three weeks (Figure 15). More insects were caught in surface traps with lures than without lures when data from all weeks were analysed together ($P < 0.0001$). There were no differences when weeks 1 –3 or 4 – 7 were analysed together. More insects were caught in buried traps with lures than without lures when data from all weeks were analysed together, for weeks 1 – 3 and for weeks 4 - 6 ($P < 0.0001$ in each case). There were no differences in the numbers of positive traps with and without lures in either surface or buried traps. Any difference may not have been evident due to the high overall number of positive traps; all of the surface traps and 236 of a possible 240 buried traps were positive for *C. ferrugineus* (Table 14a and b).

Figure 15. Total numbers of *Cryptolestses ferrugineus* caught in surface and buried traps in the grain bulk during trial 3



C. ferrugineus - PC™ Floor Traps

Few *C. ferrugineus* were caught in floor traps. There were no differences in either the number of traps which were positive for *C. ferrugineus* (Table 14c) or the numbers of insects caught. The total number of insects caught in traps with lures was 11 and 10 were caught in traps without lures.

Table 14a. Trial 3. Numbers of surface traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	10	9	8	9	7	9	27	25	52
	No lure	10	6	5	7	7	5	21	19	40
S. granarius	Lure	9	9	5	6	10	6	23	22	45
	No lure	9	10	7	9	10	6	26	25	51
C. ferrugineus	Lure	10	10	10	10	10	10	30	30	60
	No lure	10	10	10	10	10	10	30	30	60

a = significantly different from traps without lures

Table 14b. Trial 3. Numbers of buried traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	8	8	7	10	10	10	23	30	53
	No lure	9	6	7	9	10	10	22	29	51
S. granarius	Lure	2	2	4	6	8	8	8	22	30
	No lure	5	4	7	5	7	7	16	19	35
C. ferrugineus	Lure	10	10	10	10	10	10	30	30	60
	No lure	10	9	10	10	9	8	29	27	56

a = significantly different from traps without lures

Table 14c. Trial 3. Numbers of PC™ Floor Traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus*

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	6	6	6	2	3	4	18	9	27 a
	No lure	4	3	4	2	4	3	11	9	20
S. granarius	Lure	3	3	3	3	3	3	9	9	18
	No lure	3	3	2	3	2	3	8	8	16
C. ferrugineus	Lure	1	1	2	1	1	2	4	4	8
	No lure	0	0	3	1	2	0	3	3	6

a = significantly different from traps without lures

TRIAL 4

Environmental monitoring

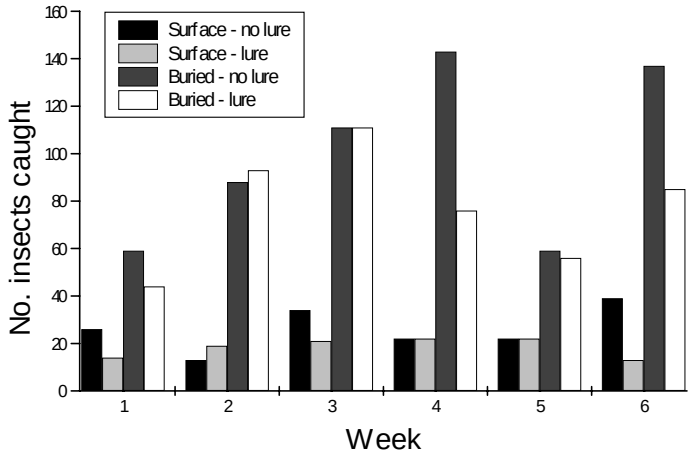
The temperatures recorded in the grain bulk were between 13°C and 20°C. Temperatures in the grain bulk were recorded for the first four weeks of the six week trial because the data loggers were initially set-up only for one six week trial (Trial 3) and ran out of capacity at the end of week four of Trial 4. The temperatures at both depths increased by approximately 1°C over the first four weeks of the trial. The moisture contents recorded in the grain bulk were between 14 % and 15.5 % and generally increased by approximately 0.5% during the trial. The temperatures recorded on the floor of the Storage Research Unit were stable between 10°C and 17°C for most of the trial. The relative humidity recorded on the floor varied between 60 and 100%.

Insect monitoring

O. surinamensis - grain bulk

Greater numbers of *O. surinamensis* were caught in buried PC™ Pitfall Traps than in surface traps throughout the trial (Figures 16). The numbers of *O. surinamensis* caught in buried traps increased over the first four weeks of the trial. This increase was not evident in surface traps. Greater numbers of *O. surinamensis* were caught in surface traps without lures than with lures during weeks one, three and six. More *O. surinamensis* were caught in traps with lures during week 2. More *O. surinamensis* were caught in buried traps without lures during the weeks one, four, five and six. Greater numbers of *O. surinamensis* were caught in buried traps with lures than without lures during week two but this was a small difference. A greater number of surface traps without lures were positive for *O. surinamensis* during each week except week five; there were more positive traps with lures during this week. There was little difference between the number of buried traps with and without lures that were positive for *O. surinamensis* (Table 15 a and b).

Figure 16. Total numbers of *Oryzaephilus surinamensis* caught in surface and buried traps in the grain bulk during trial 4



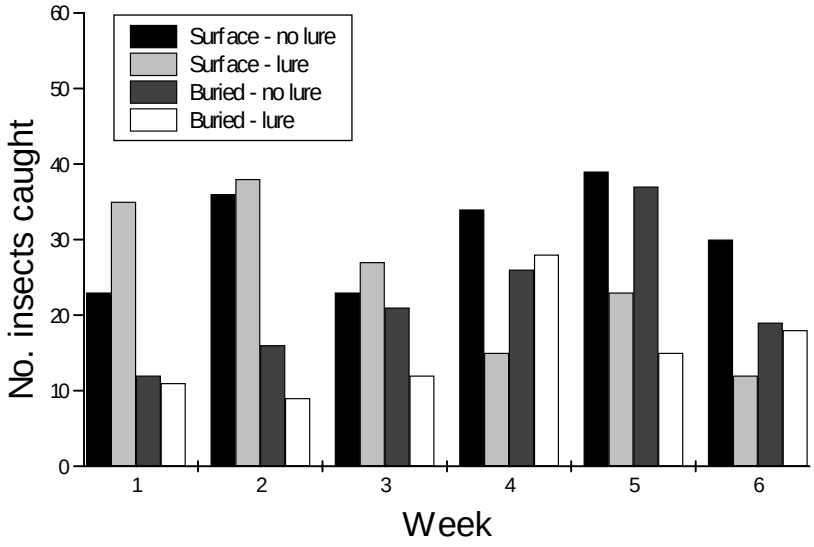
O. surinamensis - PC™ Floor Traps

Greater numbers of *O. surinamensis* were caught in traps with lures than without during each week of the trial except week two (Table 15c). Traps with and without lures were positive for *O. surinamensis* on every week of the trial. The number of positive traps with lures was greater than the number without lures during weeks one, three, four and five. There was no difference during the other weeks.

