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**Efficacy of diatomaceous earths, applied as structural treatments, against stored product insects and mites**

by

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## **ABSTRACT**

Diatomaceous earth (DE), a silicon-based dust that can desiccate insect and mite pests, acts by removal of water-proofing waxes found in the cuticle. Three DE products marketed for stored product protection were launched in the UK from 2001 onwards. Following on from previous research, that enabled recommendations for use on grain, this project aimed to investigate their use to control residual pests in empty grain store structures.

Initial laboratory experiments using methods based on an international protocol, tested UK DE products against a range of insect, mite and moth species. DE treatment was applied either as a dry dust, or an aqueous slurry. From the results of the first phase of testing, the most tolerant species and the most appropriate doses were identified. These were adults of the grain weevil (*Sitophilus granarius*), the cosmopolitan food mite (*Lepidoglyphus destructor*) and larvae of the Mediterranean flour moth (*Ephestia kuehniella*), with doses of 10-20 g/m<sup>2</sup> against the weevil/ moth and 1-3 g/m<sup>2</sup> (dry dust) or 20 g/m<sup>2</sup> (slurry) against the mite, depending upon DE product. The second phase of experiments involved more in-depth testing of the DEs against these most tolerant species on a more challenging substrate, wood. This time efficacy was tested over a longer 12 week period to indicate the DE's persistence and the most appropriate dose for field validation. As a result of this, it was decided that dry-dust treatments should be evaluated at a dose of 10 g/m<sup>2</sup> for a storage season (harvest to early summer).

Trials took place in two identical stores containing a nest of six 20 tonne steel bins, into which ca. 100,000 insects were released in July 2003. The effect of treatment was assessed by monitoring the number of insects trapped, and by counting the number killed on contained bioassays. This exercise was repeated again with a second release of insects in March. The trial concluded that under the most ideal situation where best practice was followed, the least tolerant species (saw-toothed grain beetle) could be killed within 2 weeks and the most tolerant (grain weevil) within 5 weeks.

The project concluded that DE is ideal for treating empty stores if used as part of an integrated strategy as follows:

- Clean out stores between harvest, ensuring no food residues remain in cracks and crevices.
- Monitor the empty store with traps.
- Apply DE as a dry dust at 10 g/m<sup>2</sup> if insects are found (only apply as a slurry where practice dictates).
- Where practical, leave for at least 5 weeks before filling with grain.
- Monitor the store after treatment.
- If large numbers are found in a particular area, investigate and, if necessary, re-clean and re-treat.

## **SUMMARY**

### **BACKGROUND**

Diatomaceous Earth (DE) is a naturally occurring substance, mined from geological deposits of fossilised diatoms. Diatoms are water-borne, single celled phytoplankton, of which there are over 25,000 species. DE dust is composed of over 90% silica and has a wide range of applications, including use as a filtration agent, tooth-paste ingredient and in invertebrate pest control. DE works by physical action, desiccating insects and mites. DE contains no chemical insecticide or knock-down agents, has low mammalian toxicity, is persistent but does not leave harmful residues, is effective against chemical-resistant species, and is stable at high and low temperatures.

The aim of this project was to investigate the use of DE to treat empty grain stores, and make recommendations to the UK industry. The project was divided into 3 work packages. Parts 1 and 2 identified the most likely doses, and the most tolerant species under controlled laboratory conditions. These findings were then validated on a practical scale during treatment of a large-scale storage facility.

### **LABORATORY EXPERIMENTS**

#### ***1. Initial evaluation of wet and dry DE applications***

The efficacy of two UK-marketed DE products was assessed under constant conditions of 15°C and 80% relative humidity (rh) using methodology based on an international protocol. Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup>, were applied as aqueous slurries at a range of doses, to give even deposits on the base of clean glass petri dishes after drying in an oven. The DEs were also applied as dry dust to glass or plastic petri dishes. To treat the glass plates (insect and moth tests) dry dust was sieved through a 250 µm mesh sieve to give an even coating. For application to the plastic dishes (mite tests) the required amount of dry DE was placed in the center of a dish onto which another was joined, and the dust was distributed by gently inverting and tapping the dishes, causing the dust to adhere by static electricity.

Twenty-four hours after treatment, 25 adult insects, mites or moth larvae were added to the dishes and exposed at 15°C and 80% relative humidity (rh) for either seven days (insects and moths) or 24 hours (mites) (five replicates per species/dose). After exposure the numbers killed were recorded and the insects were transferred to jars containing 50g of wheat at 16% moisture content (mc) and returned to experimental conditions for a seven day recovery period, after which mortality was re-counted. The mites were assessed similarly, although they were transferred to specially constructed cells containing a small amount of mite food for a recovery period of 24 hours only. After the recovery period, mortality was re-assessed (Table 1).

**Table 1. Mean % mortalities (corrected for control mortalities) of insects, moth and mites exposed to 3 doses of dry dust and slurry treatments of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> after seven day (insects/moth) and 24 hour (mites) exposures followed by recovery periods at 15°C/80% rh**

Treatment	Dose (g/m <sup>2</sup> )	Insect species				Dose (g/m <sup>2</sup> )	Mite species		
		Rust-red flour beetle – <i>T. castaneum</i>	Grain weevil - <i>S. granarius</i>	Saw-toothed grain beetle – <i>O. surinamensis</i>	Mediterranean flour moth – <i>E. kuehniella</i>		Cosmopolitan food mite – <i>L. destructor</i>	Flour mite – <i>A. siro</i>	
<b>Slurry</b>	Silico-sec	5	94.4	82.4	100	71.8	2.5	0	1.6
		10	88	93.6	100	68.1	5	9.3	98.7
		20	77.6	88	100	50.9	10	17.3	95.2
	Diasecticide	5	0.8	3.2	0	9.9	2.5	0	83.8
		10	0	2.4	0	0	5	0	98.5
		20	0	0	5.3	0	10	0	87.8
<b>Dry dust</b>	Silico-sec	5	100	96.8	100	95.4	0.5	95.6	100
		10	100	96.8	100	100	1	97.3	100
		20	99.2	94.4	100	98.2	2	93.2	100
	Diasecticide	5	0.8	5.6	100	30.9	0.5	36.5	50.3
		10	2.4	1.5	98.7	78.3	1	62.5	62.6
		20	5.6	1.6	97.3	84.5	2	86	95.8

From this initial screening it was concluded that –

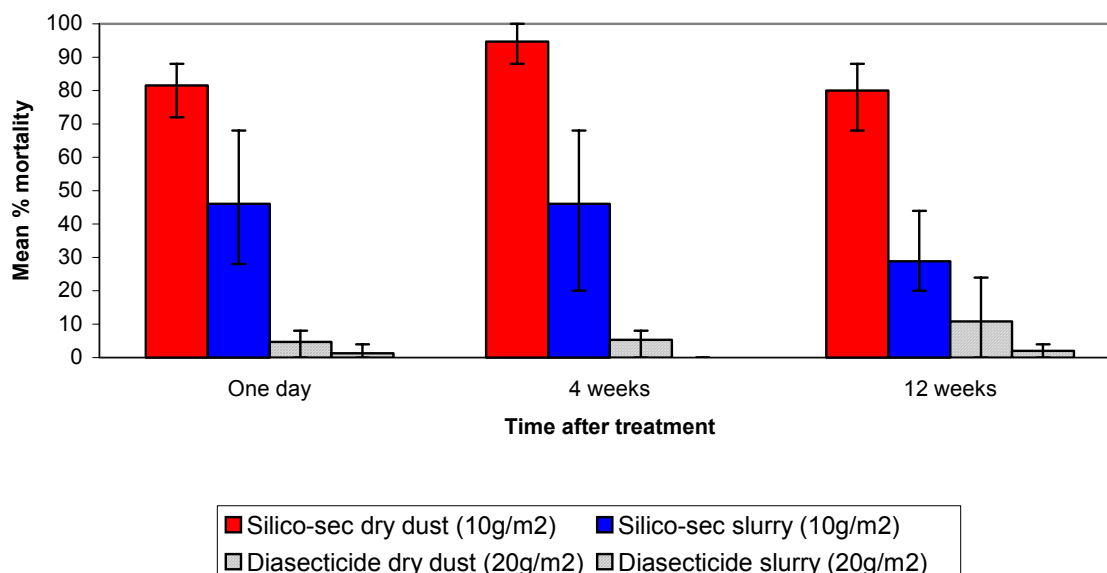
- Dry dust treatments were much more effective than slurry treatments.
- The grain weevil (*Sitophilus granarius*) and the cosmopolitan food mite (*Lepidoglyphus destructor*) were the most tolerant species of insect and mite tested.
- Ranking of the different species for DE tolerance agreed with other published findings.
- Silico-sec<sup>®</sup> was more effective than Diasecticide<sup>™</sup>.
- There was a lack of dose response in some instances.
- Identification of the most tolerant species and appropriate doses formed the basis of the second phase of testing.

## 2. Assessment of wet and dry DE applications on wooden surfaces

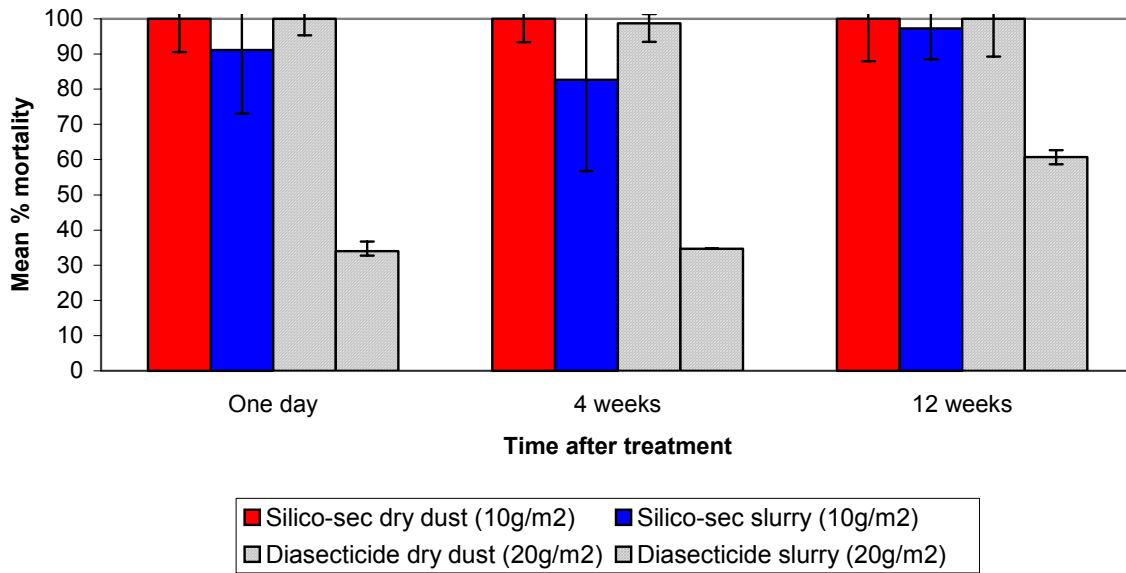
The two DE products, Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup>, were reassessed on a more 3-dimensional surface, believed to be most challenging to DE efficacy. The chosen substrate was wood; the more convoluted surface enabled insects to right themselves after a fall in the shortest time, therefore reducing the amount of DE picked up.

Methodology for treating the wooden substrates was the same as in the first phase of testing, although all dry dust treatments were applied by passing the dust through a sieve onto the treated surface. A sufficient number of surfaces were treated so that exposure of the pests could commence either one day, four weeks or 12 weeks after treatment. The most tolerant insect and mite from the first phase of testing, and the moth (that had proven more tolerant than the insects) were exposed for seven days (insect and moth) or 24 hours (mites), followed by a recovery period as before. Since mortality was assessed at intervals over a longer 12 week period, these tests gave a better indication of the length of protection that DE can offer (Figures 1-3).

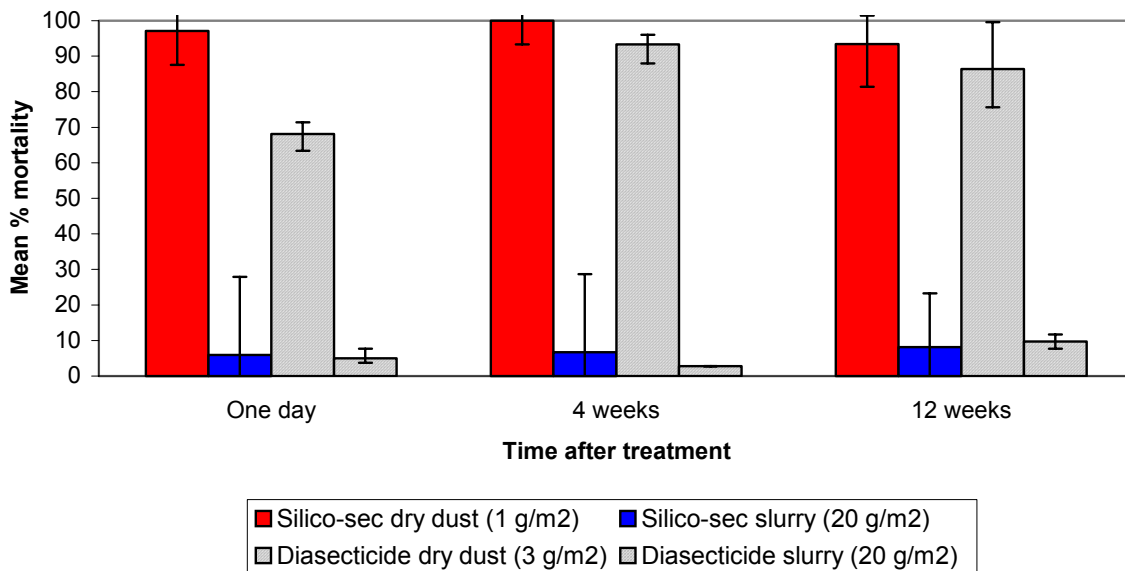
**Figure 1. Mean % mortalities (corrected for control mortalities) of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> against adults of the grain weevil (*S. granarius*) on wooden surfaces, when exposed after one day, 4 weeks or 12 weeks at 15°C/80% rh.**



**Figure 2. Mean % mortalities (corrected for control mortalities) of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> against larvae of the Mediterranean flour moth (*E. kuehniella*) on wooden surfaces, when exposed after one day, 4 weeks or 12 weeks at 15°C/80% rh.**



**Figure 3. Mean % mortalities (corrected for control mortalities) of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> against adults of the cosmopolitan food mite (*L. destructor*) on wooden surfaces, when exposed after one day, 4 weeks or 12 weeks at 15°C/80% rh.**



This second phase of testing showed that -

- In all cases the dry dust gave the best efficacy - eg. against the grain weevil (*S. granarius*), the Silico-sec<sup>®</sup> dust applied at 10 g/m<sup>2</sup> was the most effective treatment with mean mortalities of 80-95% recorded over the experimental period.
- Overall Silico-sec<sup>®</sup> was the most effective treatment against all the pest species.
- The same levels of protection were provided over the whole 12 week experimental period.

