

**GRDC**

Grains  
Research &  
Development  
Corporation

# Ground Cover

NOVEMBER – DECEMBER 2010

GRAIN STORAGE SUPPLEMENT

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## A strategic approach to storage



Go to *Ground Cover TV DVD* for more information on silo hygiene, sealed silo pressure testing, cooling aeration in silos and storage insects.



## Action required to keep phosphine as an option



BY DR JODY HIGGINS, MANAGER NEW GRAIN PRODUCTS, AND TOM McCUE, MANAGER EXTENSION AND GROWER PROGRAMS, GRDC

Grain storage is at the interface between the grower and the end-user. Therefore, it is especially important that growers not only implement best storage practice but do this in relation to the needs and demands of the market.

The combination of increased resistance, more on-farm storage and the retention of a zero tolerance of live insects in shipments of export grain means that all parts of the grain value chain need to understand and apply best management practice for grain storage.

Throwing a few phosphine tablets in an unsealed store and hoping for the best has never been an acceptable approach to treating grain for insects and, at best, only kills adult insects.

With levels of resistance increasing and greater integrity demands from international and domestic markets, this off-label practice must be stopped. If not, phosphine is likely to at least become unavailable to growers, or at worst become totally ineffective at controlling grain insect pests.

It is estimated that 80 per cent of Australia's grain production is currently treated with phosphine. Yet increasing

levels of resistance in grain storage insects to phosphine and other insecticides has been identified.

Is a \$7 million export market worth risking due to thoughtless and careless on-farm grain storage practices?

Managing grain during storage is just as important as managing the crop during the growing season. While most growers have stored seed and stockfeed, many are now storing large proportions of their harvest in order to capitalise on markets.

The GRDC is investing at all levels to help ensure high-quality grain storage on-farm and in bulk storage can be maintained.

As partner in the Cooperative Research Centre for National Plant Biosecurity (CRCNPB), the GRDC is supporting a range of post-harvest integrity research projects. These include projects that are improving our understanding of the ecology of stored grain pests, developing molecular tools and tests to rapidly identify resistant insects and investigating new and alternative control treatments.

Much of this work is building on previous research investments, many of which have produced clear and practical information on how best to manage stored grain with the current tools.

A comprehensive extension program has been established to harness and deliver this information to ensure grain growers have every opportunity to access and apply best management practice.

Practical, hands-on workshops are being delivered by grain storage specialists in each of the three GRDC regions. Whether a grower attends a workshop or not, these specialists can be contacted by individuals to help plan and introduce best practice in on-farm grain storage.

A dedicated website has been established where growers can source all the information they need about implementing excellent on-farm grain storage. A suite of new fact sheets linked to the extension program can also be sourced from the website ([www.storedgrain.com.au](http://www.storedgrain.com.au)).

By investing in this important area the GRDC aims to support growers in tackling the challenges of implementing excellent storage of grain be it for seed, stockfeed or human consumption.

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### ARTICLES AND PHOTOGRAPHS BY EMMA LEONARD, UNLESS OTHERWISE STATED



Many of the articles in this *Ground Cover* supplement have been sourced from the Cooperative Research Centre for National Plant Biosecurity's magazine *Plant Biosecurity: collaborative research initiatives*. An electronic copy is available online at [www.crcplantbiosecurity.com.au](http://www.crcplantbiosecurity.com.au)

COVER PHOTO: PAUL JONES



## A TARGETED MISSION TO IMPROVE ON-FARM STORAGE

**A new extension program is helping growers easily access best practice information on grain storage**

ON-FARM GRAIN STORAGE is not a ‘fill and forget’ activity. Like every other part of the process to produce high-quality grain, storage requires management and attention to detail if grain is to leave the store with the same quality as it entered.

With the deregulation of the marketing system, more growers are considering on-farm storage. This coincides with increasing resistance of grain storage insects to phosphine, the most widely used grain fumigant.