S. granarius - grain bulk

Greater numbers of *S. granarius* were caught in surface PC™ Pitfall Traps than in buried traps during weeks 1-3 (Figure 17). The numbers of *S. granarius* caught in buried traps increased from weeks one to five and then decreased again during week six. This increase was not evident in surface traps (Table 15 a and b). Greater numbers of *S. granarius* were caught in surface traps with lures during weeks one to three. However, greater numbers of *S. granarius* were caught in surface traps without lures from week four onwards. Greater numbers of *S. granarius* were caught in buried traps without lures during each week of the trial except week four. More *S. granarius* were caught in buried traps with lures during week four. There was little difference in the number of surface traps with and without lures that were positive for *S. granarius* during the trial, although there were slightly more positive traps without lures during weeks four to six. A greater number of buried traps without lures were positive for *S. granarius* during each week of the trial except week four.

Figure 17. Total numbers of *Sitophilus granarius* caught in surface and buried traps in the grain bulk during trial 4



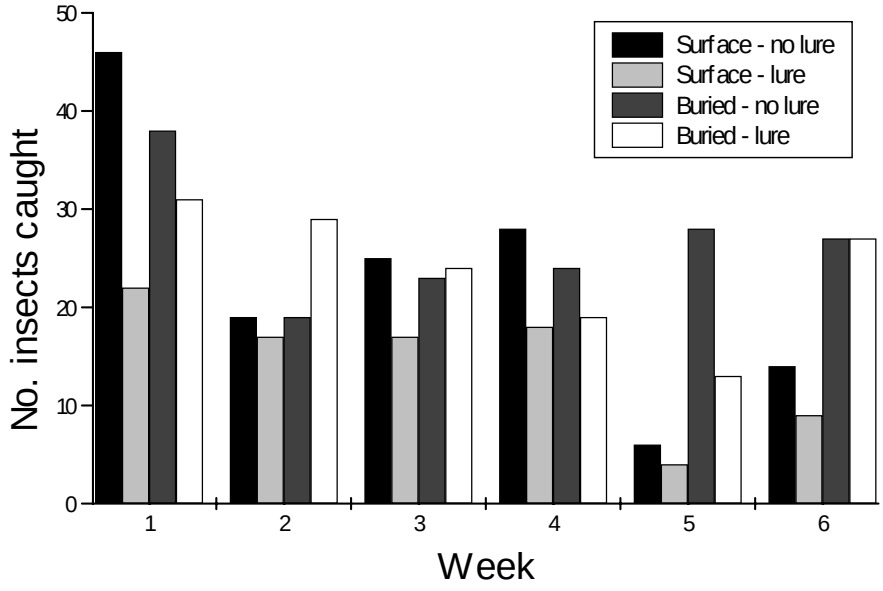
S. granarius - PC™ Floor Traps

More *S. granarius* were caught in traps with lures than without lures during weeks one, three and four (Table 15c). Traps with lures were positive for *S. granarius* for four weeks of the trial. Traps without lures were positive for *S. granarius* for five weeks of the trial.

C. ferrugineus - grain bulk

Greater numbers of *C. ferrugineus* were caught in buried PC™ Pitfall Traps than surface traps during the last two weeks but there was little difference during the first four weeks (Figure 18). Greater numbers of *C. ferrugineus* were caught in surface traps without lures than with lures for each week of the trial. Greater numbers of *C. ferrugineus* were caught in buried traps with lures during weeks two and three. During the trial the number of surface traps without lures that were positive for C. ferrugineus increased over the number of positive surface traps with lures. There was a small but consistent, except for week two, difference in the number of buried traps that were positive for *C. ferrugineus*; there were more insects in traps without lures (Table 15 a and b).

Figure 18. Total numbers of *Cryptolestes ferrugineus* caught in surface and buried traps in the grain bulk during trial 4



C. ferrugineus - PC™ Floor Traps

Very few *C. ferrugineus* were caught in the floor traps. During the trial only two *C. ferrugineus* were caught: one in a trap with a lure and one in a trap without a lure (Table 15c).

Table 15a. Trial 4. Numbers of surface traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	7	6	9	6	10	7	22	23	45
	No lure	8	8	10	8	7	10	26	25	51
S. granarius	Lure	8	10	10	8	9	5	28	22	50
	No lure	8	10	10	9	10	7	28	26	54
C. ferrugineus	Lure	10	8	8	8	4	4	26	16	42
	No lure	10	8	9	9	5	7	27	21	48

Table 15b. Trial 4. Numbers of buried traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus* in the grain bulk

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	9	9	10	10	10	10	28	30	58
	No lure	10	10	10	10	9	10	30	29	59
S. granarius	Lure	8	7	6	8	8	7	21	23	44
	No lure	5	9	8	8	8	6	22	22	44
C. ferrugineus	Lure	9	10	7	8	7	9	26	24	50
	No lure	10	7	9	10	8	10	26	28	54

a = significantly different from traps without lures

Table 15c. Trial 4. Numbers of PC™ Floor Traps positive for *Oryzaephilus surinamensis, Sitophilus granarius* and *Cryptolestes ferrugineus*

	Week	1	2	3	4	5	6	1 to 3	4 to 6	1 to 6
O. surinamensis	Lure	4	3	3	5	4	3	10	12	22
	No lure	3	3	2	4	3	3	8	10	18
S. granarius	Lure	2	2	1	2	0	0	5	2	7
	No lure	1	2	0	1	1	2	3	4	7
C. ferrugineus	Lure	0	0	0	0	0	1	0	1	1
	No lure	0	0	1	0	0	0	1	0	1

Sampling of volatiles in headspace within PC™ Pitfall Traps containing lures by HS-SPME

Very large amounts of volatiles are initially given off by the type 1 (rubber ring) lure and these amounts declined very rapidly with time (Table 16). Therefore, it was not possible to measure the effect over distance for the six week period. The volatiles were not detectable 1.25 m from the trap but were detectable 5 cm from the trap after the first week. Acetic acid, 3-methylbutanol and nonanal were detected from the grain and from the lure. After the second week, the amounts of these compounds detected from grain were greater than detected inside the traps.

The volatiles detected from inside surface traps in trial 3 are shown in Table 17. There was a general pattern of slow decline in release rate of volatiles from week 1 to week 6. At week 6 all six components were still detectable in significant quantities. At week 2 the release rate was low due to the low temperatures during that week. At week 3 the release of volatiles was high due to the increased temperatures, which increased the release rate of volatiles. In addition to the six components added to the lure several others were detected which originated from the lure dispenser: 2-methylpentane, 3-methylpentane, 2,2,4,6,6-pentamethylheptane, and triacetin. The main volatile detected from the surface of the grain was hexanal, which is an oxidative breakdown product of fats.

In trial 4, the release rates of the six components was approximately tenfold less than in trial 3, as expected from the tenfold lower loading (results not shown). The amounts of volatiles detected were so low that they were usually close to the limit of detection, and for weeks 5, and 6 often below the limit of detection for most components. In trial 4 the predominant volatile detected was 2,2,4,6,6-pentamethylheptane.

For buried traps sampled during trial 4 the decline in release rate of volatiles with time roughly matched that of the surface traps except that the concentrations of volatiles inside the buried traps was between 10x and 30x higher than inside surface trap. This is because volatiles can escape more easily from surface traps compared to buried traps, where the grain above traps slows down the diffusion of volatiles away from the trap and causes their concentration to build up. This effect increases as the volatility decreases. The number of grain volatiles and their concentration was also greater under the surface of the grain compared to just above the surface for the same reason.