It was concluded that for slurry treatments higher doses and longer exposure times than tested here would be required, and that even these may not be completely effective. Therefore, focusing on the results against the most tolerant species, the grain weevil (*S. granarius*), 10 g/m<sup>2</sup> Silico-sec<sup>®</sup>, applied as a dry dust treatment, was chosen as the most appropriate dose for field validation. However for Diasecticide<sup>™</sup>, the decision was more difficult, since even a higher dry dust dose gave poor control of the most tolerant species tested, and there were concerns about trying doses >20 g/m<sup>2</sup>, due to the amount of airborne dust that would be produced by treatment. It was therefore decided that rather than increasing dose, the solution may be to see if recommendations based on longer exposure times would be appropriate.

## FIELD EVALUATION

The aim of the trial was to test the two products against the least and most tolerant insect species from laboratory study, and to make final recommendations that could be applied regardless of DE used or pest species.

Unfortunately, insufficient quantities of Diasecticide<sup>™</sup> were available for the trial, so this product was withdrawn from the study and substituted by a third UK product, Demeter.

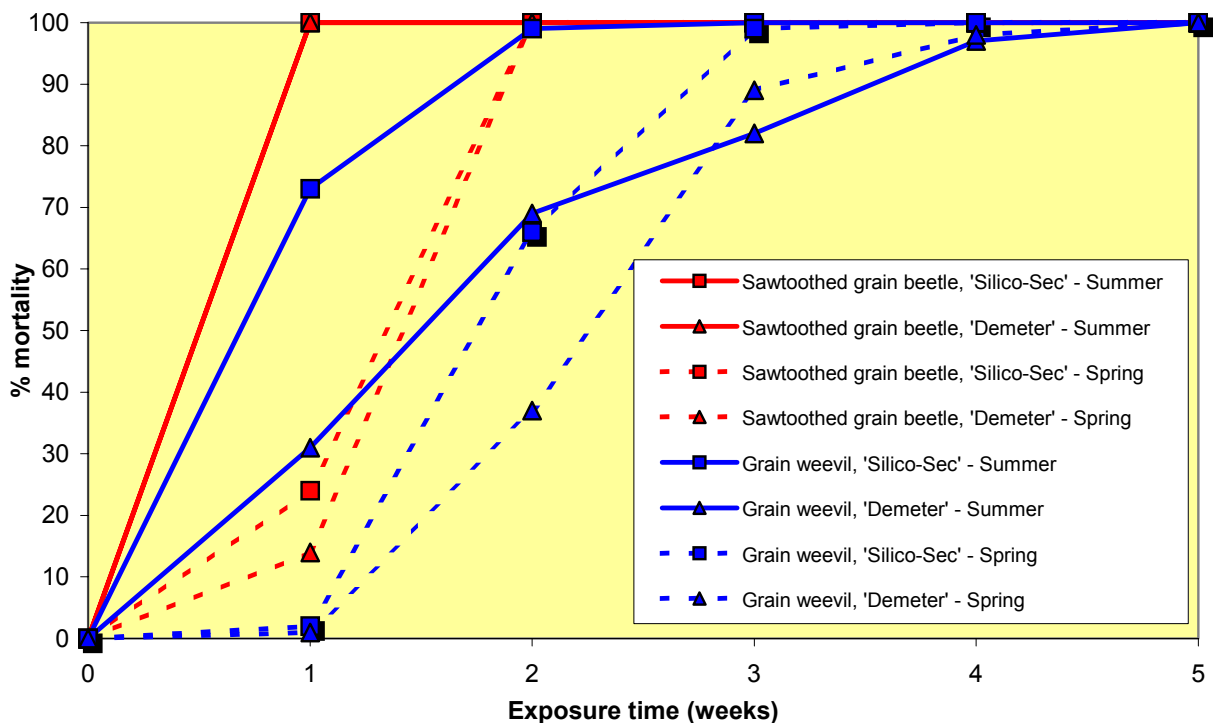
Trials took place in 2 identical stores (bin areas), each containing a nest of six, 20 tonne steel bins. Prior to treatment, both bin areas underwent intensive cleaning and the background insect population was monitored with traps (n=12) for 2-3 weeks from the beginning of July 2003. Sixty-thousand grain weevils (*S. granarius*) and 30,000 saw-toothed grain beetles (*O. surinamensis*) were introduced into each bin area a week before treatment in July 2003.

Silico-sec<sup>®</sup> was applied to bin area 1 and Demeter to bin area 2 as dry dust to the walls and floor of the store and to the sides of steel-grain bins using a hand-powered knap-sack duster. Measurement of the amount of dust at monitoring positions across the floors after treatment confirmed that there was no significant difference between the two treatments although both bin areas were slightly under-dosed at ca. 8 g/m<sup>2</sup>.



Assessments were made of free-roaming insects and also of insects contained on bioassay plates that had been exposed to treatment. In March, a second batch of insects was released, and bioassays were repeated using additional plates retained from the original treatment. The bioassays showed that for both products, complete control was achieved after 5 weeks exposure, both immediately after treatment and 36/37 weeks later demonstrating longevity of treatment (Figure 5).

**Figure 4. Field validation – weeks to kill the least and most tolerant insects, when exposed to two DE products immediately after treatment (summer) and 36/37 weeks later (spring).**



However, control of the free-roaming population took 2-3 months longer. This illustrates that in practice, control may be more variable depending upon the individual structure, and the nooks and crevices present that may allow pests to harbour away from treatment. Therefore where possible, DE should be blown into these voids using a suitable applicator, to give complete coverage.

Building upon work from a previous HGCA funded study, an electrostatic duster was compared to the conventional duster to see whether improvements in adherence to vertical surfaces could be achieved when treating simulated wall cavities. In contrast to the increased adherence normally found when treating external structures, there was no significant improvement. Although both cavities had been treated evenly,

the heaviest dust deposits were observed on the floor and measurement of the dust adhering to vertically suspended steel plates were ca. 10 times lower than the intended dose of 10 g/m<sup>2</sup>, illustrating the problems associated with treating vertical surfaces.

It was also of interest that the number of free-roaming insects surviving the store treatments was very low, with many dying prior to dust application. It was presumed that the thorough store cleaning before the experiment start had deprived them of essential food and water, and underlines the importance of hygiene.

#### GUIDLINES FOR DE STRUCTURAL TREATMENTS

Based on the results of this project and after consultation with the DE manufacturers/distributors and end-users, a number of simple guidelines were drawn together. These were recently published in a new HGCA topic sheet - No. 79, "Insect and mite control in empty grain stores using DE" (Summer 2004). Summarised under 'Action':

1. Clean store thoroughly before treatment.
2. **Preferably apply DE as a dry dust** at 10 g/m<sup>2</sup> to store structures at least five weeks before storing grain.
3. Use an appropriate duster, eg electric-powered or knap-sac, with the appropriate nozzle.
4. **Only apply DE as water-based slurry where necessary**, eg vertical sides, or where there is a need to minimise air-borne dust.
5. Use longer exposure and much higher dose with slurry, eg 20 g/m<sup>2</sup> for 'Silico-Sec'.
6. **Always** use correct dust mask.
7. **Always** check market acceptability before using DE, especially if treated surfaces come into contact with grain.

## **TECHNICAL DETAIL**

### **GENERAL INTRODUCTION**

In the UK, synthetic pesticides have traditionally been used to treat the structure of empty grain stores between harvests to control residual invertebrate pest populations. However, the number of pesticides currently registered for this use is decreasing. In addition, concerns over food and environmental safety, the development of resistant pest populations to some conventional insecticides and the continued requirements for pest-free grain, has necessitated the need to identify alternative compounds.

Of the many alternatives that have been researched, diatomaceous earth (DE) is a natural product with excellent environmental and consumer safety profile, and has attracted increasing interest for pest control in the last 10 years.

### **DIATOMACEOUS EARTHS**

#### ***Origins and composition***

DE is a naturally occurring substance composed of almost pure silicon dioxide and is mined from geological deposits of fossilised diatoms. Diatoms are water-borne, single celled chrysophyte phytoplankton, similar to some species of brown algae (Plate 1.). There are more than 25,000 species of diatoms with no two having the same morphology (Round et al., 1992). Diatoms extract silicon from water, from which they produce a hydrated amorphous silica skeleton.

Plate 1. A diatom as viewed under a microscope

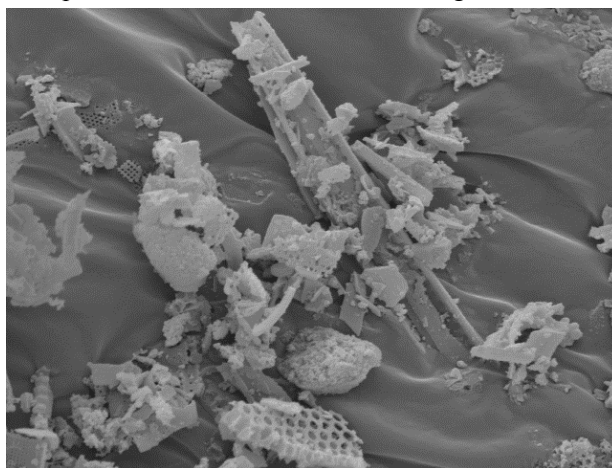


Over centuries the tiny shells of the dead diatoms, would have accumulated and compressed into a soft chalky rock. The fossilised layers mined today, originate from species that died about 20 to 80 million years ago, mostly in the Eocene and Miocene epochs (Korunic, 1998). DE deposits are found world-wide and range in thickness from a few centimetres to several hundred metres. DE deposits contain ca. 50% moisture, and of the remaining solids, 86-94% is silica ( $\text{SiO}_2$ ), with the rest being mainly alumina and alkalis from clay.

### ***Usage***

For commercial use, the mined DE deposit (often referred to as Diatomite) is dried and milled to form a very fine dust of particle size 0.5-100 $\mu\text{m}$  (Plate 2.). Current world production of DE was most recently estimated at 2m t per annum, with estimated world reserves standing at 800m t (Crossley, 2000). The USA supplies the majority of DE, with other significant global producers including China, Japan, Spain, Denmark, Former-USSR, France, Mexico, South Korea, and Australia (in descending order). DE has a wide range of applications, including use as a filtration agent (water, liquors, juices, commercial fluids), a “filler” (paints, paper, rubber) an absorbent and is also found in products such as detergents, deodorisers, tooth-pastes, anti-caking agents and animal feed additives. Use as an insecticide is an emerging market and accounts for a small fraction of use.

Plate 2. DE particles as viewed under a scanning electron microscope



### ***Mode of action and pesticide efficacy***

Unlike conventional pesticides, DE is ‘inert’ in nature and works by physical, rather than chemical action. The mode of action (MOA) is by gentle abrasion and sorption of cuticular waxes, stripping away the insects’ water-proofing and resulting in desiccation when insects crawl among dust-coated kernels (Ebeling, 1971). Due to their composition and MOA, DEs are often referred to as ‘siliceous dusts’, ‘inert dusts’ or ‘desiccant dusts’. These broader groups of compounds also include various clays, wood ashes and man-

made silicas that are similar in action to DEs. Desiccant dusts are often cited as being the earliest pesticides used by man, and observations of animals taking dust baths to rid themselves of mites and parasites is believed to have led the Chinese to start using DE in pest control over 4000 years ago (Allen, 1972). Although not as effective as man-made silicas that have a higher oil carrying capacity (Ebeling, 1971), recent advances in 'formulation' through selection of best particle size, enhancement by addition or coating with silica aerogel etc. has renewed international interest in their use for pest control in the last decade.

For grain storage, DEs have proven efficacy when directly admixed to grain at 0.5 – 3 g/kg (Desmarchelier and Dines, 1987; Subramanyam et al., 1994) and applied as structural treatments to empty storage facilities at 2 – 6 g/m<sup>2</sup> (Bridgeman, 1994, 2000; Wright, 1990; Desmarchelier et al., 1992; McLaughlin, 1994). Unlike conventional organophosphorous based insecticides, DEs do not cause an immediate knock-down effect and are comparably slow acting. DEs are also mildly abrasive, and this has caused worry about use from some sectors with regards damage to machinery. Although this concern is often mentioned in the literature, the authors can find no published data to support this and it is of note that the DE Dryacide<sup>®</sup> is routinely used to treat grain-handling equipment in Australia. Despite these perceived disadvantage, DEs offer considerable advantages since they have low mammalian toxicity, do not leave harmful residues, are effective against chemical-resistant species, give long periods of protection and are stable at high and low temperatures (Subramanyam et al., 1994; McLaughlin, 1994). Differences in susceptibility have been observed in a wide range of insects with tolerances varying between species, strains and life stages (Fields and Korunic, 2000; Mewis and Ulrichs, 2001; Rigaux et al., 2001).

The efficacy of DEs is also known to be affected by storage conditions, being more effective against insects held at higher temperatures and lower humidity (Le Patourel, 1986; Fields and Korunic, 2000). For this reason, it has been necessary to develop dosing and treatment strategies specific to the UK's cool maritime climate. In previous research, high doses were required to achieve control on grain stored under typical UK conditions of low temperature and high humidity (Collins et al, 2001, Cook and Armitage, 2000). However, high DE doses are undesirable as they have a negative effect on the bulk density and cause problems with grain flow (Jackson and Webley, 1994). To overcome this, the use of DE is only recommended when applied to the top 30 cms depth of grain, when used as part of CSL's integrated strategy based on cooling and drying (Cook and Armitage, 2002). Not only does this allow the dose applied to be diluted on outloading, but it was also found that a low surface dose comparable to the 1 g/kg dose used in drier countries, could be used as a preventative treatment. These recommendations are available in the revised HGCA grain storage guide (Armitage & Wildey, 2003). Until now, no UK specific recommendations have been available for treatment of empty stores.