To help ensure that growers are aware of grain storage best practice and its implementation, a comprehensive extension program is being delivered. This program is tailored to the three GRDC regions as there are different issues in each.

At the heart of the extension program is clear, practical information based on extensive Australian research conducted over the past 20 years.

The extension program consists of three elements: workshops, support material including a dedicated website and team members supporting research staff to ensure a valuable two-way flow of up-to-date information.

Grain storage workshops are being run by Philip Burrill (Queensland Department of Employment, Economic Development and Innovation), Chris Newman (Department of Agriculture and Food, Western Australia) and independent consultant Peter Botta. Each presenter is keen to demonstrate that successful grain storage is about much more than just phosphine. Indeed, many insect control problems can be minimised with excellent hygiene and well-managed aeration cooling.

Issues covered at the workshops include monitoring and identifying pests, key hygiene sites, aeration cooling, effective phosphine fumigation, selection of storages and practical farm storage strategies that apply an integrated approach to controlling pests and maintaining quality.

The production of fact sheets, which support the information delivered at the workshops and which can be used as a standalone resource, is being managed by Chris Warrick of the Kondinin Group. The first five fact sheets deal with the most pressing issues – pest control, identification of stored grain pests, hygiene and structural treatments, aeration cooling and pressure testing sealable, gas-tight silos.

These fact sheets can be found at the new Stored Grain Information Hub website ([www.storedgrain.com.au](http://www.storedgrain.com.au)). The site has nine grain storage topic headings containing material from a range of sources.

The Pests and Diseases Image Library – PaDIL ([www.padil.gov.au](http://www.padil.gov.au)) – provides access to high resolution images to help with identification. This online library is an Australian Government initiative, with support provided by the Department of Agriculture, Fisheries and Forestry and Plant Health Australia, a non-profit public company. □

GRDC Research Code DAQ00158

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Learning how to correctly and safely store grain on farm is essential to ensure quality, insect and residue-free grain is delivered.

**MANY INSECT CONTROL PROBLEMS CAN BE MINIMISED WITH EXCELLENT HYGIENE AND WELL-MANAGED AERATION COOLING.**

# Successful grain storage

New grain storage fact sheets bring together the practical outcomes developed from recent research **By Philip Burrill, Chris Newman, Peter Botta and Chris Warrick**

## Meticulous hygiene

A bag of infested grain can produce more than one million insects during a year, which can walk and fly to other grain storages where they will start new infestations.

Meticulous grain hygiene involves removing any grain residues that can harbour pests and allow them to breed.

Insects are active in warm weather during spring and summer, breeding then flying to any new sources of grain. So any residual grain left in harvesters, bins, augers and so on creates a haven for over-wintering insects and a direct route into the following year's grain. Insects look for food, shelter and warmth.

Carryover seed wheat or barley is a favoured site.

Thorough grain hygiene combined with structural treatments, such as inert dusts and slurries, can play a key role in reducing the number of stored grain pests.

After harvest, grain handling and harvesting equipment should be cleaned to reduce breeding locations for insects. Silos and other storages should be cleaned as soon as they are empty. A physical clean followed by water will remove grain, trash and dust. Select a warm day to ensure the inside of the silo dries quickly. Emphasis must be placed on areas where grain can be trapped.

Once clean, a structural treatment can be applied.

Always check with potential grain buyers prior to applying grain insecticides either as structural treatments or as grain protectants.

In most cases, an inert dust such as diatomaceous earth (DE) (amorphous silica, commonly known as Dryacide®) can be applied, either as a dust or a slurry to treat storages and handling equipment for residual control.

If applied correctly with complete coverage in a dry environment, inert dusts can provide up to 12 months protection in an

empty store, killing most species of grain insects and with no risk of building resistance.