Floor traps: in trials 3 and 4 the decline in release rate of volatiles with time roughly matched that of the surface traps except that the amounts of volatiles released was between 10x higher than inside surface trap because the amounts of volatiles in the lure were 10x higher. One of the floor traps was located near a door and the airflow over the trap was greater than that over the other traps sampled. The amount of volatiles detected by SPME was about 10x less for this trap than for the other floor traps sampled.

Discussion

Insect movement in the grain bulk and on the floor would have been expected at the temperatures recorded during all of the trials. The lowest temperature recorded during these trials was 9°C. *C. ferrugineus* move around at temperatures as low as 5°C (Jian et al., 2003; Fleming et al., unpublished data) and *O. surinamensis* and *S. granarius* as low as 2.5°C (Fleming et al., unpublished data). The temperature decreased during trial one, increased during trial three and was stable during trial four. The moisture contents of the grain measured in the floor store were typical of stored grain.

The type 1 lure (rubber ring), currently supplied by Russell IPM for use in the PC™ Floor Trap, released too many volatiles and released the majority of them at the beginning of the trial rather than at the steady rate

over the duration of the trial which is required. This lure did increase trap catches of *O. surinamensis* and *C. ferrugineus* over empty traps but decreased the catch of *S. granarius* compared with control traps. Since fewer *S. granarius* were caught in traps with lures than in control traps, the type 1 lure is possibly repellent to this species. The initial repellent effect may have been due to the very high amount of volatiles released by the lure but there may have been a component of the lure that was causing this effect. Trial 2 therefore examined the effect of the lure containing the matrix and BHT only. Traps without lures caught significantly more insects than control traps even when the positions of the traps were switched. Therefore, it is probable that something in the formulation was repellent to *S. granarius*. Given the unstable volatile release rate and problem of repellence to *S. granarius* it was concluded that this type of lure was not suitable for use in the traps under grain store conditions.

The type 2 lure (vial) was an improvement on the first lure in both the PC™ Pitfall Traps in the grain bulk and in PC™ Floor Traps. In the grain bulk, this lure greatly increased the trap catch over the control traps of *C. ferrugineus*: more were caught in surface and buried traps but there was no difference in the number of positive traps due to nearly all of the traps with and without lures containing insects. The lure increased the number of *O. surinamensis* caught in surface traps; although there were no statistically significant differences, the numbers of insects caught in traps with lures was greater for five of the six weeks of the trial. Similarly, with the exception of one week, the number of positive traps with lures was always greater than or equal to the number of positive traps control traps. The effect of the lure on trap catches of *S. granarius* was less clear: there were no statistically significant differences in the numbers of insects caught or positive traps. However, for surface traps, more insects were caught in traps with lures for the first two weeks then fewer were caught in traps with lures during the remainder of the trial. The reverse was true for buried traps: fewer *S. granarius* were caught in traps with lures for the first three weeks then more were caught in traps with lures for the remainder of the trial. These effects are probably due to changing amounts and ratios of the volatile components released from the lure. In the PC™ Floor Traps, the second lure made a great improvement to the catches of *O. surinamensis* and *S. granarius* but too few *C. ferrugineus* were caught to make a comparison.

The type 2 vial lure containing lower amounts of attractants was worse than the original type 2 lure formulation. This lure did not make any meaningful difference to the trap catches of any of the three species in the grain bulk. In the floor traps, there was no improvement in trap catch over the control traps for *O. surinamensis* and *C. ferrugineus* but there was a slight increase in trap catch of *S. granarius*.

The release rates of volatiles from the second lure decreased over time but the amounts of volatiles released after six weeks was much higher than with the first lure. The release rate was much more stable and sufficient volatiles were being released after six weeks to attract insects. The triacetin detected from the vial lure originates from the filler used in the lure. The 2,2,4,6,6-pentamethylheptane originates from the plastic vial and is added in the manufacturing process of many plastics as a solvent/dispersal agent for the oxide

polymerisation catalyst. The origin of the 2- and 3-methylpentanes is not known but may arise from the use of ether as a solvent to add the attractant components to the lure. Any effect on the insects of the additional volatiles detected, other than the six components added to the lure, is not known. Neither are the effects of any compounds arising from reactions within the lure or with grain volatiles known. The ideal would be a lure that only emits the six components added and nothing else. This is unrealistic because even the amounts of the six components themselves are very low. The vial lure gave a much more controlled release rate than the rubber ring lure used in Trial 1.

This is the first time that the volatile headspace in and around lures in traps has been sampled *in situ*. It has proven to be a valuable tool in determining the release rate of volatiles from lures under realistic conditions. However, the maximum detectable distance from the trap at which the components are detected by SPME is likely to be smaller than the distance at which the insects can detect the components. This is inferred from earlier laboratory bioassays (Wakefield et al., unpublished data). The main factors determining the maximum distance from a lure that volatile components are detectable are: volatility of the component, loading, temperature and air movement. If a component is very volatile it escapes from the trap easily but if it is less volatile it will be released from the lure but remain within the trap.

Conclusion

On the basis of these trials in the Storage Research Unit at CSL and under the particular environmental conditions recorded the best food volatile based lure for further testing is the type 2 vial lure containing cellulose acetate filler and attractants (hexanoic acid, 3-methylbutanol, 3-octen-2-one, nonanal and E-2-nonenal and 0.25mg of 4-ethylacetophenone). However, in general, the increases in trap catches were modest and the lure was more effective in the PC™ Floor Traps than in the grain bulk. Lower trap catch with a multi-species lure than with pheromone lures may be the pay-off for having a lure which attracts more than one species, for example the *O. surinamensis* lactone lure increased the trap catch in PC™ Traps in the grain bulk increased the trap catch ten fold (Wakefield et al., unpublished data).

Table 16. SPME-GCMS of headspace within surface PC™ Pitfall Traps in the grain bulk and at 5 cm away from the traps and background grain (1.25 m away) during Trial 1 (area counts)

	Week 1		Week 2		Week 3		Week 4	
	inside	5 cm	inside	5 cm	inside	5 cm	inside	background grain
acetic acid	3,483,061	2,334,721	3,073,244	2,272,174	370,342	339,107	166,834	380,199
3-methylbutanol	3,195,998	954,984	764,569	240,219	61,197	98,550	16,205	21,589
hexanoic acid	3,470754	255	2,960,582	2,125	27,364	150	731	ND
3-octen-2-one	2,824,176	196,371	67,638	30,467	17,747	16,266	ND	ND
nonanal	10,421,841	111,378	1,489,116	97,375	75,414	61,802	13,718	56,961
E-2-nonenal	15,427,314	12,240	1,577,937	30,482	32,754	ND	ND	ND
3-ethylacetophenone	130,629	ND	54,803	ND	ND	ND	ND	ND
4-ethylacetophenone	18,877,432	6,080	10,108,378	18,091	400,463	5,838	7,151	ND
triacetin	12,745,179	20,941	12,686,236	64,414	1,073,341	ND	259,614	ND
BHT	26,067,646	ND	32,668,048	1,566	768,435	313	148,631	ND