### ***Environmental impact, consumer and operator safety***

A comprehensive review on DE safety and environmental impact was made by Desmarchelier and Allen (2000). Comprised mainly of silica dioxide, DE poses no threat to the environment since 50% of the earth's crust is silica (eg. sand) or a silicate (eg. soil). Being inert, there are no harmful chemicals produced that can poison wild-life and DE has a very low mammalian toxicity. In fact, silica is naturally present in foods and water and its intake in the diet is relatively high. In addition, silica does not accumulate in mammals, but is excreted, as silicate in the urine. DE is therefore classified as non-toxic and registered as a food additive in USA and EU (E551c). Furthermore, impact on the consumer is also lessened, as little if any DE residue ends up in the finished product. Parkin (1944) suggested that industrial processes used for cleaning wheat, removes inert dusts. For example, Desmarchelier and Dines (1987) removed 98% of the DE Dryacide<sup>®</sup>, applied at 1 g/kg to wheat using a pilot mill (1 t capacity). They also found that less than 3% of Dryacide<sup>®</sup> from un-cleaned wheat carried over into the flour, with no effect to flour quality. This was later repeated using a larger pilot mill (3 t capacity), where > 99% of Dryacide<sup>®</sup> was removed from wheat (Desmarchelier et al., 1996).

The main health concern with DE, as with many airborne particulates, is from breathing in the dust. The scale of risk posed by DE dust depends upon the form of DE used. When considering respirable risk, DE can be classed under 2 categories depending upon the type of silica contained; - 'crystalline' or 'amorphous'. Most DEs are amorphous (non-crystalline silica), although DE that has been subjected to geomorphic changes may contain crystalline silica. 'Calcined' DE, produced by heating with sodium chloride or sodium carbonate and often used for filtering by brewers, also contains high levels of crystalline silica. The problem with crystalline forms of silica is that they are dangerous if inhaled and can scar fibroid tissue of the lungs, whereas amorphous forms are not regarded as fibrogenic and are often referred to as lung irritant, rather than hazardous. For this reason DE products for pest control normally contain <1% crystalline silica, making them safe to the operator providing the simple precaution of wearing a dust mask is taken. The type of mask required is dependent upon the particle size of the product, and this is normally detailed on the product label, although those recommended are normally the same type used to protect against grain dust. In the UK, the Health and Safety executive (HSE) have set the long-term (8-hour) occupational exposure standard (OES) for DE at 1.2 mg/m<sup>3</sup> (Anon, 2002).

### ***Current legislation for storage use***

Diatomaceous earths have been registered for storage use in USA, Canada, Australia, Japan, Indonesia, Germany, Austria, Switzerland and Saudi Arabia. In the UK three DE products are currently available and have been actively marketed for stored product protection since 2001; these are Silico-sec<sup>®</sup>, Demeter and Diasecticide<sup>™</sup>. However, none of these products have been registered under the Control of Pesticides Regulations, 1986 UK (COPR). Products that work by physical mode of action are exempt from registration,

as set out in COPR regulation 3 (2). All 3 companies have written to PSD/HSE stating that their products fall under this classification and on this basis their products have not been required to undergo registration.

However as this report goes to press, current regulations are being superseded by two new EC Directives, the Plant Protection Products Directive (PPPD), 91/414/EEC, concerned with mainly agricultural pesticides and the Biocidal Products Directive (BPD), 98/8/EC, concerned with non-agricultural pesticides and biocides. These aim to harmonise the registration process across the EU, to allow for a system of mutual recognition of regulatory decisions where a product registered in one Member State may be authorised by a second Member State, provided agronomic, climatic and environmental factors are similar. Companies have had to notify their intent to support their products through this registration process. The following interpretation of PPPD has been made after seeking informal clarification with Pesticide Safety Directorate (PSD) -

- It is the active ingredient (AI) that must be notified to the BBA, Germany under Annex I (basic notification).
- A two-step notification had to be completed by February, 2003.
- Notification of an AI by a company automatically covers any other products that also have the same AI, regardless of manufacturer.
- If a product's AI has not been notified, the product cannot be used after 25/7/03.
- As long as notification has taken place, the products can continue to be used in the marketplace under existing national rules until the review has been finished.
- This EU review must be completed by 2008, and some products may not be looked at until as late as 2006.

Progress with the implementation of directive 91/414 and detail of subsequent revision was recently summarised at the BCPC International Congress 2003 (Smeets, 2003; Flynn, 2003)

“Kieselgur” (diatomaceous earth) is listed in Annex I but also falls under Annex II/3 as a substance for stored product protection. The UK marketed products Silico-sec<sup>®</sup> and Demeter have been notified under Annex I by the manufacturers Agrinova and Agil as part of the Render 4 project (summarised in - Verschwele and Pingel, 2003), according to Article 4 and Article 5 (2) (a) of Commission Regulation (EC) No. 1112/2002. DE has also been supported as an active ingredient (AI) by FiBL, Amu-systeme and Denka.

In summary, all 3 products marketed in the UK can continue to be used until the review has been completed and it is anticipated that DE will be available as registered products thereafter.

### ***End-user acceptability***

DEs were warmly received by the UK farming community as a replacement for OP dusts, the latter having been withdrawn in 2001. However, in common with any new concept there has been a mixed response to the use of DE from end-users. As this report goes to press –

- Organic - DEs have been accepted as a restricted product under the British Soil Association's certified input scheme for organic cereals and hence can be used by Organic growers under the rules of the scheme.
- Milling wheat – NABIM are currently advising their member NOT to accept DE treated wheat. NABIM's technical committee is currently in dialogue with DE companies and in house trials are planned to address outstanding concerns.
- Malting Barley – MAGB have advised that the maltsters will only accept DE treated barley if the DE product is on the British Beer and Pub Association approved list of products. Demeter and Silico Sec<sup>®</sup> are currently listed.
- Oilseed rape - The preventative grain surface treatment recommendation has been accepted by SCOPA.
- Feed grain – Feed members of AIC advise that there are no restrictions for DE treatment of animal feed at the levels recommended for the UK, but treatments must be recorded on grain passports.

However, in contrast to direct admixture to grain, all the end-users that have been consulted are happy with the concept of DE structure treatments. Never the less, growers should always check market acceptability if there is any risk that treated surfaces are to come into contact with stored grain intended for malting or milling.

### **PROJECT AIMS**

The objective of this HGCA study was to devise appropriate structure treatment strategies suitable for UK conditions. Since it is known that there are differences in efficacy for different DE products, this study aimed to set doses at a level where the same doses and exposure times were applicable for all products. The experiments reported here were firstly undertaken to determine the effective doses of the DE products marketed in the UK against a range of storage pests in the laboratory, followed by validation on a large-scale under fluctuating field conditions against the most and least tolerant insects found. An industry steering group that included representatives from the DE companies met at intervals throughout the project to ensure recommendations would be practical for the industry.



## Part 1: INITIAL LABORATORY EVALUATION OF WET AND DRY DE APPLICATIONS

D.A. Collins & D.A. Cook

### INTRODUCTION

In commercial practice, DEs are applied to structures using slurry (wet) and dry blown methods. In Australia, Dryacide<sup>®</sup> is applied as a dry dust to grain-handling machinery, ducts and vertical silos, and slurries are applied to horizontal grain stores (Golob, 1997). Slurries are useful where there is a need for personnel to avoid exposure to the very dusty atmospheres created by the dry blown method, however some dusts tend to have reduced efficacy when applied as slurries (McLaughlin, 1994; Gowers and Le Patourel, 1984). As a result, recommended rates of Dryacide<sup>®</sup> are 2 g/m<sup>2</sup> when applied as a dry dust and 6 g/m<sup>2</sup> as a 10% aqueous slurry.

This first phase of work, aimed to assess slurry and dry dust treatments against insects, mites and a moth using laboratory methods based on guidelines developed by an international DE working group (Fields et al., 2003).

### MATERIALS AND METHODS

#### *Insects and mites*

CSL laboratory strains of –

Insects:

- Rust-red flour beetle - *Tribolium castaneum* (Herbst)
- Grain weevil – *Sitophilus granarius* (L.)
- Saw-toothed grain beetle – *Oryzaephilus surinamensis* (L.)

Mites:

- Flour mite - *Acarus siro* L.
- Cosmopolitan food mite - *Lepidoglyphus destructor* (Schrank).

Moth:

- Mediterranean flour moth - *Ephestia kuehniella* Zeller

Mixed sex adults of *T. castaneum*, *S. granarius* and *O. surinamensis* aged 3-5, 2-4 and 1-3 weeks old respectively were used. Larvae of *E. kuehniella* were removed from cultures set up with adults 4 weeks previously and mixed sex adult mites approximately 3 days old were used. Beetle species were maintained in a controlled environment (CE) room set at 25°C and 70% relative humidity (rh) in darkness, with the moths kept in a CE room at the same temperature but at 60% rh and with a 16 hour light : 8 hour dark lighting regime. The mites were maintained at 15°C and 90% rh in darkness.

Prior to exposure, the insects were acclimatised to the experimental condition of 15°C by moving culture jars to CE rooms set at decreasing temperatures in 5°C steps for 48 hours.

### ***Diatomaceous earths***

Silico-Sec<sup>®</sup>, a pure DE containing 90% SiO<sub>2</sub>, and Diasecticide<sup>™</sup>, a pure DE of freshwater origin containing 83.68% SiO<sub>2</sub>, both with less than 1% crystalline silica content, were obtained from the UK distributors.

Slurry and dry dust applications of each DE were assessed. Three doses of each DE application and an untreated control were assessed, with five replicates of each treatment. Since it was anticipated that higher doses would be required, doses started from a low dose, comparable to that recommended for drier countries, and increased x2 with each upwards step. Lower doses were used for the mites since it was known from previous CSL work that they would be less tolerant than the insects.

### ***Insect bioassays***

Prior to treatment, the sides of clean glass petri dishes (137 mm diameter, 25 mm high) were coated with 'Fluon' (PTFE) for those used to contain *S. granarius* and *O. surinamensis* and white paraffin for *E. kuehniella*, to prevent the insects escaping from the treated surfaces.

For the slurry application, the required amount of DE was placed in the centre of a clean glass petri dish. The DE was then mixed with 1.5 ml of water by rotating and tilting the dish by hand to obtain an even deposit over the base of the dish. A gloved fingertip was used to assist in the dispersion of the deposit to give what was judged by eye to be an even coating. The water was evaporated by placing the dish in an oven (~ 80°C) and rotating it approximately every 5 minutes until all the water had evaporated.

For the dry dust applications the required amount of dust was sprinkled evenly over the base of a glass petri dish by sieving through a 250µm mesh sieve. This differs from the methodology proposed by Fields et al. (2003), which recommends using plastic petri dishes and distributing the dust between two dishes using the static electricity generated by shaking and tapping. This is because in the experiments described here, the high doses assessed did not adhere to, or distribute evenly over, plastic dishes.

The treated dishes were put into the experimental conditions of 15°C and 80% rh overnight before addition of the insects. Twenty-five insects of each species were then placed into separate dishes and the tops of the dishes were covered with filter papers. After 7 days the numbers of live, knocked down and dead insects were recorded. An insect was considered knocked down if it was on its back and unable to right itself even if aided with a small brush. It was recorded as dead if no visible movement was detected.

All the insects were then transferred to 50 g of wheat in 120 ml jars, and the jars were sealed with filter paper lids. The jars were left in the experimental conditions for a 7 day recovery period after which time the numbers of live, knocked down and dead insects were again assessed.

### ***Mite bioassay***

For the slurry application, the methodology was as in the insect bioassays except that the bases of the glass petri dishes measured 48 mm diameter, 18 mm high, and the tops 57 mm diameter, 12 mm high; and the DE was mixed with 0.25 ml of water.

For the dry dust application, the required amount of DE was placed in the centre of a plastic petri dish (base, 53 mm diameter, 12 mm high; top, 56 mm diameter, 7 mm high). The petri dishes were joined, top to top, or base to base, with a strip of 'Parafilm'. The dishes were then shaken and tapped to distribute the dust evenly between both dishes. Static electricity caused the DE to adhere to the surfaces. The dishes were then taken apart.

The treated dishes were put into the experimental conditions of 15°C and 80% rh overnight before addition of the mites. Twenty-five mites of each species were then transferred to separate dishes using a single haired brush. For the fast moving *L. destructor*, the dishes were placed over frozen blocks of concrete in order to slow mite movement. Those transferred to bases were covered with another treated base and those transferred to tops were covered with another treated top. Both halves were then joined together with 'Parafilm'. After 24 hours the numbers of live and dead mites were recorded.

All the mites were then transferred to recovery cells consisting of a chamber made of a piece of Whatman no. 1 (42.5 mm diameter) filter paper. The filter paper was moulded to create a depression, 2 mm deep and 33 mm in diameter and secured using 'Pritt stick' glue to a glass square (75 x 75 x 4 mm), in the centre of which a hole 35 mm had been drilled. A small amount of mite food (wheatgerm and yeast) was added to the chamber which was then covered with a thin glass square (75 x 75 x 2 mm) and secured with bulldog clips. The numbers of live and dead mites were then recounted after a further 24 hours in the experimental conditions.

### ***Analysis of data***

The mean percentage mortalities were calculated for each pest species and treatment and were then subjected to angular transformation ( $\sin^{-1} \sqrt{p}$ ). The transformed data were statistically compared using analysis of variance (ANOVA) at the 5% probability level with individual comparisons made using Tukey's pairwise comparisons.

## RESULTS

### ***Insects***

Insect mortalities increased after the recovery periods because individuals recorded as 'knocked down' after the initial exposure, were unable to recover from the effects of the treatment and subsequently died.