## OH&S

**Always have another person close by when entering an empty silo for cleaning. If possible use water pressure equipment, air blowers etc to prevent the need to enter the silo. Read the safety directions on the label before handling and applying structural treatments.**

## Check grain

Good hygiene also includes regular inspection of all stored grain, including seed and stockfeed grain, so any pest infestations can be controlled before pests spread.

Stored grain should be inspected at least once a month for pests and evidence of damage, moisture and temperature.

Sieving is the most effective method of detecting grain pests. Sieve samples from the top and bottom of stores to detect low levels of insects early. Insect probe traps pushed into the grain surface are also an easy method of detecting storage pests in grain sheds or silos.

Sieving samples on to a white tray makes it easier to see small insects. Holding the tray in the sunlight warms the insects and encourages movement, aiding identification.

Even if only one or two storage pests are detected in a storage then treatment is usually carried out as soon as possible, particularly in the warmer months as populations can increase very rapidly.

In winter and when grain temperatures are less than 15°C due to well-managed aeration, treatment can be delayed until temperatures increase or until marketing.

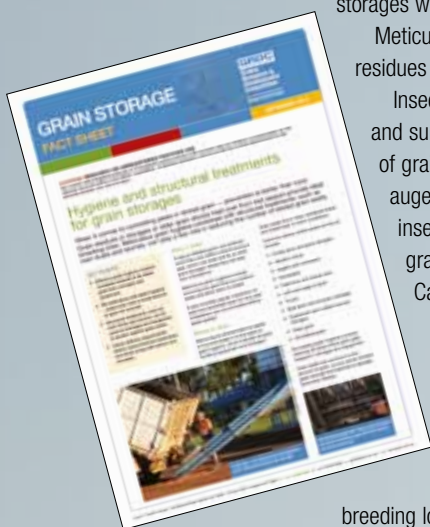
Use a grain temperature probe to check grain temperature. Freshly harvested wheat or barley usually has a temperature of about 30°C, which is an ideal breeding temperature for storage pests.

The optimum grain temperature for insect reproduction is between 25°C and 30°C. Research has shown that the rust-red flour beetle's life cycle is completed in four weeks at 30°C, but at 22°C the life cycle is slowed to 11 weeks, significantly decreasing population growth and insect activity. Breeding ceases at 20°C (see Table 1).

Aim for grain temperatures of less than 23°C during summer and less than 15°C in winter.

## OH&S

**Think safety when accessing the top of silos. Caged safety ladders and handrails should be installed. Safety harnesses may be required for various tasks. Silos containing mouldy wet grain can contain toxic gases or have a low oxygen atmosphere, so extra care must**



**Table 1 Low temperature can limit insect reproduction**

Insect type	Temperature at which reproduction is limited
Rust-red flour beetle	20°C
Bruchid – cowpea weevil	20°C
Lesser grain borer	18°C
Saw-toothed grain beetle	17.5°C
Flat grain beetle	17.5°C
Rice weevil	15°C

be taken. Personal safety monitors that sound an alarm are available for testing the atmosphere for a range of hazards, including potentially dangerous phosphine levels.

## Identify pests

Growers need to know which pests they have found because this will determine the treatments required. For example, the lesser grain borer (*Rhyzopertha dominica*) is a serious pest in most regions of Australia, but can now only be reliably controlled with one or two products due to resistance.

A clean glass container helps to identify grain pests. Live insects are placed into a warm glass container (above 20°C so they are active but not too hot or they will die). Weevils and saw-toothed grain beetles can walk up the walls of the glass easily, but flour beetles and lesser grain borers cannot.

Look closely at the insects as they walk up the glass: weevils have a curved snout at the front, but saw-toothed grain beetles do not.

## Aeration

Freshly harvested wheat and barley usually has a temperature of about 30°C, which is an ideal breeding temperature for storage pests.

During a trial where barley was harvested at 28°C and 16.0 per cent moisture, grain temperature rose to 40°C within hours of being put into storage, eventually reaching 46°C after two days. With no aeration, these high temperatures develop rapidly, causing serious damage to grain quality, germination and vigour. High grain moisture conditions also rapidly lead to the development of moulds.