ND = no data

Table 17. SPME-GCMS of headspace within surface PC™ Pitfall Traps in the grain bulk during Trial 3 (area counts)

Week	**1**	**2**	**3**	**4**	**5**	**6**
Grain surface temperature (˚C)	19.5	13.0	25.0	20.0	17.0	21.0
Compounds added to the lure:						
3-methylbutanol	1,893,194	243,339	4,998,370	2,077,994	1,060,958	1,048,388
hexanoic acid	451,020	10,572	490,945	95,508	28,386	35,427
3-octen-2-one	4,675,998	236,135	7,077,646	1,817,397	613,371	626,441
nonanal	4,197,883	268,043	8,254,082	2,091,680	725,293	547,123
E-2-nonenal	9,503,954	160,760	5,631,451	1,078,554	320,147	286,866
4-ethylacetophenone	347,469	25,455	617,584	88,788	25,976	34,747
Additional compounds detected:						
2-methylpentane	17,369,349	2,536,739	15,227,753	4,436,764	2,010,671	915,466
3-methylpentane	6,462,322	891,682	8,219,336	2,099,372	832,803	349,574
acetic acid	1,122,424	402,792	1,547,175	521,852	209,647	445,995
hexanal	1,251,418	266,033	1,599,767	561,636	465,765	254,256
terpene	363,613	81,201	453,649	90,550	53,801	172,851
2,2,4,6,6 pentamethylheptane	4,036,090	246,353	3,327,965	976,697	352,140	332,714
triacetin	40,285	3,316	313,050	60,651	40,587	52,178

MILESTONE 5. Conduct laboratory tests to confirm that simplified lure is effective against the agreed additional species

Introduction

Although work has focused on just three primary pests of major importance, to ensure the widest applicability of the lure, its effectiveness will need to be measured against a much wider range of pests. The outcome of Step 3.1 was a list of such additional species to be tested which had been agreed with representatives of the grain and milling trades. Field strains of the additional species were tested when they were available as well as laboratory strains. In addition, it was agreed that field strains of the three primary pests of major importance (*O. surinamensis, S. granarius* and *C. ferrugineus*) would also be tested. Two additional species, *Stegobium paniceum* and *Ptinus tectus,* were also tested since they were available.

Materials and Methods

Insects

Insect species and strains tested are shown in Table 18. All of the field strains were collected and supplied by Igrox Ltd. except *O. surinamensis* Daventry, *C. ferrugineus* Daventry and *S. granarius* Dorset which were already available at CSL. Each field strain was identified on arrival and cultured according to standard procedures.

Table 18. Insect species and strains tested

Species	Lab Strain	Field Strain
O. surinamensis	n/a	Huntingdon Daventry Norfolk
C. ferrugineus	n/a	Daventry
S. granarius	n/a	Dorset
O. mercator	lab ref. S. stock	
T. castaneum	FSS II S. Stock	Norfolk
T. confusum	lab. Susc.	
R. dominica	Salisbury ex. S. stock	
S. oryzae	Droxford S. stock	
S. zeamais	ex. s. stock	
A. advena	Susc. S. stock	Staplehurst
S. paniceum	n/a	Scotland
T. stercorea	Datchet	
P. tectus	PICL	
L. bostrychophila	n/a	

Bioassay

Various unsuccessful attempts were made to test the lure in PC™ Floor Traps in arenas. The amount of volatiles was too great for the available headspace and no distinction could be made between traps with lures and control traps without lures. Therefore, the pitfall bioassay was used for all of the tests. This is the same bioassay which was used in Step 1.3. The environmental conditions were also identical to those used in Step 1.3: 20 ± 1°C, 50 ± 3% r.h. The modification of the 5 mm lip was used for the following species: *S. granarius, S. oryzae, S. zeamais* and *A. advena.* The lures could not be used in the pitfall bioassays due to the large amounts of volatile compounds in a relatively small headspace so a test mixture containing all of the attractive components of the lure was prepared in pentane. The mixture contained 100 ng of each of the following in 5 µl: hexanoic acid, 3-methylbutanol, 4-ethylacetophenone, 3-octen-2-one, nonanal and E-2-nonenal. The negative control was 5 µl of pentane and this was tested in a separate room. The test mixture was tested at 5 µl per arena and the positive control was 1 µg of carob-peanut extract in 5 µl pentane.

Ten replicates of each test and control were carried out with each strain of each species. The numbers of insects that responded were square root arcsine transformed and the differences between the responses to the pentane control, test mixture and carob-peanut extract control compared using One-way ANOVA followed by Tukey's pairwise comparisons.

Results

There was no disagreement between tests with laboratory strains and recently collected field strains of the same species. A summary of the responses of the species and strains is shown in Table 19. Four of the additional species tested responded to both the test mixture and the carob-peanut extract. These were: *O. mercator, T. confusum, S. oryzae* and *L. bostrychophila.* Four of the species responded to the carob-peanut extract but not to the test mixture. These were: *R. dominica, S. zeamais, A. advena* and *T. stercorea.* Three of the additional species tested did not respond to either the test mixture or to the carob-peanut extract. These were: *T. castaneum, S. paniceum* and *P. tectus* . The latter two were not prioritised for testing by trade representatives.

All of the field strains of the three primary pests of major importance (*O. surinamensis*, *S. granarius* and *C. ferrugineus*) responded to both the test mixture and to the carob-peanut extract. The responses of three of the species (*O. surinamensis, O. mercator* and *S. oryzae*) to the carob-peanut extract were significantly greater than their responses to the test mixture.

Table 19. Summary of behavioural responses to test mixture and carob-peanut extract

	Lab Strains			Field Strains		
Species	**Strain**	**Responded to test mixture**	**Responded to carob-peanut extract**	**Strain**	**Responded to test mixture**	**Responded to carob-peanut extract**
O. surinamensis	n/a			Huntingdon Daventry Norfolk	Yes Yes Yes	Yes Yes Yes
C. ferrugineus	n/a			Daventry	Yes	Yes
S. granarius	n/a			Dorset	Yes	Yes
O. mercator	lab ref. S. stock	Yes	Yes			
T. castaneum	FSS II S. Stock	No	No	Norfolk	No	No
T. confusum	lab. Susc.	Yes	Yes			
R. dominica	Salisbury ex. S. stock	No	Yes			
S. oryzae	Droxford S. stock	Yes	Yes			
S. zeamais	ex. s. stock	No	Yes			
A. advena	Susc. S. stock	No	Yes	Staplehurst	No	Yes
S. paniceum	Lab. strain	No	No	Scotland	No	No
T. stercorea	Datchet	No	Yes			
P. tectus	PICL	No	No			
L. bostrychophila	Lab. strain	Yes	Yes			

Discussion

Eleven additional species were tested with a volatile mix corresponding to the volatiles added to the lure. Nine of these had been prioritised for testing by representatives of the grain and milling trades. Four of the nine prioritised species (*O. mercator, T. confusum, S. oryzae* and *L. bostrychophila*) responded to the test mix in pitfall bioassays. Together with *O. surinamensis, S. granarius* and *C. ferrugineus* (including field strains of these species) this brings the total number of species found to respond to the lure volatiles to seven.