Against *T. castaneum*, the Silico-sec<sup>®</sup> dry dust was the most effective treatment with mean mortalities of 99-100% recorded at the three doses, following the recovery period (Table 1.1). The Silico-sec<sup>®</sup> slurry produced mean mortalities of 78-94%, but the Diasecticide<sup>™</sup> treatments were ineffective at the doses.

Against *S. granarius*, there was no significant difference in the mortalities produced between the doses of Silico-sec<sup>®</sup> dry dust and slurry, with means ranging from 82-97% after the recovery period. The Diasecticide<sup>™</sup> treatments were again ineffective, with mortalities recorded of less than or equal to 6%.

A high rate of death (30-39%) was recorded with control insects of *O. surinamensis* after the seven day exposure period, which increased following the recovery period to 40-53%. Complete mortality was recorded with each dose of the Silico-sec<sup>®</sup> dry dust and slurry, with 98-100% recorded with the Diasecticide<sup>™</sup> dry dust. There was no significant difference in the mortalities obtained with the Diasecticide<sup>™</sup> slurry and the untreated controls.

Relatively high control mortalities of 22% were also recorded with *E. kuehniella*, however, the Silico-sec<sup>®</sup> dust was again the most effective treatment with mean mortalities of 96-100%. The top two doses of the Diasecticide<sup>™</sup> dry dust and the Silico-sec<sup>®</sup> slurry treatments were next in order of effectiveness, although there was a good deal of overlap in the mortalities recorded with the different treatments.

### ***Mites***

Against *A. siro*, complete mortality was achieved with all three doses of the Silico-sec<sup>®</sup> dry dust following the initial 24 hour exposure period (Table 1.3.). The Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> slurries at 5 and 10 g/m<sup>2</sup> (Table 1.4.), and the Diasecticide<sup>®</sup> dry dust at 2g/m<sup>2</sup> produced mean mortalities ranging from 92-99%, after the recovery periods, which did not differ significantly from the Silico-sec<sup>®</sup> dry dust results.

The Silico-sec<sup>®</sup> dry dust was also the most effective treatment against *L. destructor* with mean mortalities of 93-97% recorded at the three doses. The Diasecticide<sup>™</sup> dry dust at 1 and 2 g/m<sup>2</sup> produced mean mortalities of 64 and 86% respectively with a reduction in efficacy and overlap in effectiveness with the other treatments.

**Table 1.1. Mean % mortality (and ranges) of insects exposed to 3 doses of dry dust and slurry formulations of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> (n=5)**

Treatment	Dose (g/m <sup>2</sup> )	Insect species							
		<i>T. castaneum</i>		<i>S. granarius</i>		<i>O. surinamensis</i>		<i>E. kuehniella</i>	
		7 day exp	7 day rec	7 day exp	7 day rec	7 day exp	7 day rec	7 day exp	7 day rec
<b><u>Dry dust</u></b>	0	0 a	0 a	0 a	0 a	30 a (16-48)	40 a (24-52)	5 a,b (0-18)	12 a,b (9-17)
Silico-sec <sup>®</sup>	5	98 d (96-100)	100 c	34 c (16-56)	97 b (92-100)	100 d	100 b	91 e,f (88-96)	96 e (92-100)
	10	97 d (87-100)	100 c	42 c (32-56)	97 b (92-100)	100 d	100 b	99 f (96-100)	100 e
	20	98 d (92-100)	99 c (96-100)	38 c (28-52)	94 b (92-100)	100 d	100 b	98 f (92-100)	98 e (92-100)
Diasecticide <sup>™</sup>	5	0 a	1 a (0-4)	4 a (0-8)	6 a (0-12)	98 c,d (96-100)	100 b	22 b,c (16-34)	39 b (21-61)
	10	0 a	2 a (0-4)	0 a	1 a (0-4)	95 b,c (92-96)	99 b (96-100)	76 d,e (68-84)	81 d,e (61-96)
	20	0 a	6 a (0-12)	1 a (0-4)	2 a (0-4)	86 b (80-96)	98 b (96-100)	82 d,e (80-84)	86 d,e (80-92)
<b><u>Slurry</u></b>	0	0 a	0 a	0 a	1 a (0-4)	39 a (32-44)	53 a (40-64)	12a,b (0-38)	22 a,b (0-43)
Silico-sec <sup>®</sup>	5	74 c (68-88)	94 b,c (92-100)	17 b (4-32)	82 b (64-100)	100 d	100 b	67 d,e (38-84)	78 d (62-84)
	10	61 b,c (48-72)	88 b (72-96)	34 c (24-40)	94 b (84-100)	100 d	100 b	49 c,d (16-72)	75 c,d (60-84)
	20	41 b (28-80)	78 b (60-100)	25 b,c (16-32)	88 b (76-100)	100 d	100 b	46 c,d (25-72)	61 c,d (36-75)
Diasecticide <sup>™</sup>	5	0 a	1 a (0-4)	2 a (0-4)	3 a (0-4)	35 a (16-56)	46 a (28-60)	12a,b (4-22)	29 b,c (15-48)
	10	0 a	0 a	0 a	2 a (0-8)	39 a (28-52)	50 a (36-60)	3 a (0-12)	7 a (0-21)
	20	0 a	0 a	0 a	0 a	34 a (28-36)	55 a (40-80)	5 a (0-9)	12 a,b (4-18)

Means within an entire column followed by the same letter are not significantly different ( $P = 0.05$ )

**Table 1.2. Mean % mortality (and ranges) of mites exposed to 3 doses of dry dust and slurry formulations of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> (n=5)**

Treatment	Dose (g/m <sup>2</sup> )	Mite species			
		<i>A. siro</i>		<i>L. destructor</i>	
		24 hr exp.	24 hr rec.	24 hr exp.	24 hr rec.
<b>Dry dust</b>	0	3 a (0-8)	5 a (0-4)	0 a	2 a (0-10)
Silico-sec <sup>®</sup>	0.5	100 d	100 d	95 e (87-100)	96 f (91-100)
	1	100 d	100 d	97 e (87-100)	97 f (87-100)
	2	100 d	100 d	81 e (4-100)	93 f (67-100)
Diasecticide <sup>™</sup>	0.5	45 b,c (13-75)	53 b (29-77)	32 b,c (14-41)	38 c,d (23-55)
	1	61 c (24-84)	64 b,c (32-89)	56 c,d (17-71)	64 d,e (32-75)
	2	94 d (86-100)	96 d (89-100)	84 d,e (65-96)	86 e,f (65-100)
<b>Slurry</b>	0	21 a,b (8-35)	31 a,b (23-40)	2 a,b (0-4)	22 a,b,c (4-43)
Silico-sec <sup>®</sup>	2.5	28 a,b,c (0-71)	32 a,b (4-81)	0 a	4 a,b (0-13)
	5	98 d (92-100)	99 d (95-100)	12 a,b,c (4-28)	30 b,c,d (4-48)
	10	97 d (88-100)	97 d (88-100)	20 a,b,c (4-40)	26 a,b,c,d (9-52)
Diasecticide <sup>™</sup>	2.5	64 c (33-76)	89 c,d (57-100)	3 a,b (0-11)	6 a,b (0-15)
	5	88 c,d (80-100)	99 d (95-100)	13 a,b (0-65)	13 a,b,c (0-56)
	10	74 c,d (37-91)	92 d (63 -100)	3 a,b (0-9)	5 a,b,c (0-14)

Means within an entire column followed by the same letter are not significantly different ( $P = 0.05$ )

## DISCUSSION

The methods used in these experiments were based on those developed by an international working group. However, modifications to the procedure were made to allow for assessing high doses of dry dusts against the insects and when testing against mites. Problems were experienced in the mite bioassays with trying to retain the mites within the petri dishes. This was particularly the case with the fast moving *L. destructor*, which tended to escape before the lids were put on. However, this was improved by placing the dishes over frozen blocks of concrete which slowed mite movement. Also, if a complete seal was not made between the two treated halves with the 'Parafilm', the mites escaped during the exposures, resulting in lower numbers recovered from those initially introduced. Large variations were also observed between some of the replicates.

Relatively high control mortalities were observed with *O. surinamensis* and *E. kuehniella*. It is known that *O. surinamensis* does not survive well where a suitable food source is lacking (Armstrong and Howe, 1963). With *E. kuehniella*, larvae were seen to be feeding on other larvae, presumably again because of the lack of a suitable food source. This resulted in lower numbers of larvae assessed at the end of the exposures than those initially introduced resulting in higher mortality values and also large variations between replicates.

Against all the pest species, the Silico-sec<sup>®</sup> dry dust was the most effective treatment, producing higher mortalities at lower doses compared to the other treatments. Against the insect species, the lowest dose of 5 g/m<sup>2</sup> was as equally effective as the highest dose of 20 g/m<sup>2</sup>; and with the mites 0.5 g/m<sup>2</sup> was as effective as 2 g/m<sup>2</sup>.

This lack of an increasing dose response has been seen in other work (Cook, 2003; Mewis and Ulrichs, 2001), and may be due to the pests only being able to pick up a limited amount of dust on their bodies, depending on their surface area. Le Patourel et al., (1989) found that the processes of pick up and loss of amorphous silica differed from those of talc dust when applied to wheat and did not follow a first order rate process. The silica adhered to the insects in such a way that it was less likely to be lost than talc during movement through the grain. Once the available area on the insect is covered, the dust is unlikely to be lost so, increasing the amount on the surface would have little effect. Likewise, in the present DE experiments, the insects exposed to 5g/m<sup>2</sup> appeared as equally covered as those exposed to 20g/m<sup>2</sup> even though more dust was available. Therefore only in situations where some of the pests have not picked up the maximum possible amount of dust, would a higher dose be required.

Diasecticide<sup>™</sup> was less effective than Silico-sec<sup>®</sup> against the more tolerant species, with higher doses required to produce equivalent mortalities. It was a much coarser heavier DE than Silico-sec<sup>®</sup> and so a smaller volume of Diasecticide<sup>™</sup> was applied per unit surface area, which may in part have explained the

lesser efficacy. For dusts that are not very absorbent, or those that contain coarse particle sizes, easy removal from the cuticle, due to insect activity, may also result in reduced mortality (Subramanyam and Roesli, 2000).

Ranking was observed in the tolerances of the different insect species to these DEs as reported by other workers with other products (Fields and Korunic, 2000; Shawir et al., 1988; Subramanyam and Roesli, 2000). *Oryzaephilus surinamensis* was the least tolerant species with *S. granarius* and *T. castaneum* the most tolerant. With the mites, *A. siro* appeared less tolerant than *L. destructor* which is consistent with other work (Collins et al., 2001; Cook and Armitage, 1996, 1999).

In agreement with previous work (McLaughlin, 1994; Fields and Korunic, 2000), reduced efficacy was observed with the slurries compared to the dry dust formulations. The slurries formed a smooth, uniform coverage over the surface of the dishes and initially the insects appeared to be able to walk over the surface without picking up much DE. As the exposure period increased, however, the surface appeared to break up, probably due to the movement of the insects, and more of the DE was picked up. Longer exposure periods may therefore be required with slurries to obtain similar mortalities to those achieved with dry dusts. However, slurries are likely to be valuable where practice dictates i.e. where there is a need to avoid exposure to dusty atmospheres.

The efficacy of DE is also known to be affected by storage conditions (Le Patourel, 1986; Fields and Korunic, 2000), with the typical UK conditions of low temperature and high humidity considered to be particularly challenging. For example, at 30°C and 50% rh, Cook (2003) recorded complete mortality of *T. castaneum* and *S. granarius* when exposed to dishes treated with 1-10 g/m<sup>2</sup> Silico-sec<sup>®</sup> for 48 hours, followed by a 7 day recovery period. In comparison, at these cooler and damper conditions of 15°C and 80% rh, complete mortality was not achieved, even with a longer, 7 day exposure period.

In conclusion, this first phase of laboratory testing identified the most tolerant insects and the most appropriate range of doses for subsequent testing on more challenging surfaces. These were adults *S. granarius*, *L. destructor* and larvae of *E. kuehniella*, with doses of 10-20 g/m<sup>2</sup> against the weevil/ moth and 1-3 g/m<sup>2</sup> (dry dust ) or 20 g/m<sup>2</sup> (slurry) against the mite, depending upon DE product.



## **Part 2: LABORATORY ASSESSMENT OF WET AND DRY DE APPLICATIONS ON WOODEN SURFACES**

**D.A. Collins & D.A. Cook**

### **INTRODUCTION**

The first phase of laboratory experiments assessed the efficacy of the DEs against a range of pest species under typical UK storage conditions on filter paper. However, efficacy on glass and plastic surfaces may be greater than on other surfaces with a more 3-dimensional construction (Gowers and Le Patourel, 1984). On flat surfaces, insects take longer to right themselves after a fall, thereby picking up more dust and replenishing dislodged dust during the activity of righting themselves (Gowers and Le Patourel, 1984). On more convoluted surfaces, insects take shorter times to right themselves, thereby picking up less dust (Gowers and Le Patourel, 1984).

Therefore, in order to provide a dose recommendation for field validation trials, the aim of these laboratory experiments was to provide a more rigorous test of efficacy by applying slurry and dry dust treatments to wood, which is a more 3-dimensional surface, against the most tolerant insect and mite species identified in the first phase of the study.

### **MATERIALS AND METHODS**

#### ***Insects and mites***

Mixed sex adults of *S. granarius* and *L. destructor*, and larvae of *E. kuehniella* were used and acclimatised to experimental conditions as before (Part 1).

#### ***Diatomaceous earths***

Slurry and dry dust applications of Silico-Sec<sup>®</sup> and Diasecticide<sup>™</sup> were assessed. The most appropriate dose for each species as selected from the first phase of testing and an untreated control were assessed, with three replicates of each treatment.

#### ***Substrate***

The substrate consisted of 4mm thick plywood which was confirmed by the suppliers as not having been treated with fungicide. The wood was supplied as 300mm and 75 mm squares for the insect and mite bioassays respectively. The surface of the wood was wiped to remove any debris and then put into the experimental conditions of 15°C and 80% rh for at least two weeks prior to treatment.