Aeration pushes ambient air through the grain, reducing temperature and evening out moisture. With an adequate aeration cooling system fitted, grain temperature can be reduced in hours and any high-moisture grain held safely for short periods of time until blending or drying can be arranged. Use a grain temperature probe to monitor grain conditions.

Grain temperature can be reduced with an aeration cooling rate of two litres per second per tonne; reducing grain moisture requires aeration drying with aeration rates above 20 litres per second per tonne.

The best results are achieved when the process is controlled as aeration needs to continue throughout the storage period. Aeration controllers will achieve the most reliable results as they are designed to automatically select the best time to run aeration fans.

Lowering the temperature and moisture of stored grain results in fewer insect pests, consequently the need for multiple chemical treatments is reduced.

Unsealed and gas-tight sealed silos can be fitted with aeration equipment provided a rooftop vent that can be locked down for fumigation is installed.

## OH&S

Ensure all electrical wiring associated with aeration equipment in storages is professionally fitted so that it is not a potential hazard.

## Fumigation

Using the right type of storage is the first and most important step towards an effective fumigation.

Only use fumigants, such as phosphine, in a pressure-tested, gas-tight sealed silo. New sealable, gas-tight silos need to meet a five-minute, half-life pressure test to be classed as sealable and meet the new Australian Standard (AS2628).

Research shows that fumigating in a storage that is anything less than this standard does not achieve a high enough concentration of fumigant for a long enough period to kill pests at all life cycle stages. Fumigation in unsealed storages is not effective and increases numbers of phosphine-resistant insects.

For effective phosphine fumigation, a minimum of 300 parts per million (ppm) gas concentration for seven days or 200ppm for 10 days is required.

Fumigation trials in silos with small leaks demonstrated that phosphine levels are as low as 3ppm close to the leaks. The rest of the silo also suffers from reduced gas levels.

Achieve effective fumigation by placing the correct phosphine rates (as directed on the new label) on to a tray and hanging it in the top of a pressure-tested, gas-tight sealable silo. Bag chains or belts are an easy-to-use alternative to tablets. Ground-level application systems also require a sealed, gas-tight silo and may be used in conjunction with a gas recirculation system on larger silos (greater than 150 tonnes capacity). This provides a faster gas distribution throughout the storage.

After fumigation, open up the silo and ventilate grain for a minimum of one day with aeration fans running, or five days if no fans are fitted.

A minimum withholding period of two days is required after ventilation before grain can be used for human consumption or stockfeed.

The total time needed for fumigating is usually between 10 and 17 days.

As a general rule, only keep a silo sealed while carrying out the fumigation. Return silo to aeration cooling to maintain grain at a cool temperature, making it less attractive to insects and to preserve grain integrity and viability.

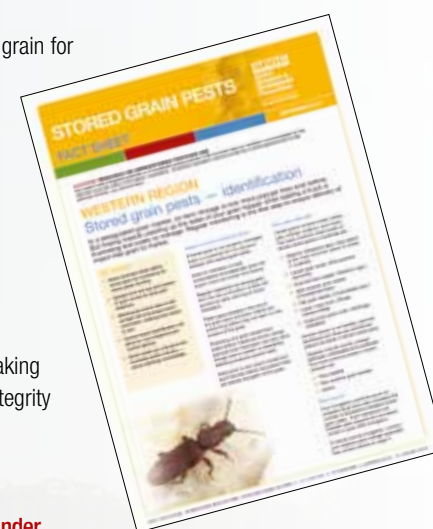
## OH&S

Use appropriate warning signs around storages under fumigation. Check product label safety directions and MSDA sheets. Keep good records of all treatments applied to grain.