A further four species responded to the carob-peanut volatiles but not to the lure volatiles (*R. dominica, S. zeamais, A. advena* and *T. stercorea*) therefore there is potential to identify which components of the carob-peanut extract are responsible for this behavioural effect. This would require additional electroantennography (EAG) and behavioural bioassays. The lure components could then be modified to include these compounds, assuming they do not repel any of the other species, so that the lure also attracts these additional species. The components of carob extract which are attractive to *A. advena* have already been identified as 2-nonanone and 2-undecanone (Wakefield *et al.,* 2005). These components are commercially available and could be added to the multi-species lure.

Since the carob-peanut extract elicited better responses from *O. surinamensis, O. mercator* and *S. oryzae* than the test mix, the same process could be used to improve the responses of these three species to the lure.

The three species which did not respond to either the test mixture or the carob-peanut extract (*T. castaneum, S. paniceum* and *P. tectus)* would require a return to the more basic search for attractive volatile extracts of foodstuffs followed by identification of the attractive volatiles by EAG and behavioural bioassays. However, of these three species, only *T. castaneum,* was prioritised by trade representatives and there is a commercially available pheromone lure for this species.

MILESTONE 6. Complete trials of chosen trap and lure combination in chosen premises

Introduction

Using the type of premises identified from milestone 2 four different premises were identified for validation of the lure in commercial premises. The premises chosen were a maltings, a flour mill, import/export warehouse and a premises with a floor stored bulk of barley. The aim of this milestone was to compare monitoring devices with and without the formulated lure over a six week period and to examine differences in numbers caught and the numbers of positive traps.

Methods

Premises

Trials were carried out in the following premises 1. Port - import/export warehouse:- a total of 31 PC™ floor traps were positioned in the vicinity of a bay 28 m x 20.4 m containing wheat within the premises. In the 5th

week of the trial the wheat was removed from the bay and replaced with rape seed. 2. Maltings:- a total of 28 PC™ floor traps were positioned in a building housing eight 80 tonne bins containing barley, wheat and roast barley 3. Flour mill:- a total of 40 PC™ floor traps were positioned in three areas, 12 in the grain intake building, 14 in the screens room and 14 in the wheat silos area 4. Grain bulk (floor storage):- a total of 40 PC™ traps, 20 on the surface and 20 buried, were positioned across the bulk (barley) which measured approx. 48.5 x 25 metres. The trials in premises 1–3 commenced in October 2005 and the trial in premises 4 commenced in March 2006. Environmental conditions (temperature and humidity) were monitored using Tinytag data loggers (Gemini dataloggers, UK) at premises 1-3 and with in-store temperature monitors at premises 4.

Trial protocol

At each premises traps, with or without the lure, were placed in alternate positions and as far as was possible these positions were equivalent in terms of position from corners, equipment etc. All trials lasted for a six week period and traps were checked each week. Any insects found were sent to CSL for identification. Traps remained in the same positions throughout the trial. Current monitoring methods used and any hygiene or treatment measures carried out during the trials were recorded.

Statistical analyses

Analyses were performed using Genstat8 unless otherwise stated. The numbers of insects in traps were analysed by repeated measures Anova (analysis of variance), with the numbers transformed to log(n+1). Sites were treated as blocks and adjacent lure and non-lure traps were treated as pairs. The proportion of positive traps was analysed by GEE (general estimating equations) using a binomial distribution and a logit link.

Results

Insects were found in traps at all four premises. The range of species found varied at each site but representatives of the major storage beetle pest species were found at all sites (Table 20a-f). *Sitophilus* spp were identified as *S. granarius* or *S. oryzae/zeamais.* For the majority of specimens no further identification of these latter two species was made but it should be noted that for the small number (N=10) of individuals identified to species all were *S. oryzae.* Comparison of traps with or without the lure showed that no significant differences ($P>0.05$) were found either in terms of the number of insects caught or the number of positive traps for any of the species found where comparisons were possible. Where a species was found at more than one site the results were combined within the analysis. At premises 4 a total of only 2 individuals were caught. These were detected in week 4 of the trial in two different traps neither of which contained a lure.

Table 20. Number of insects found and number of positive traps at premises 1 to 3 for each of the six weekly assessment periods

A. Premises 1. Port. Number of insects found.

Total number caught		Traps: No Lure=15, Lure=16						
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
A. advena	No Lure	4	10	17	8	1	0	40
	Lure	5	28	13	16	0	0	62
S. oryzae/zeamais	No Lure	0	43	13	17	0	0	73
	Lure	2	2	0	1	0	0	5
Cryptolestes spp	No Lure	0	11	1	0	0	0	12
	Lure	1	0	0	0	0	0	1
O. surinamensis	No Lure	14	25	21	20	0	0	80
	Lure	10	29	19	29	1	0	88

B. Premises 1. Port. Number of positive traps.

Total positive traps		Traps: No Lure=15, Lure=16						
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
A. advena	No Lure	3	7	6	2	1	0	19
	Lure	3	9	4	3	0	0	19
S. oryzae/zeamais	No Lure	0	1	2	1	0	0	4
	Lure	1	2	0	1	0	0	4
Cryptolestes spp	No Lure	0	1	1	0	0	0	2
	Lure	1	0	0	0	0	0	1
O. surinamensis	No Lure	6	6	4	8	0	0	24
	Lure	4	10	6	9	1	0	30

C. Premises 2. Maltings. Number of insects found.

Total number caught		Traps: No Lure=14, Lure=14						
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
T. castaneum	No Lure	0	0	1	0	1	0	2
	Lure	0	1	2	1	0	0	4
T. confusum	No Lure	0	1	1	2	0	0	4
	Lure	0	0	4	0	1	0	5
S. granarius	No Lure	39	22	121	0	5	0	187
	Lure	23	22	48	12	16	3	124
S. oryzae/zeamais	No Lure	23	30	44	0	9	0	106
	Lure	16	5	32	10	10	0	73
Cryptolestes spp	No Lure	0	0	1	0	0	0	1
	Lure	0	0	1	0	0	0	1
O. surinamensis	No Lure	3	1	3	1	0	0	8
	Lure	1	0	4	2	0	0	7

D. Premises 2. Maltings. Number of positive traps.

	Total positive traps			Traps: No Lure=14, Lure=14				
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
T. castaneum	No Lure	0	0	1	0	1	0	2
	Lure	0	1	2	1	0	0	4
T. confusum	No Lure	0	1	1	1	0	0	3
	Lure	0	0	2	0	1	0	3
S. granarius	No Lure	8	7	7	0	2	0	24
	Lure	8	6	8	4	4	1	31
S. oryzae/zeamais	No Lure	5	6	5	0	1	0	17
	Lure	6	3	7	4	4	0	24
Cryptolestes spp	No Lure	0	0	1	0	0	0	1
	Lure	0	0	1	0	0	0	1
O. surinamensis	No Lure	3	1	3	1	0	0	8
	Lure	1	0	2	1	0	0	4