### ***Insect bioassays***

The sides of plastic petri dishes (135mm diameter, 17mm high) were coated with 'Fluon' (PTFE), for those used to contain *S. granarius*, and white paraffin for *E. kuehniella*, to prevent the insects escaping from the treated surfaces. Two dishes were prepared for each species for each plywood square so that four dishes were used on each square with a total of 12 dishes used for each treatment.

The methods of application were as before (part 1). For the slurry application, the required amount of DE was placed in the centre of a wooden square. The DE was then mixed with 10 ml of water and spread over the surface using a gloved fingertip to give what was judged by eye to be an even coating. The water was evaporated by placing the square in an oven (~ 80°C) until all the water had evaporated.

For the dry dust applications, the required amount of dust was sprinkled evenly over the wooden surface by sieving through a 250µm mesh sieve. The treated squares were then stored in the experimental conditions of 15°C and 80% rh until needed for the one day, 4 week and 12 week post-treatment assessments.

At each assessment period, 25 insects of each species were placed under separate upturned petri dishes (Plate 2.1). After 7 days exposure the numbers of live, knocked down and dead insects were recorded. An insect was considered knocked down if it was on its back and unable to right itself even if aided with a small brush. It was recorded as dead if no visible movement was detected.

**Plate 2.1. Insects exposed to treatment on wooden square (Silico-Sec<sup>®</sup>, slurry)**



The insects from each replicate were then transferred to 50 g of wheat in 120 ml jars, and the jars were sealed with filter paper lids. The jars were left in the experimental conditions for a 7 day recovery period after which time the numbers of live, knocked down and dead insects were again assessed.

### ***Mite bioassay***

The methodology used for the treatments was the same as that used in the insect bioassays except that for the slurry applications, 1 ml of water was used due to the smaller size of the squares.

For each dry dust treatment, the inner surface of three glass rings (60 mm diameter, 20 mm high) were coated with white paraffin to prevent mites escaping off the treated surface. A single ring was then fixed onto each wooden surface using 'Pritt stick' glue (Plate 2.2). Twenty-five mites were then placed inside rings on separate squares using a single haired brush. A filter paper was then glued to the top of the ring.

For each slurry treatment, two treated squares were used for each replicate. An EPDM (E-A-P International) 'washer' (33 mm diameter, 2 mm high) was glued to the treated surface of one square and 25 mites were placed within the washer on each surface (Plate 2.2). The other square was then placed on top of the washer with the treated surface facing downwards and the two squares were held together with bulldog clips.

**Plate 2.2. Mite arenas - (A) dry dust treatments (B) slurry treatments.**

**(A)**



**(B)**



After 24 hours exposure, the numbers of live and dead mites were recorded. All the mites were then transferred to recovery cells consisting of a chamber made of a piece of Whatman no. 1 (42.5 mm diameter) filter paper. The filter paper was moulded to create a depression, 2 mm deep and 33 mm in diameter and secured using 'Pritt stick' glue to a glass square (75 x 75 x 4 mm), in the centre of which a hole 35 mm had been drilled. A small amount of mite food (wheat germ and yeast) was added to the chamber which was then covered with a thin glass square (75 x 75 x 2 mm) and secured with bulldog clips. The numbers of live and dead mites were then recounted after a further 24 hours in the experimental conditions.

### ***Analysis of data***

The mean percentage mortalities were calculated for each pest species and treatment and were then subjected to angular transformation ( $\sin^{-1} \sqrt{p}$ ). Mortality data was not corrected for the control mortalities as the

untreated controls were statistically compared to the treatments. The transformed data were statistically compared using analysis of variance (ANOVA) at the 5% probability level with individual comparisons made using Tukey's pairwise comparisons.

## RESULTS

### *Insects*

Insect mortalities increased after the recovery periods because individuals recorded as 'knocked down' after the initial exposure, were unable to recover from the effects of the treatment and subsequently died. Against *S. granarius*, the Silico-sec<sup>®</sup> dry dust was the more effective treatment, with mean mortalities of 80-95%, whereas the Silico-sec<sup>®</sup> slurry produced mean mortalities of 29-46% following the recovery period, at the different times after treatment. For this species, the Diasecticide<sup>™</sup> dry dust and slurry treatments were ineffective with a maximum mean mortality of 11% which, in general, did not differ significantly from the untreated controls, even though twice the dose compared to that of Silico-sec<sup>®</sup> was assessed.

**Table 2.1. Mean % mortality of *S. granarius* exposed to dry dust and slurry treatments of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> when applied to wood, after seven days exposure followed by a seven day recovery period, one day, four weeks and 12 weeks after treatment (n=6).**

Treatment	Dose (g/m <sup>2</sup> )	Time after treatment					
		One day		4 weeks		12 weeks	
		7 day exp	7 day rec	7 day exp	7 day rec	7 day exp	7 day rec
Control	0	1 a (0-4)	3 a (0-8)	0 a	0 a	2 a (0-8)	1 a (0-8)
Silico-sec <sup>®</sup> dry dust	10	35 c (20-64)	82 c (72-88)	56 c (40-68)	95 d (88-100)	43 c (28-56)	80 c (68-88)
Diasecticide <sup>™</sup> dry dust	20	2 a (0-4)	5 a (0-8)	1 a (0-4)	5 b (0-8)	3 a,b (0-4)	11 a (0-24)
Control	0	0 a	0 a	0 a	1 a,b (0-4)	1 a (0-4)	2 a (0-4)
Silico-sec <sup>®</sup> slurry	10	17 b (4-40)	46 b (28-68)	14 b (0-28)	46 c (20-68)	8 b (4-12)	29 b (20-44)
Diasecticide <sup>™</sup> slurry	20	0 a	1 a (0-4)	0 a	0 a	1 a (0-4)	2 a (0-4)

Means within an entire column followed by the same letter are not significantly different (p>0.05)

The dry dust treatments were equally effective against *E. kuehniella* with mean mortalities of 99-100% after the recovery periods at the different times after treatment (Table 2.2). The slurry treatments killed 83-97% and 34-61% of insects for Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> respectively after the recovery periods. However, relatively high control mortalities ranging from 22-31% were also recorded.

**Table 2.2. Mean % mortality of *E. kuehniella* exposed to dry dust and slurry treatments of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> when applied to wood, after seven days exposure followed by a seven day recovery period, one day, four weeks and 12 weeks after treatment (n=6).**

Treatment	Dose (g/m <sup>2</sup> )	Time after treatment					
		One day		4 weeks		12 weeks	
		7 day exp	7 day rec	7 day exp	7 day rec	7 day exp	7 day rec
Control	0	12 a (5-19)	22 a (10-33)	26 a,b (8-44)	27 a,b (16-46)	7 a (0-13)	31 a (22-38)
Silico-sec <sup>®</sup> dry dust	10	100 d	100 c	99 e (96-100)	100 d	99 d (96-100)	100 c
Diasecticide <sup>™</sup> dry dust	20	93 c (84-100)	100 c	89 d (84-100)	99 d (96-100)	96 c,d (92-100)	100 c
Control	0	12 a (0-31)	22 a (10-30)	9 a (0-24)	13 a (0-29)	6 a (0-13)	37 a (8-55)
Silico-sec <sup>®</sup> slurry	10	75 b (58-88)	91 b (83-100)	55 c (40-76)	83 c (68-96)	88 c (80-100)	97 c (92-100)
Diasecticide <sup>™</sup> slurry	20	14 a (5-25)	34 a (24-50)	30 b (17-54)	35 b (21-54)	21 b (5-33)	61 b (29-77)

Means within an entire column followed by the same letter are not significantly different (p>0.05)

### **Mites**

Against *L. destructor*, the dry dusts were significantly more effective than the slurries, with no significant difference in efficacy between the two products after the recovery periods, even though the Diasecticide<sup>™</sup> was applied at three times the dose of Silico-sec<sup>®</sup> (Table 2.3). However, 24 and 20% of mites died in the controls four and 12 weeks respectively after treatment although these were significantly lower than in the DE treatments. Both slurry treatments were ineffective with 10% or less mites killed, which did not differ significantly from the untreated controls.

**Table 2.3. Mean % mortality of *L. destructor* exposed to dry dust and slurry treatments of Silico-sec<sup>®</sup> and Diasecticide<sup>™</sup> when applied to wood, after 24 h exposure followed by a 24 h recovery period, one day, four weeks and 12 weeks after treatment (n=3)**

Treatment	Dose (g/m <sup>2</sup> )	Time after treatment					
		One day		4 weeks		12 weeks	
		24 hr exp	24 hr rec	24 hr exp	24 hr rec	24 hr exp	24 hr rec
Control	0	3 a (0-5)	10 a (4-22)	3 a (0-4)	24 a (8-36)	0 a	20 b (13-36)
Silico-sec <sup>®</sup> dry dust	1	94 c (91-96)	97 b (96-100)	94 b (83-100)	100 b	91 c (89-95)	93 c (91-95)
Diasecticide <sup>™</sup> dry dust	3	46 b (25-59)	68 b (44-86)	82 b (81-85)	93 b (85-100)	72 b (62-87)	86 c (81-96)
Control	0	0 a	3 a (0-5)	0 a	4 a	0 a	0 a
Silico-sec <sup>®</sup> slurry	20	4 a (0-9)	6 a (0-10)	0 a	7 a (0-12)	0 a	8 a,b (4-14)
Diasecticide <sup>™</sup> slurry	20	2 a (0-5)	5 a (0-10)	1 a (0-4)	3 a (0-4)	0 a	10 a,b (0-23)

Means within an entire column followed by the same letter are not significantly different (p>0.05)

## DISCUSSION

Relatively high control mortalities were observed with *E. kuehniella* throughout the experiment and with *L. destructor* at 4 and 12 weeks with the dust treatments. Moth larvae were seen to be feeding on other larvae which was presumably due to the lack of a suitable food source; and in some replicates, not all the mites were recovered during the assessments. These factors resulted in lower numbers recorded at the end of the exposures than those initially introduced, resulting in higher mortality values and also large variations between replicates. However, these factors did not significantly affect the comparison as the statistical analysis showed the DE treatments to produce significantly greater mortalities compared to the controls.

As in the first phase of experiments, the Silico-sec<sup>®</sup> dry dust was the more effective treatment against all the pest species producing higher or equivalent mortalities at a lower dose compared to the other treatments. A dose of 10 g/m<sup>2</sup> produced 100% and >80% mortality of *E. kuehniella* and *S. granarius* respectively with 1 g/m<sup>2</sup> producing >93% mortality of *L. destructor*. The slurries were also less effective than the dry dusts, forming a smooth uniform coverage over the surface of the wood, which allowed the pests to walk on the surface without picking up much of the deposit.

Efficacy was consistent throughout the experimental period with, in general, no significant decline in effectiveness over the 12 weeks. Bridgeman (1994) reported that a Dryacide<sup>®</sup> slurry treatment of 6-8 g/m<sup>2</sup> to a storage structure under Australian conditions, provided effective insect control for at least 11 weeks, with McLaughlin (1994) suggesting that DEs can provide protection for at least 12 months at the appropriate concentration. The long term persistence afforded by the DEs would therefore reduce the need for further treatments.

Compared to the first experiments on glass and plastic, the Silico-sec<sup>®</sup> treatments were less effective against *S. granarius* on wood and Diasecticide<sup>™</sup> was even less effective than Silico-Sec<sup>®</sup> as noted in 'Part 1'. This is likely to be due to the insects being able to right themselves more easily on wood after a fall and therefore picking up less dust during the righting process. However, against *E. kuehniella*, all the treatments were similar to or more effective on wood than on glass and plastic. The larvae appeared to produce a lot of webbing, which incorporated DE particles, while moving over the surface which may have produced greater contact of the larvae with the dust causing higher mortalities. In contrast, against *L. destructor* the treatments were similar to, or less effective than the first phase of testing when applied to wood. During the assessments, mites were found within the grain of the wood which may have served as protection from the effects of the DEs.

The most challenging surface, wood and the most tolerant pest species were assessed in these experiments, to provide information on effective doses in a worst case scenario under the difficult cold and wet conditions pertaining in the UK. Any dose recommendations would need to be broad enough to encompass a range of potential infestation situations. It was concluded that for slurry treatments, higher doses and longer exposure times than tested here would be required, and that even this may not be completely effective. Therefore, focusing on the results against the most tolerant species *S. granarius*, a dose of 10 g/m<sup>2</sup> Silico-sec<sup>®</sup>, applied as a dry dust treatment, was decided to be the most appropriate for further investigations under practical, farm-scale conditions. However for Diasecticide<sup>™</sup>, the decision was more difficult, since even this high dry dust dose gave poor control of the most tolerant species tested, and there were concerns about trying doses >20 g/m<sup>2</sup>, due to the amount of airborne dust that would be produced by treatment. It was therefore decided that rather than increasing dose, the solution may be to see if recommendations based on longer exposure times would be appropriate. Therefore the same dose was to be tested for both products.

### **Part 3: FARM-SCALE EVALUATION OF DE TREATMENT ON AN EMPTY STORAGE FACILITY**

**D. A. Cook, L.E. Collins & D.A. Collins**

#### **INTRODUCTION**

The objective of the final phase of the project was to validate the dry dust dose 10 g/m<sup>2</sup> recommended by laboratory experiments in order to make practical recommendations to the industry. Unfortunately, there was not enough of the DE product, Diasecticide™ left at CSL for large scale testing, and difficulties were encountered in re-supply. However, during the course of the project, the authors had become aware of a third DE product called Demeter, that had also been launched onto the UK market, and large stocks of this material had been obtained. Therefore having put back the second treatment by a week, the decision was made that treatment could not be delayed any longer, and this third product, was introduced to the trial for testing along side Silico-sec®.

The aim of the trial was to test the two products against the least and most tolerant species from the laboratory studies, and to make final recommendations that could be applied regardless of DE used or pest species.

#### **MATERIALS AND METHODS**

##### ***Diatomaceous earths***

Silico-Sec® and Demeter, both freshwater DE based products containing 90% SiO<sub>2</sub> with less than 1% crystalline silica content, were obtained from the UK distributors.

It was intended that each of these products would be used to treat one of two identical storage areas at the same dose in each.