GRDC Research Code DAQ00158

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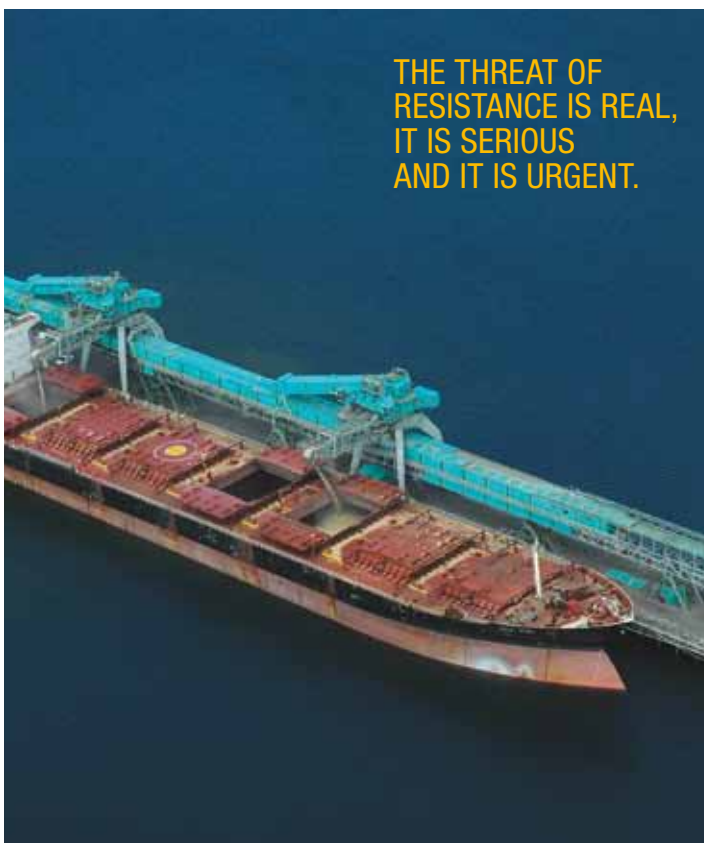
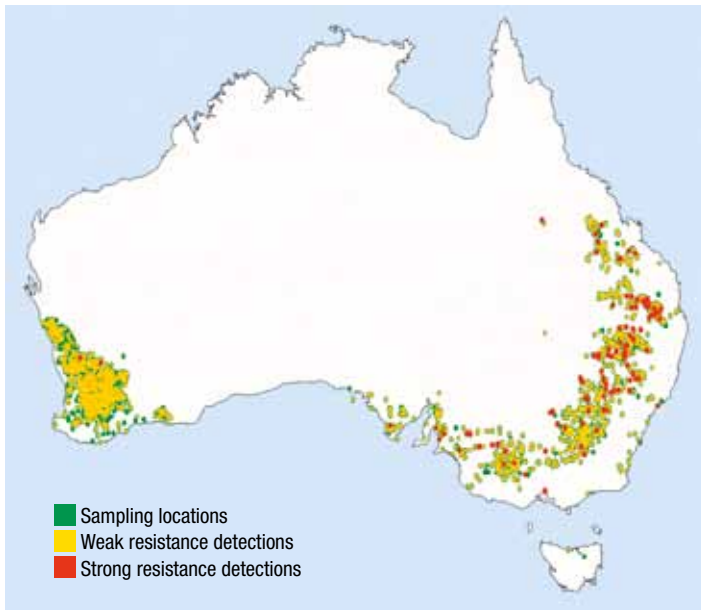
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# THE IMPORTANCE OF INSECT-FREE STORED GRAIN

A collaborative approach is being taken to maintain Australia's high grain-hygiene standards **By Pat Collins**

**Figure 1** Australian phosphine resistance situation – resistance detections in the past five years



AUSTRALIA EXPORTS ABOUT 60 per cent of its grain production annually into the highly competitive international marketplace. To ensure continued market access we provide the highest quality product, including freedom from insect infestation. Freedom from insect infestation is so important that all grain shipments are inspected before they leave port to ensure they meet the 'nil tolerance' standard. This same standard is also now demanded by most domestic markets.