E. Premises 3. Flour mill. Number of insects found

	(All sites)	**Total number caught**			Traps: No Lure=20, Lure=20			
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
T. castaneum	No Lure	0	0	0	0	0	0	0
	Lure	0	0	0	1	1	0	2
S. granarius	No Lure	9	5	7	6	0	3	30
	Lure	79	25	1	14	2	4	125
S. oryzae	No Lure	14	23	16	9	0	0	62
	Lure	4	9	24	9	4	0	50
Cryptolestes spp.	No Lure	0	0	1	0	0	0	1
	Lure	0	0	2	0	0	0	2
O. surinamensis	No Lure	0	1	2	1	0	0	4
	Lure	0	0	0	2	0	0	2

F. Premises 3. Flour mill. Number of positive traps

	(All sites)	**Total positive traps**			Traps: No Lure=20, Lure=20			
Species	Trap	Week1	Week2	Week3	Week4	Week5	Week6	Overall
T. castaneum	No Lure	0	0	0	0	0	0	0
	Lure	0	0	0	1	1	0	2
S. granarius	No Lure	4	2	2	1	0	2	11
	Lure	4	2	1	3	1	2	13
S. oryzae/zeamais	No Lure	4	4	5	4	0	0	17
	Lure	3	3	6	3	2	0	17
Cryptolestes spp.	No Lure	0	0	1	0	0	0	1
	Lure	0	0	1	0	0	0	1
O. surinamensis	No Lure	0	1	2	1	0	0	4
	Lure	0	0	0	2	0	0	2

Discussion

It was found that over the total trapping period, when traps with and without the lure were compared, there were no significant differences for any of the species found either in the number of insects or the number of

positive traps. It will be noted that in general the number of insects present were fairly numerous and it is possible that at lower insect densities the sensitivity of traps with the lure would be apparent. The trials in commercial premises have confirmed that the PC™ Floor trap and PC™ trap are extremely effective in detecting the presence of storage insects. However, it would appear that further optimisation of the lure is required before the benefits of the use of the lure can be shown to be cost-effective.

MILESTONE 7. Refine protocol for use of trap and lure combination, validate in experimental grain storage facility and promote to trade

The results obtained from the trials in commercial premises and in the CSL Grain Storage Research Unit demonstrated that although in some circumstances the detection of insects was improved by the addition of the lure this was not always significant and the degree of enhancement was not the same for the different species. Given that further refinement of the lure and validation is required it was not possible to produce and refine a protocol for trap and lure use. It is also felt that because of this need the lure is not yet ready to be commercialised. However, during the course of the project several technology transfer opportunities have been realized for example articles have appeared in International Food Hygiene and Crops magazines and the project has been promoted at both the Grain and Cereals events.

During the course of this project techniques in spatial analysis to examine insect distribution have been used to look at insect movement in the grain bulk in relation to traps with and without lures. This type of analysis can be used to determine optimal distance for the spacing of traps and can also be used to indicate positions of infestations and for the setting for thresholds for control. This type of examination will therefore be used in further refinement of the lure to produce a robust protocol for use of the trap and lure both in grain bilks and in premises. As an example the use of spatial analysis techniques to examine whether the distribution of insects changed over the duration of the trial from the original uniform distribution at the introduction of the population to a patchy distribution.

The coordinates of the traps and the weekly total trap counts for each trap position for *O. surinamensis, S. granarius* and *C. ferrugineus* were analysed using Spatial Analysis by Distance IndicEs (SADIE) version 1.22 software (Perry, 1998). This was used to identify clusters (patches and gaps) in the insect count data using red-blue techniques (Perry *et al.,* 1999). The outputs were plotted as contour maps using Surfer version 8 software. The red areas on the maps are patches of high numbers of insects, the blue areas are gaps in insect distribution and the white areas are areas of uniform distribution.

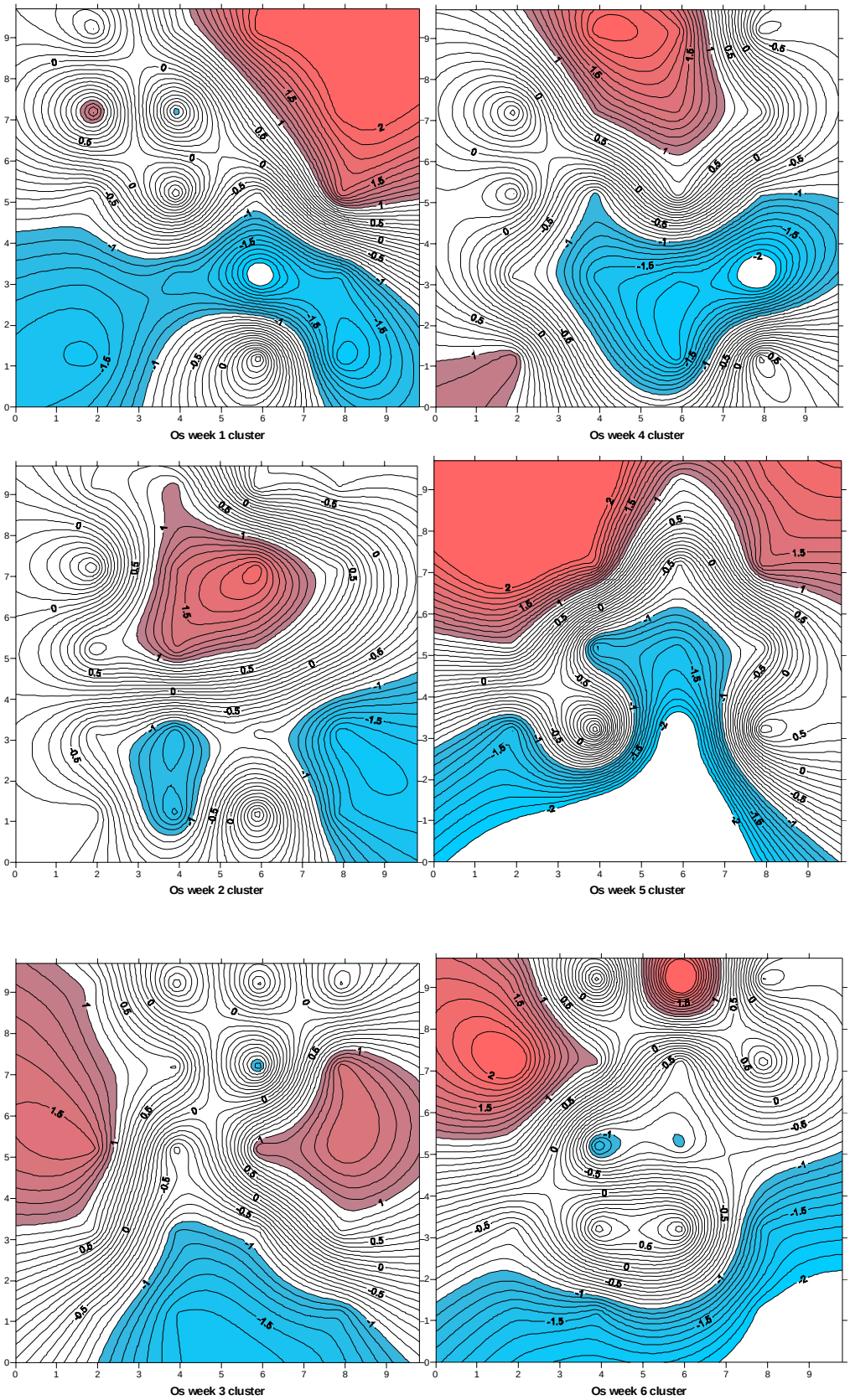
The maps show that the insects did not remain uniformly distributed. Patches of *O. surinamensis* and *C. ferrugineus* developed on the plateau of the grain bulk and gaps developed at the bottom of the slope (Figure 19). These clusters became larger over the six weeks of the trial. Patches and gaps were also present

in the distribution of *S. granarius*. These were in different places each week and did not increase in size (Figure 19).