##### ***Insects***

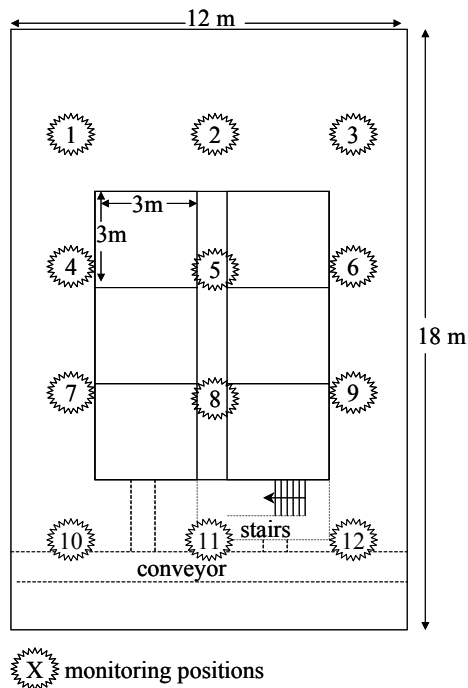
CSL laboratory strains of *S. granarius* and *O. surinamensis* were bred at constant laboratory conditions of 25°C and 70% rh in 2 large batches. The timing of these was to ensure that sufficient mixed age/sex adults were ready for release into the stores in mid-summer 2003 and early-spring 2004. It was intended that for each release, 100,000 of each species would be introduced into each store. However, for reasons unexplained, the first batch of insects was not as productive as expected and ca.120,000 *S. granarius* and ca.60,000 *O. surinamensis* were available. For the later Spring release, a greater number of insects were available and totalled ca.135,000 for *S. granarius* and ca.200,000 for *O. surinamensis*.



### ***Test site – CSL experimental grain store***

The test site was CSL’s experimental grain store, located at Sand Hutton, York, North Yorkshire. The store comprised two identical flat stores of 400 t capacity each and two identical undercover bin areas, of 120 t capacity in each. For this trial, the undercover bin areas were used, allowing comparison of the two different DE products, one applied to each. Each bin area measured 12 x 18 m and 3 storeys high with galvanised steel external walls along two opposite sides and separated from other parts of the store by fibre-cement internal walls. Within each area was a ‘nest’ of six 3 x 3 x 4 m galvanised steel open topped bins, serviced by bottom fed conveyors with stairs to the upper gantry at one end. The stores were cleaned using a combination of sweeping and pressure washing to ensure the structure and building fabric free from food and other dust residues. The environmental conditions of each bin area were monitored at floor level. A data logger (Eltel, 1000 series, model 1045) was located in the centre of each store, connected to thermocouples that measured temperature at each of the 12 monitoring positions, spaced at equidistant distances from the walls (Figure 3.1). In addition, rh was recorded at a single position in the centre of each store using a ‘Tiny talk’ logger (TK-0302).

**Fig. 3.1. Configuration of each bin store area and monitoring positions.**



### ***DE store treatment***

It was intended that each DE product be applied at 10 g/m<sup>2</sup> to the two bin areas, so that bin area 1 was treated with Silico-sec<sup>®</sup> and bin area 2 was treated with Demeter. Treatments took place 1 week apart on 21/7/04 and 28/7/04 for Silico-Sec<sup>®</sup> and Demeter respectively. For each treatment, the DE was applied to the outsides

and undersides of each nest of bins (Plate 3.1), the entire concrete floor area, and surrounding store walls to a height of 4 m, using a hand operated duster, model 'Echo D700'.

**Plate 3.1. Treating bin area 1, with the DE product Silico-Sec<sup>®</sup>.**



Plates of known surface area and weight were placed at each monitoring position and used to calculate dose variability by comparison of pre and post-treatment weight, immediately after treatment.

During the first DE treatment, the level of airborne dust was so great that visibility was reduced with the air taking on the appearance of thick fog. Although this quickly dissipated after treatment, it was decided that it would be useful to measure the amount of respirable dust during the second treatment with Demeter.

Therefore during the second treatment, respirable dust was monitored according to HSE method 14/3 (Anon, 2000) at a central point in the store using a cyclone respirable sampler, in conjunction with sampling pump (SKC, model 224-PCEX3) calibrated to run at 2.2 l/min. The measurement took place over the 2.25 hour treatment period, and the amount of respirable dust calculated from pre and post-treatment weights of the internal membrane filter. Twenty-four hours after treatment, the measurement was repeated. In addition images of the filter membranes were taken using a Philips XL20 scanning electron microscope (SEM), to confirm whether or not DE particles had been sampled.

#### ***Monitoring of introduced free-roaming insects***

During the trial, each weekly monitoring period was defined - Monday to Sunday, and free-roaming insect populations were assessed during the final 72 hour period. Three weeks before the first DE treatment, at the end of wc = 30/6/03, PC<sup>TM</sup> floor traps (also known as I-SPy Insect Indicators) incorporating lures were

placed at the 12 monitoring positions in each store and the “background” populations were monitored; this was repeated the following week. All insects trapped were returned to the stores.

On the 14/7/03, one week before the first DE treatment, ca.60,000 *S. granarius* and ca.30,000 *O. surinamensis* were released into each store. Prior to the release, the insects were removed from culture and batched into 24 equal lots of each species as measured by volume. These were transferred to glass jars that were closed with filter paper tops, which were moved into, and divided between the two stores for 48 hours to acclimatise to ambient conditions. The insects were released in equal quantities at the 24 monitoring positions. At the end of the week, the free-roaming populations were assessed as before.

From 28/7/03, additional traps were added to the 4 corners of the bin stores, and against the base of the longest 2 walls. Trap evaluations were repeated at weekly intervals after treatment in each store, until 100% control was observed in the contained bioassays as described in the following section. Thereafter, the free roaming populations were re-assessed on a monthly basis.

The residual action was assessed 37 weeks after treatment by a second release in early spring under more challenging cooler/damper conditions. This second assessment was timed to take place once the ambient temperature had started to warm-up above insect movement thresholds. Insects were released as before, but this time ca.67,500 - *S. granarius* and ca.100,000 - *O. surinamensis*, were introduced into each store. Populations were monitored weekly and then monthly as before, and the trial was terminated on 7/6/04.

### ***Bioassays with contained insects***

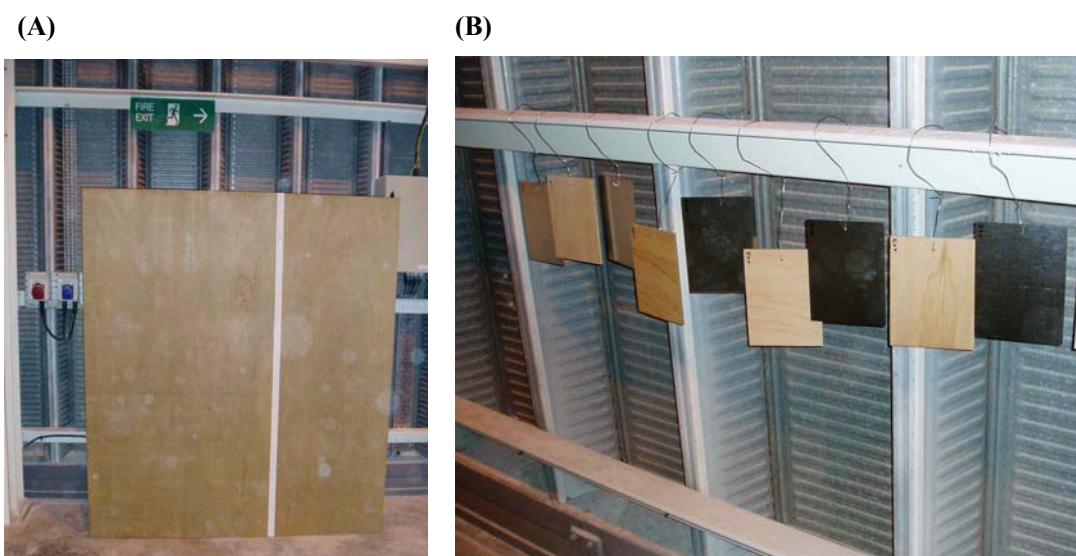
Surplus insects from the same batches of insects used in the 2 store releases were used to set up contained bioassays. At each monitoring position, two sets of paired bioassays were placed corresponding to; set (a), “summer assessment” and set (b), “spring assessment”. Each paired bioassay consisted of two 14 cm glass petri-dishes that had previously been coated with ‘Fluon’ (PTFE) to prevent insect escape. For each pair, one of the dishes was covered with its lid, and the other left uncovered during DE treatment, to give treated and untreated (control) dishes for comparison. Thus there were 4 bioassay dishes at each monitoring point, giving a total of 96 dishes. One hundred insects of each species were added to each dish of set (a) immediately after DE treatment, and set (b) were re-covered and left empty until the spring assessment. Insects were added to the latter either 36 or 37 weeks as before, when the second batch of free-roaming insects was introduced to the stores. It was decided that the two different products should be assessed together despite being separated by treatment time of a week, to ensure both underwent the same environmental conditions. On each occasion, the insects were acclimatised to store conditions for 24 hours before being added to the dishes. Approximately 1 g of coarsely ground wheat was added to each bioassay dish with the insects, to minimise control mortality.

### ***Dead space treatments using electro-static duster***

At the end of the trial, an electrostatic duster was compared to the hand-powered duster used in the original store treatments to treat simulated cavity spaces. The electrostatic duster was provided by Agil, and is marketed to improve adherence of DE to vertical sides. This simple device is based on a modification of a standard electric powered blower, where a plastic bucket with secured lid containing the DE, is attached to the outlet of the blower. At the attachment point towards the base of the bucket side, an internal t-pipe ensures that the air is blown around the inside of the bucket upwards, creating a vortex, and charging the dust as it rubs against the plastic sides. The ‘charged’ dust is then blown from a long plastic pipe attached to the top, further enhancing the static charge.

Four wall cavities were simulated by enclosing sections of external wall, with plywood boxes open at the back, measuring 2 m high x 2 m wide x 0.5 m deep, two in each bin area (Plate 3.2). This created cavities of 2 m<sup>3</sup> volume with internal surface areas 12 m<sup>2</sup> each, and with a small gap left at the wall interface to allow DE treatment. Within these cavities were suspended wooden and galvanized steel plates measuring 15 x 20 cm (n=5) hung vertically by galvanised steel wire from a steel beam running the length of the external walls (Plate 3.2). These had been pre-weighed in snap-seal plastic bags. For each pair of cavities, one was treated with the hand-powered duster used in the original store treatment and the other by electrostatic duster using the corresponding DE product for the bin area in which they were contained. The crevices were dosed at 10 g/m<sup>2</sup> for Silico-sec and 9 g/m<sup>2</sup>, for Demeter, confirmed by the amount of dust used during treatments.

**Plate 3.2. Dead space assessment – (A) closed off wall section simulating wall cavity; (B) steel and wooden plates contained within cavity.**



Twenty-four hours after treatment, the plates were recovered by carefully inserting them back into the plastic bags before detaching the plates, sealing the bags, and re-weighing to allow calculation of the amount of DE that adhered.

### ***Analysis of data***

Comparisons were made using analysis of variance (ANOVA) at the 5% probability level, whilst students t-test Spearman Rank Correlation Coefficient tests (using Minitab v.13) were used to examine the trap catch against the environmental data for statistically significant correlations. For the contained insect bioassays, data was corrected using the method of Abbott (1925) where control mortality was greater than 5%.

## **RESULTS**

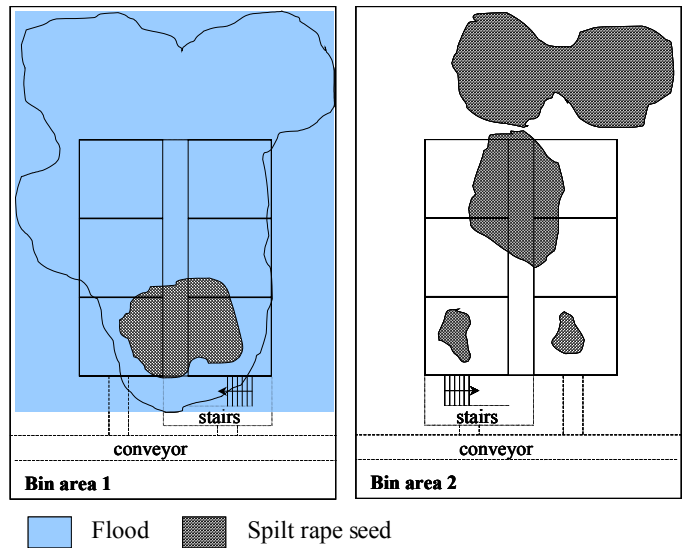
### ***Store Conditions***

Since this trial aimed to assess the performance of DE under practical conditions, normal store operations continued during the life of the experiment. The empty bins were filled with rape seed (area 1 & 2), and wheat (area 2) during August 2003. During conveying, rape seed was spilt on to the floor directly beneath the bins in both areas on a number of occasions (Figure 3.2). This was heaviest in bin area 2, where in contrast to the thin layers of residue elsewhere, it formed a layer 20 cm deep in the centre. Fortunately these spills did not interfere with the traps or bioassays and so were deliberately left to challenge the efficacy of the treatments by providing untreated food refuges. During the Christmas break (24/12/04 - 5/1/04) a burst pipe caused flooding of bin area 1, several centimetres deep. This took approximately a month to dry up, and again did not compromise the bioassays. Also, during January, an additional thin layer of rape seed was spilt on the floor in the upper part of bin area 2. Therefore, since parts of the treatment in bin area 1 had turned to slurry, and some of the rape seed had started sprouting, it was decided that both stores would undergo spot cleaning and careful re-treatment only of these cleaned areas, on 17/3/04, prior to the second insect release and spring assessment.