Australian growers are at a major disadvantage compared with our overseas competitors such as Canada or the European Union because while their grain goes into storage in cooler weather, we harvest at the beginning of summer and store grain over the hottest period of the year. This results in conditions ideal for insect population growth, producing a very high insect pest pressure.

Our industry uses non-chemical methods to reduce insect pest populations, but currently these alone do not provide the standard of freedom required by both international and domestic markets.

Since the mid-1990s, phosphine use has become central to successful insect management in the grains industry – it is cheap, can be used on most commodities, results in low or no residue, is environmentally benign and is accepted by international markets.

This almost complete reliance on the use of phosphine places us in a very risky position. There is no practical replacement for phosphine; no other fumigant or contact insecticide can match its combined attributes.

However, the continued effectiveness of phosphine is now threatened by the development of resistance in insect pests. This places in jeopardy continued access to premium markets for Australian grain.

Although high levels of resistance were first detected in 1997, its frequency has increased and very strong levels of resistance are now appearing in some species.

The threat of resistance is real, it is serious and it is urgent. In response to this threat, the GRDC has, either directly or as part of its role in the Cooperative Research Centre for National Plant Biosecurity (CRCNPB), invested in a range of research and extension projects. These projects are aimed at managing resistance today and also looking to the long term, providing the knowledge we need to effectively manage resistance into the future.

Further information can be found on the CRCNPB website ([www.crcplantbiosecurity.com.au](http://www.crcplantbiosecurity.com.au)). □

GRDC Research Code NPB00004

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# STORAGE PESTS BEAT AROUND THE BUSH

Ecology studies have discovered stored grain insects many kilometres from the nearest silos, raising important questions about survival and their preferred food sources **By Greg Daghli**

THE LESSER GRAIN borer, a prime pest of stored grain, is just as likely to be found in native vegetation and farm paddocks as it is in the grain silos, a new study into the insect's behaviour has found.

The unexpected finding poses real challenges for efforts to control the insect. With increasing concern about phosphine resistance there was a need to understand the ecology of these insects if we are to manage them better.

The ecological research is the first to establish in any detail where these grain pests are located outside of grain silos.

This project is focusing on the ecology of the lesser grain borer (*Rhyzopertha dominica*) and rust-red flour beetle (*Tribolium castaneum*). These pests were selected for the study as bulk grain handlers identified these species as the most problematic in terms of developing resistance to fumigants.

This project is part of the CRC for National Plant Biosecurity's Post-Harvest Integrity Research Program for the grains industry, which focuses on insect resistance to phosphine.

The initial trapping of lesser grain borers and rust-red flour beetles began in September 2008 and concluded in December 2009. Traps were set in southern Queensland between Condamine and Moonie. In southern NSW, Dr Mark Stevens, principal research scientist at Yanco Agricultural Institute, has collaborated on the project, setting traps in the Leeton district.

Traps were placed adjacent to farm silos, in cereal paddocks at least one kilometre from grain silos, or in native vegetation. There were two additional sites at regional grain depots.

Pheromone traps were set for both insects at all sites. The traps were put out for one week every four to six weeks, and the insects caught were sorted and frozen for further analysis. More than 40,000 lesser grain borers and 5000 rust-red flour beetles were trapped during the study, with numbers in individual traps varying greatly. The greatest number of lesser grain borers caught in a single trap was 1419, in NSW, and Queensland recorded the highest number of rust-red flour beetles in a single trap (387).

Trapping shows that these beetles can be found across the rural landscape. In particular, the lesser grain borer's distribution is more widespread than we initially believed. The borers have been consistently caught in traps that are significant distances from grain storages and, in some cases, in greater

numbers compared with traps adjacent to silos.

On one occasion, 887 lesser grain borers were caught in a single trap in a Queensland national park, at least five kilometres from the nearest farm. Rust-red flour beetles were concentrated around silos, but were still caught in traps away from farm storages.