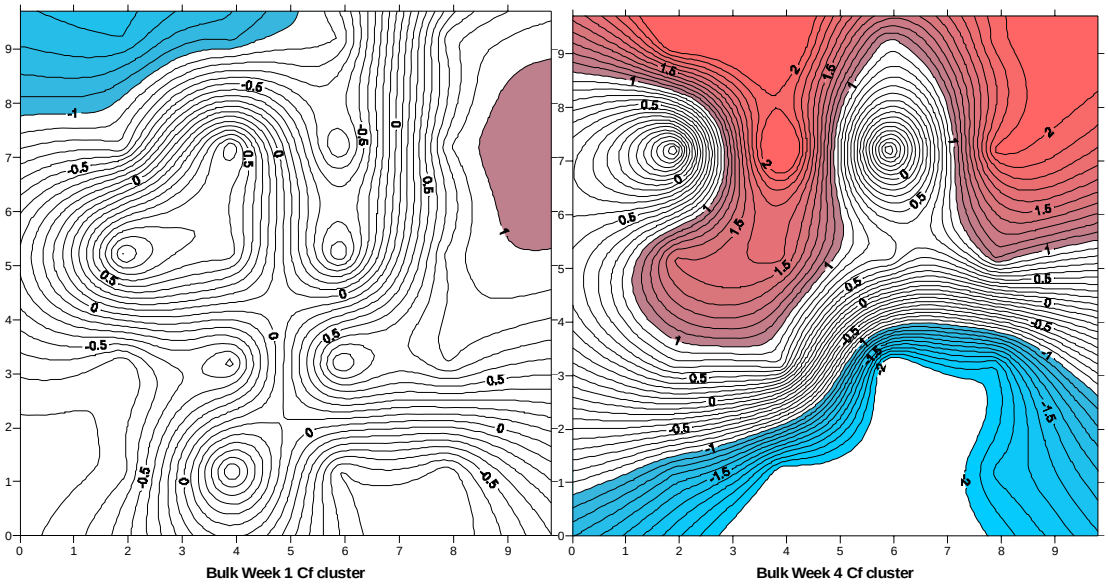
These results show that the distribution of the three species did not remain uniform over the duration of the trial and that *O. surinamensis* and *C. ferrugineus* clustered in the same areas whereas *S. granarius* formed smaller clusters which varied in location with time. The patches and gaps may be due either to insect movement or differential insect mortality in different locations within the grain bulk.

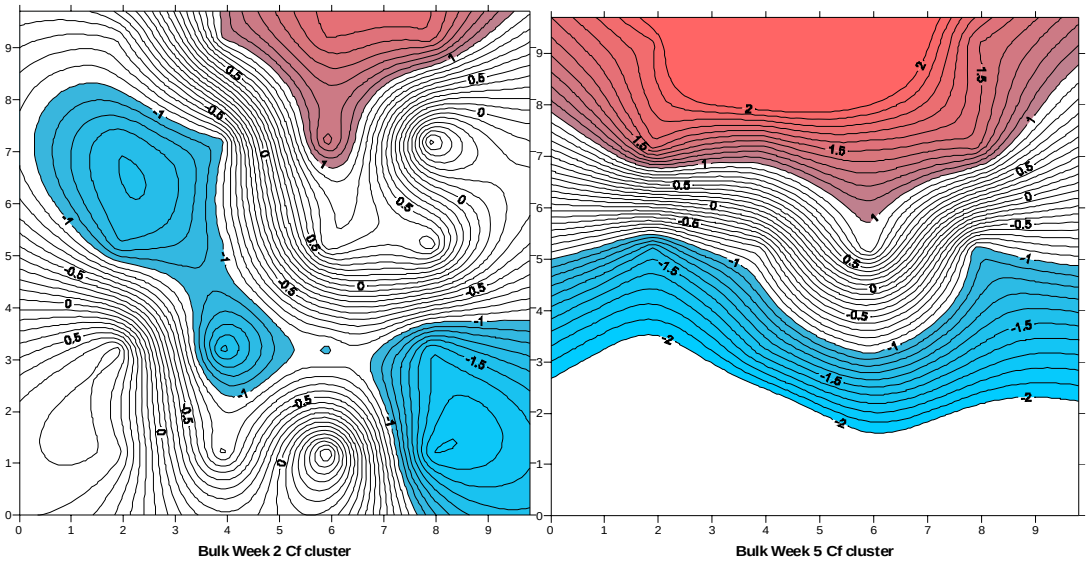
These, and similar, techniques can be used to visualise the areas in which infestations are present and in this way can target areas for treatment. The same techniques can be used after treatment to identify any residues of infestation. Through the use of spatial models optimal spacing for traps can be determined. Effective monitoring tools are key if these techniques are to be used to their full potential and the progress made within this project in the development of a multi-species lure and techniques for examining release of volatiles from lures has provided a significant step towards this goal.

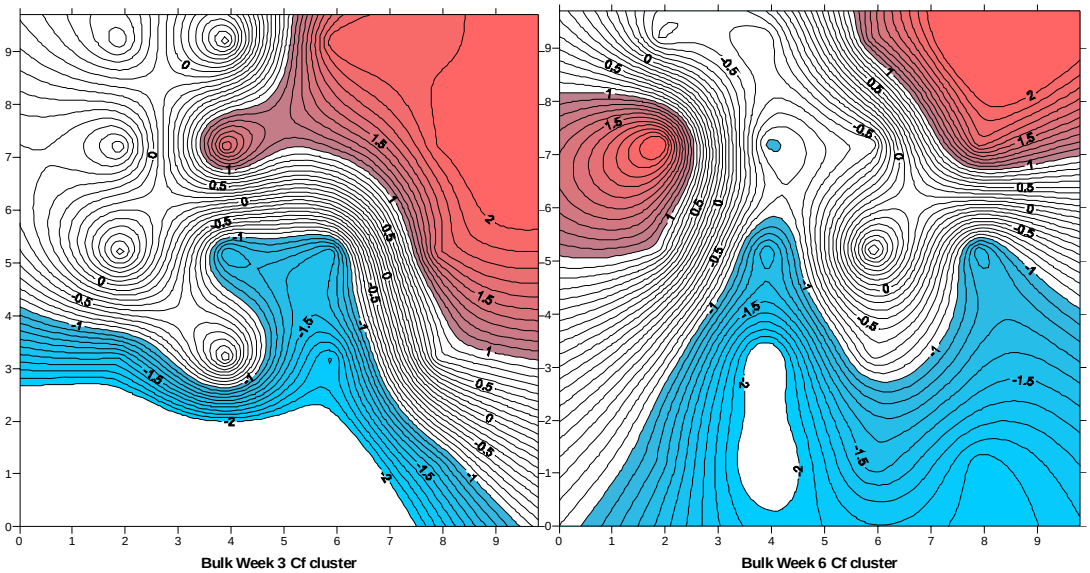
Figure 19. *O. surinamensis* distribution