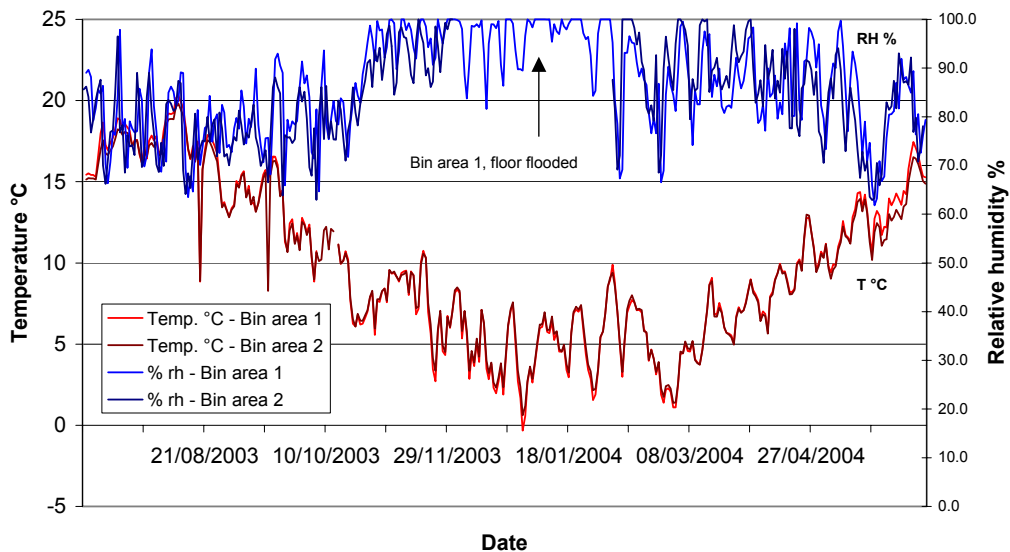
Environmental conditions of low temperature and high humidity were monitored for most of the trial. Mean daily temperatures peaked at ca. 20°C at the start of the trial, dropping quickly to 10°C by mid-October, to 5°C by late November, and to 0°C by 30/12/03 (Figure 3.3). With the exception of a few mild spells in February, store conditions did not start warming up until March, reaching 10°C by late April and 15°C by the beginning of June 04. Mean daily humidity fluctuated greatly throughout the trial, peaking at 100% frequently throughout the winter months and dropping below 65% rh on a few occasions in summer and

spring. However, when averaged across the whole trial, the mean rh was ca. 85% for both stores, illustrating typical UK store conditions.

**Fig. 3.2. Position of spilt rape seed during conveying (August) and flood caused by burst water pipe (end-December).**



**Fig. 3.3. Mean daily temperature and relative humidity in 2 identical bin stores treated with Silico-Sec® and Demeter.**



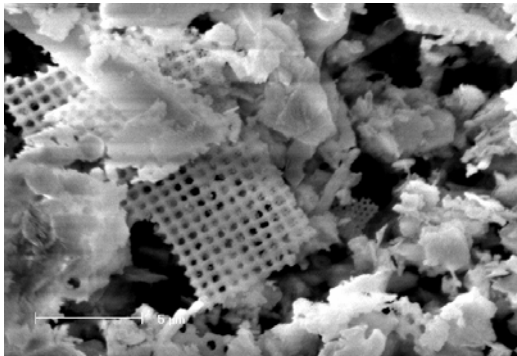
### ***DE store treatment***

Mean DE doses measured on the floor were 7.7 g/m<sup>2</sup> for Silico-sec<sup>®</sup> and 8.5 g/m<sup>2</sup> for Demeter (n=12). Both treatments were very variable and ranged 3.7 – 14.1 g/m<sup>2</sup> for Silico-sec<sup>®</sup> and 5 – 17.1 g/m<sup>2</sup> for Demeter. There was no significant difference between the mean dose for either treatment (p=0.57).

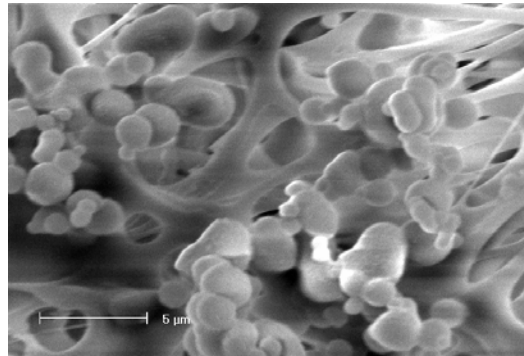
Comparison of pre and post treatment weights of the internal membrane filters used to sample respirable dust during the second DE treatment showed airborne levels at 86.7 mg/m<sup>3</sup> for the 2.25 hour period. Twenty-four hours later, airborne respirable dust had decreased to 2 mg/m<sup>3</sup>. However, examination of these membrane filters by SEM confirmed that the small amount of material collected 24 hours later were actually mould spores (Plate 3.3).

**Plate 3.2. SEM images of material sampled during monitoring of respirable dust – (A) during treatment, DE particles clearly visible; (B) 24 hrs after treatment, only mould spores visible.**

(A)



(B)



### ***Monitoring of introduced free-roaming insects – summer release***

Within 48 hours of the summer insect release and prior to DE treatment, high mortality of both species was seen. A large number of insects died in their tracks, whilst moving away from the release points (Plate 3.4). Many of those that did not die were subsequently observed, congregating at the bottom of store structures. Thereafter, low numbers of insects were trapped, and in addition to the two species of insect introduced, naturally occurring psocids (*Lepinotus reticulatus* and *Lachesilla pedicularia*) and moth larvae (*Plodia interpunctella*) were also detected.

**Plate 3.4. Movement of insects away from the release point, and accumulation at the base of an internal wall, pre-treatment, July 2003.**



Trap catches for the summer assessment were correlated against temperature, humidity and time to determine if DE treatment had an effect. Too few *S. granarius* were captured in bin area 1 to undertake statistical tests for any correlations (Figure 3.4). In both bin areas the mean temperatures recorded during insect monitoring decreased significantly with time after insect release (bin area 1:  $r_s = -0.782$ ,  $P = 0.008$ ; bin area 2:  $r_s = -0.875$ ,  $P < 0.001$ ). The average rh recorded during insect monitoring increased significantly with time after insect release in bin area 2 ( $r_s = 0.710$ ,  $P = 0.021$ ), although there was no significant correlation for bin area 1.

In both bin areas the numbers of *O. surinamensis* decreased significantly with time after insect release (bin area 1:  $r_s = -0.648$ ,  $P = 0.043$ ; bin area 2:  $r_s = -0.817$ ,  $P = 0.002$ ). There was no significant correlation between the number of *S. granarius* captured in bin area 2 and time after insect release. Naturally occurring numbers of *L. reticulatus* trapped decreased significantly with time in bin area 2 ( $r_s = -0.855$ ,  $P = 0.001$ ).

In both bin areas the numbers of *O. surinamensis* trapped decreased significantly with declining temperature (bin area 1:  $r_s = 0.706$ ,  $P = 0.023$ ; bin area 2:  $r_s = 0.688$ ,  $P = 0.019$ ). There was no significant correlation between the number of *S. granarius* captured in bin area 2 and temperature, and the number of *L. reticulatus* trapped decreased significantly with temperature in bin area 2 ( $r_s = 0.819$ ,  $P = 0.002$ ).

The number of *O. surinamensis* trapped decreased significantly as the average rh increased in bin area 1 ( $r_s = -0.686$ ,  $P = 0.028$ ). There was no significant correlation between rh and the numbers of *O. surinamensis* or *S. granarius* captured in bin area 2. The number of *L. pedicularia* trapped decreased significantly as the average rh increased in bin area 2 ( $r_s = -0.651$ ,  $P = 0.042$ ).



Fig. 3.4. Total number of insects, psocids and moth larvae trapped in the summer, immediately after treatment with Silico-Sec® (bin area 1) and Demeter (bin area 2). Mean temperature and humidity for each 72 hour monitoring period.

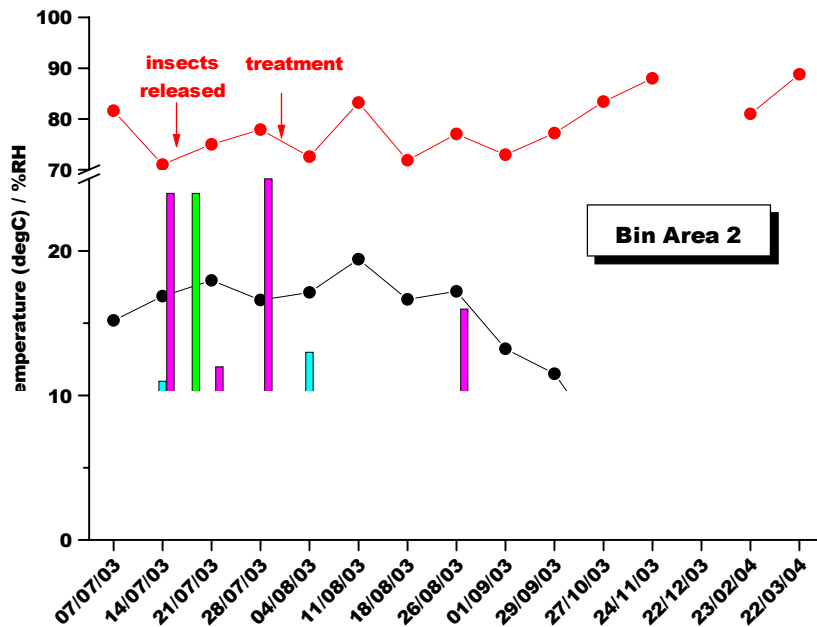
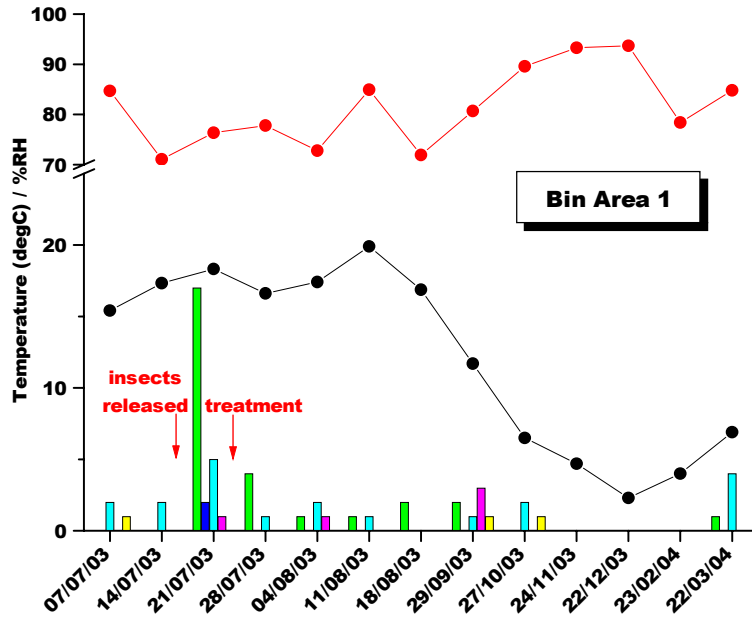
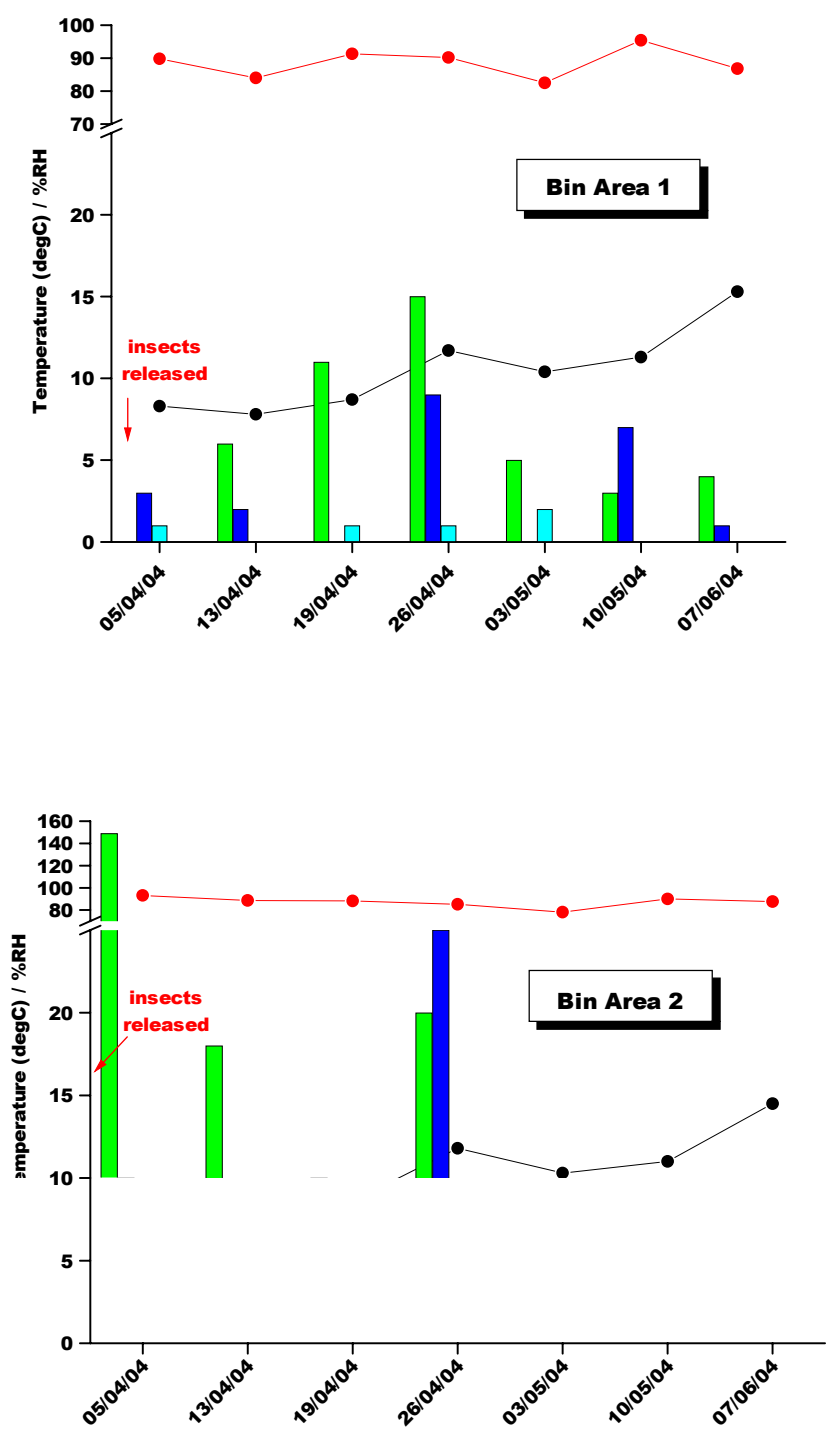


Fig. 3.5. Total number of insects, psocids and moth larvae trapped in the spring, 36/37 weeks after treatment with Silico-Sec® (bin area 1) and Demeter (bin area 2). Mean temperature and humidity for each 72 hour monitoring period.