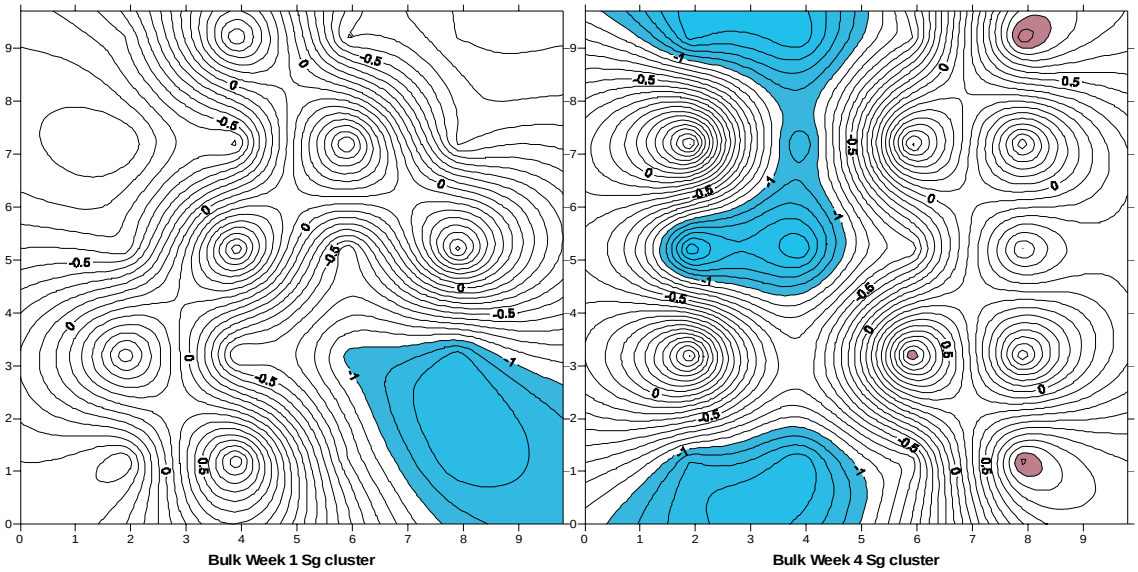
C. ferrugineus distribution

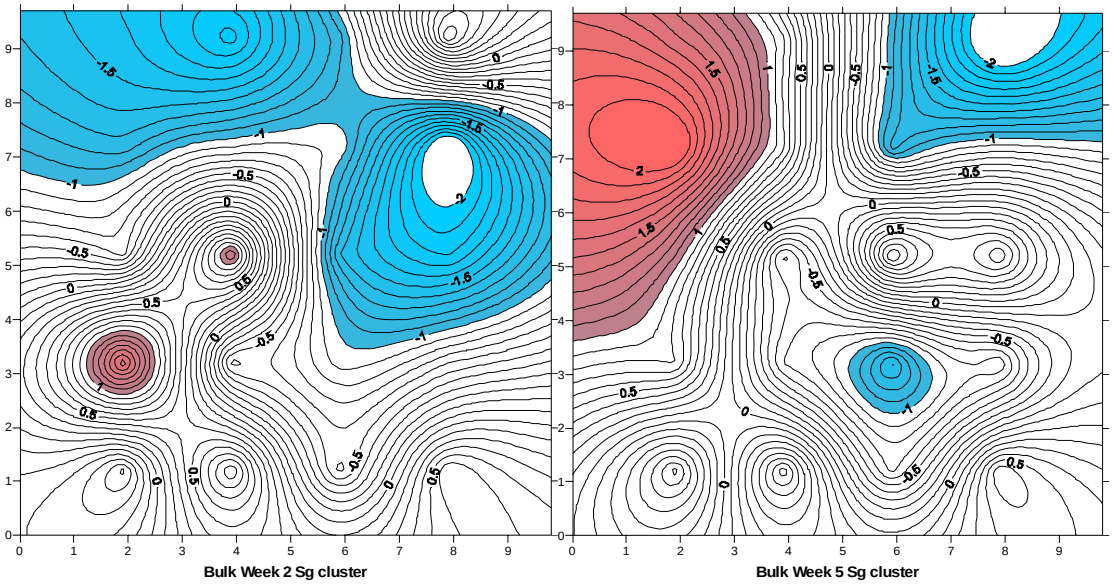


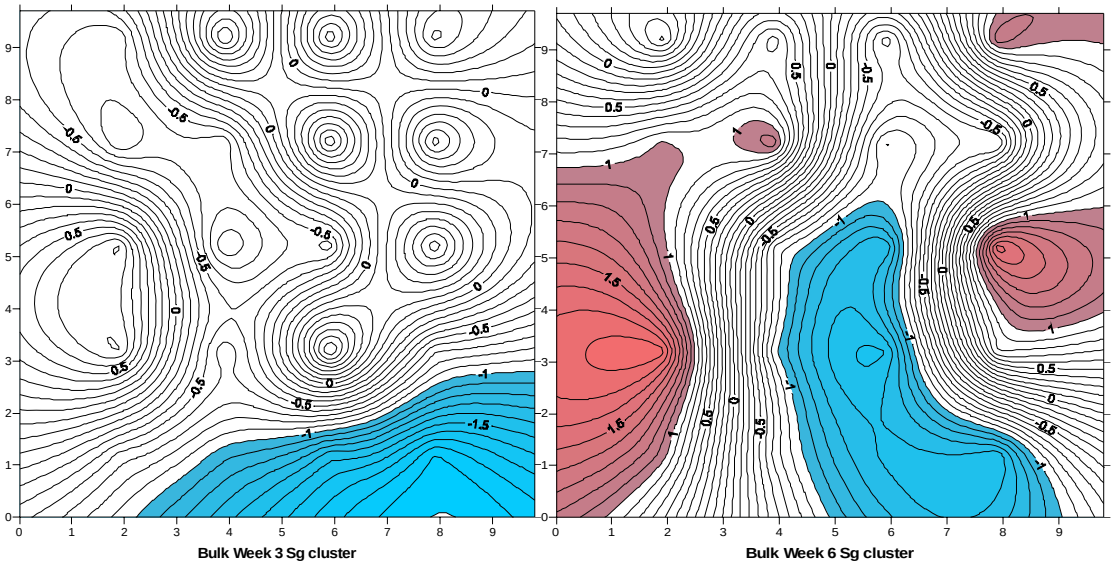




S. granarius distribution







OVERALL CONCLUSION

- A mixture of compounds attractive to 7 insect pest species in laboratory bioassays was identified
- The attractant compounds are readily available
- The compounds were formulated into a lure dispenser and the lure is practical and easy to use
- The lure was validated in both laboratory and field trials in commercial premises
- The release of volatiles from the lure was quantified under practical conditions and release rates from the lure dispenser were established
- Trials in the CSL Grain Storage Research Unit and in commercial premises showed a modest increase in trap catch over control traps
- Further refinement of the lure components is required for optimisation of the lure including examination of different ratios of the components
- Significant progress towards a multi-species lure for storage insect pests was made

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