**Monitoring of introduced free-roaming insects – spring release**

Again, too few *S. granarius* were captured in bin area 1 to undertake statistical tests for correlations. In both bin areas the mean temperatures recorded during insect monitoring increased significantly with time after insect release (bin area 1:  $r_s = 0.893$ ,  $P = 0.007$ ; bin area 2:  $r_s = 0.883$ ,  $P = 0.008$ ) (Figure 3.5). There was no significant correlation between the average rh recorded for either bin area.

In both bin areas the numbers of *O. surinamensis* trapped decreased significantly with time after insect release (bin area 1:  $r_s = -0.821$ ,  $P = 0.023$ ; bin area 2:  $r_s = -0.883$ ,  $P = 0.008$ ). There was no significant correlation between the number of *S. granarius* captured in either of the bin areas and time after insect release. The number of *L. reticulatus* trapped decreased significantly with time after insect release in bin area 2 ( $r_s = -0.757$ ,  $P = 0.049$ ). There was no significant correlation between the average temperatures and rhes recorded during insect monitoring and the numbers of any of the species captured.

**Bioassays with contained insects**

When freshly applied, both products achieved 100% control against *O. surinamensis* after 1 week’s exposure under summer conditions (Table 3.1). The exposure time needed for control of *S. granarius* was longer, taking 3 weeks for Silico-Sec® and 5 weeks for Demeter. However it is of note that after 3 weeks, mortality with Demeter was very high at >80%. Despite starting 1 week apart, there was only 1-2°C difference in mean weekly temperatures for the 2 stores for the corresponding weeks exposure time, and little difference in rh.

**Table 3.1. Bioassay results – time taken for mortality (Abbotts corrected for control mortality; n=12 except where marked thus\* n=8) of contained insects when exposed on dishes treated with Silico-Sec® (bin area 1) and Demeter (bin area 2), immediately after treatment during summer (21/7/03- 24/8/03).**

Weeks Exposure	BIN AREA - 1		2		1		2	
	% Mortality (n=12)				% RH (n=1)		Temp. °C (n=12)	
	<i>O. surinamensis</i>		<i>S. granarius</i>					
1	100	100	73	31	78	77	17.2	17.0
	-	-	(56-86)	(1-65)			(17-17.4)	(16.6-17.3)
2			100	69	79	80	17.3	18.9
			(98-100)	(22-95)			(17.1-17.5)	(18.4-19.3)
3			100	82*	80	74	19.3	17.6
			-	(21-99)			(18.8-19.3)	(17.3-17.9)
4				97*	75	76	17.8	16.1
				(85-100)			(17.5-18)	(16.2-16.6)
5				100*	77	76	16.3	14.4
				-			(16.5-16.9)	(13.8-14.9)

The pattern of mortality in the spring, for insects exposed to dishes that had been treated 36/37 weeks earlier, was very similar to the summer (Table 3.2). However, the mortalities were lower during the first 3 weeks of exposure and this time it took 2 weeks to completely control *O.surinamensis* with both products, and 100% control of *S. granarius* with Silico-Sec<sup>®</sup> took a week longer at 4 weeks.

**Table 3.2. Bioassay results – time taken for mortality (Abbotts corrected for control mortality; n=12 except where marked thus\* n=10) of contained insects when exposed on dishes treated with Silico-Sec<sup>®</sup> (bin area 1) and Demeter (bin area 2), 36/37 weeks after treatment during spring (26/3/04- 1/5/04).**

Weeks Exposure	BIN AREA -							
	1	2	1	2	1	2	1	2
	% Mortality (n=12*)				% RH (n=1)		Temp. °C (n=12)	
	<i>O. surinamensis</i>		<i>S. granarius</i>					
1	24*	14	2*	1	90	95	7.3	7.3
	(0-62)	(0-49)	(0-6)	(0-3)			(7.1-7.6)	(7-7.7)
2	100*	100	66*	37	84	88	7.4	7.3
	-	-	(53-82)	(15-56)			(7.1-7.6)	(7-7.6)
3			99*	89	83	88	9.0	8.9
			(94-100)	(74-96)			(8.7-9.3)	(8.6-9.4)
4			100*	98	89	86	9.0	8.9
			-	(83-100)			(8.8-9.1)	(8.7-9.2)
5				100	94	88	11.6	11.6
				-			(11.3-11.8)	(11.3-11.9)

In summary both species could be controlled within 5 weeks, regardless of product, the length of time since DE had been applied, or the time of year.

#### ***Dead space treatments using electro-static duster***

The doses achieved on the steel plates by both methods of application were extremely low and calculated to be 5 – 15% of that intended (Table 3.3 & 3.4). In contradiction, on wood, the post treatment weights indicated they were treated with around 6 times more than intended. However, visual observation of the amount of dust adhering to the wood in comparison to the amount adhering on the steel plates did not bear this out and it was probable that unexpected fluctuations in the ambient humidity had caused the wooden plates to absorb water from ambient therefore adding to the weight change.

Therefore, on the assumption that the wooden plates had absorbed moisture in equal quantities, comparison of weight change showed that the hand operated duster gave significantly higher dosing of Silico-sec<sup>®</sup> than the electrostatic duster, with no significant difference between the Demeter treatments on either substrate (p=0.05).

**Table 3.3. Comparison of the amount of DE deposited on vertically hung steel plates by electrostatic duster and hand powered duster, during treatment of simulated wall cavities.**

Treatment method	Mean weight change (g)	Mean calculated dose (g/m <sup>2</sup> )	Mean weight change (g)	Mean calculated dose (g/m <sup>2</sup> )
	<b>Silico-sec<sup>®</sup></b>		<b>Demeter</b>	
Electrostatic duster	0.024 <i>a</i> (0-0.05)	0.4 (0-0.8)	0.06 <i>a</i> (0.01-0.1)	1 (0.2-1.7)
Hand duster	0.064 <i>b</i> (0.04-0.08)	1.1 (0.2-1.3)	0.08 <i>a</i> (0.04-0.14)	1.3 (0.7-2.3)
<i>Students T-test (n=5)</i>	<i>P = 0.020</i>	-	<i>P = 0.533</i>	-

Means within an entire column followed by the same letter are not significantly different (p>0.05)

**Table 3.4. Comparison of the amount of DE deposited on vertically hung wooden plates by electrostatic duster and hand powered duster, during treatment of simulated wall cavities.**

Treatment method	Mean weight change (g)	Mean calculated dose (g/m <sup>2</sup> )	Mean weight change (g)	Mean calculated dose (g/m <sup>2</sup> )
	<b>Silico-sec<sup>®</sup></b>		<b>Demeter</b>	
Electrostatic duster	3.23 <i>a</i> (3.1-3.4)	53.9 (51.8-56.8)	3.45 <i>a</i> (3.28-3.62)	57.6 (54.8-60.5)
Hand duster	3.74 <i>b</i> (3.57-3.99)	62.4 (59.6-66.6)	3.37 <i>a</i> (3.22-3.5)	56.3 (53.8-58.5)
<i>Students T-test (n=5)</i>	<i>P = &lt;0.000</i>	-	<i>P = 0.408</i>	-

Means within an entire column followed by the same letter are not significantly different (p>0.05)

## DISCUSSION

After treatment, insect numbers declined in both stores for the period summer to early winter, taking approximately 3 months until insects were no longer trapped. The total number of each species trapped on any occasion was very low and <10 in all cases, with the exception of naturally occurring *L. reticulatus* and

*L. pedicularia* (psocids) trapped on 2 occasions during August in bin area 2. This decline in insect numbers corresponded with a fall in temperature, with significant correlation for *O. surinamensis* as average temperatures dropped below the lower movement threshold (chill-coma) of 6 – 7°C for this species (Evans, 1983). Taken alone, therefore, the trapping records might only have reflected a decline in insect activity with falling temperature rather than reflecting the efficacy of the DE treatments. However, after the second release of insects in early spring, there was a significant fall in numbers despite a rise in temperature from 8°C to 15°C. By the end of the trial, 2.5 months later, there were no insects trapped in bin area 2, with only one or two trapped in bin area 1.

Confirmation that the reduction in insects trapped was mainly due to the DE treatment comes from the corroborating evidence from the contained bioassays. When insects were exposed immediately after treatment, and 36/37 weeks later in the spring, 100% control of *O. surinamensis* was achieved within two weeks exposure and *S. granarius* within 5 weeks, regardless of product. This efficacy of insects retained on treated surfaces also illustrates the importance of other factors such as store design. These contained bioassays were representative of the perfect treatment scenario, where insects are unable to move off the treated surface. In reality, structure treatments are rarely perfect and often treatments fail to penetrate into cracks and crevices, allowing insects to move off treatment and take refuge in these untreated dead spaces. Movement off the treated surface would reduce the amount of DE picked up by an escaping insect and affect efficacy. This probably explains why it took longer for the free-roaming insects to decline, compared to the bioassays.

Effective dead space treatments will therefore be essential to the adoption of DE for protecting grain store structures. During a previous HGCA-LINK project (Bell et al., 2004; Cook, 2003), DE was assessed in conjunction with heat for the treatment of cracks and crevices in the structures of flour mills. This included the evaluation of various DE applicators for treating cavities only a few centimetres thick, such as those between internal dry-lined walls. Whilst assessing suitability, conventional dusters were judged too powerful for targeted treatment and a smaller CO<sub>2</sub> powered applicator, marketed for treating wasp nests, was further validated during mill trials. So the treatment of larger cavities, more typical of those found in grain stores, during this trial, built usefully upon the previous work. Since the CO<sub>2</sub> powered applicator did not have a large enough dust carrying capacity, this experiment presented an excellent opportunity to assess the electrostatic duster against a conventional duster.

Firstly, during treatment the electrostatic duster was much easier to use; movement of the hand-powered duster's cranking handle was restricted against the store side and the applicator was difficult to manoeuvre. Visual examination of the inside of the cavities after treatment, showed that for both applicators an even coating was applied to the internal walls, although this was heaviest on the floor. Quantitative assessment of the vertically hung steel and wooden plates showed that the electrostatic duster did not significantly increase

adherence to vertical surface in this instance. This was surprising since Agil's in-house trials have found this duster to improve adherence to the sides of steel bins (Hyden, personal communication). It is of note however, that these treatments were much closer to the target surface than the recommended 1-2 m. Finally, the low doses achieved on the steel plates illustrate the difficulties in getting dry dust applications to adhere to vertical surfaces.

The results from this trial compare well to a pilot trial 2 years earlier in the same store (Cook et al., 2003). In the earlier trial, the DE Silico-sec<sup>®</sup> was applied to the external surface and structure of the bins and their under-floor space in bin area 1 to control an infestation of *O. surinamensis* that migrated from a 20 t bulk of 'heating' insect infested wheat, during mid-winter. Quite by chance, the DE had been applied to the structure at a comparable dose of 9.6 g/m<sup>2</sup>, the authors having under-dosed from an intended application rate of 20 g/m<sup>2</sup>. In this trial it took 3 weeks for 100% control of insects retained on bioassays, and significant reductions in free-roaming numbers were observed.

In comparison to published work in Australia, much higher doses were required in these UK trials. For example, Dryacide<sup>®</sup> applied at 2 g/m<sup>2</sup> (to give a wall dose of 1 g/m<sup>2</sup>) as a dry dust to empty bins, gave dramatic reduction of psocid populations 3 weeks after treatment (Desmarchelier *et al.*, 1992). Three of the six treated bins remained psocid free for up to 17 months and the treatment also prevented infestation by Coleoptera for two years at 15 - 24°C and 60 - 65% r.h. When applied as a slurry to empty storage "sheds at 6 - 8 g/m<sup>2</sup> the percentage of traps infested with Coleoptera before and after treatment fell from 18% to 3%, an 84% reduction in the proportion of infested traps (Bridgeman, 1994). Although environmental data were not reported on, conditions would have been much drier and warmer than in these UK trials.

The monitoring of respirable dust levels during store treatment is the first published data of health surveillance during DE treatment that the authors are aware of. The fact that the OEL was exceeded underlines the importance of wearing suitable personal protective equipment (PPE). This was not unexpected, but more interestingly airborne dust had dropped to safe levels within 24 hours of treatment.

When considering guidelines for DE structure treatment, the importance of hygiene and monitoring as part of an integrated pest management program should not be under-valued. Thorough store cleaning at the beginning of the trial played an important factor in the success of the treatments. This was evident from the heavy mortalities observed visually during the first release of insects before store treatment. It is probable that the cleaning deprived them of essential food and water and placed them under additional stress. In addition, the presence of food may reduce the efficacy of DE as in some instances it will present opportunity for insects to burrow into an untreated medium and dislodge previously picked up dust, escape further exposure, as well as allowing replenishment of lost water through feeding (Arthur, 2000). It is also important to note that the risk from residual pests is not just related to the numbers that are present. A single egg

bearing female is all that is needed to infest stored grain, and so monitoring with floor traps is essential to give early warning of pests and allow timely treatment.

## CONCLUSIONS

In conclusion, the results from this trial suggest that DE is an ideal candidate for treating grain store structures if used as part of an integrated strategy. Ideally, they should be used in line with best practice as summarised in the revised HGCA grain storage guide (Armitage and Wildey, 2003) –

- Clean out stores between harvest, ensuring no food residues remain in cracks and crevices.
- Monitor the empty store with traps.
- Apply DE as a dry dust at 10 g/m<sup>2</sup> if insects are found (only apply as a slurry where practice dictates).
- Where practical, leave for at least five weeks before filling with grain.
- Monitor the store after treatment.
- If large numbers are found in a particular area, investigate and, if necessary, re-clean and re-treat.

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