It is not yet clear whether the lesser grain borer is surviving on unknown host plants or whether they are travelling long distances to the vegetation sites. If the findings demonstrate this species is reproducing and developing on native host plants then a whole new set of challenges about insect control arise.

However, a more likely explanation is that beetles are flying great distances from infested grain and being caught in the traps.

Further analysis of the insects preserved from traps using molecular techniques may help to answer the question of travel. Population genetics will identify how closely related insects found around silos are to those found in paddocks and further afield. A close genetic relationship in all populations could indicate insects are travelling much longer distances than previously thought; a more distant relationship could indicate that separate populations are surviving on alternative host plants. Results available so far show a strong relationship between rust-red flour beetles trapped in the Queensland study area.

There have been differences in the trapping results between the two states, with NSW traps catching more insects when they are around, but for shorter periods. In the two months following a heatwave in southern NSW in January 2009, when temperatures reached 40°C or higher for more than a week, the number of insects of both species trapped declined dramatically.

In Queensland, warmer than normal temperatures in late winter coincided with a major increase in insects trapped, compared with previous months. These findings suggest weather conditions may have a significant bearing on insect populations and further research will investigate these links.

Other aspects of the project include identifying the levels of phosphine resistance in different insect populations, although early results suggest little difference between silo and paddock-collected lesser grain borers. □

As grain insects have been found across the landscape, the importance of good hygiene to reduce the attraction of grain storage areas is important.

TRAPPING SHOWS THAT THESE BEETLES CAN BE FOUND ACROSS THE RURAL LANDSCAPE.

GRDC Research Code NPB00004 (CRCNPB project CRC50089)  
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## SNIFFING OUT INSECTS

A biosensor to smell the presence of grain pests is being developed – but don't hold your breath, it's at least six years away **By Alisha Anderson**

An insect can only identify an odour if it has the receptors for that odour. Measuring the change in electrical signal in the insect's antennae when exposed to different odours identifies an insect's ability to detect an odour.

PHOTO: CSIRO

IF A GRAIN storage insect wants to find another, especially a mate, it uses its highly sensitive olfactory receptors to sniff out the pheromones released by other insects. Researchers are now combining a range of disciplines to develop a biosensor, the Cybernose<sup>®</sup>, to sniff out pests in stored grain.

A biosensor is a device for detecting and measuring very small quantities or changes in a biochemical or chemical substance such as insect pheromones.

Researchers at CSIRO Food Futures Flagship in collaboration with researchers at the South Australian Research and Development Institute are working on this CRC for National Plant Biosecurity project. The project hopes to deliver a range of Cybernose<sup>®</sup> devices suited to identifying the presence of grain storage pests.

The use of a Cybernose<sup>®</sup> for stored grain pests is a spin-off from a CSIRO project to develop a biosensor with a range of applications including the measurement of wine aroma, monitoring for food safety and detecting explosives. There are strong synergies between the CRC collaboration and CSIRO's broader research in this area.

The research team is developing knowledge of olfactory (scent organ) receptors of grain pests, in particular red flour beetle (*Tribolium castaneum*).

Commencing in September 2009, the initial part of the project is focused on finding receptors in the red flour beetle that respond to known pheromones.

For example, insects have a very sensitive system for detecting sex pheromones, so receptors are dedicated to that purpose. By using a biosensor fitted with the receptors that detect the sex pheromones researchers would be able to identify the presence of red flour beetle in stored grain.

One of the two postdoctorates working on this project, Dr Bradley Stevenson, is using an

olfactometer to test what chemicals attract the insect.

Air carrying different chemicals is blown down two arms of a Y-shaped tube. Insects are introduced at the bottom of the Y and make their way up the tube. When they make a choice at the junction, information on the volatiles that are attractive to red flour beetle is gathered.

Electrophysiology, measuring the change in electrical signal in the insect's antennae when exposed to different odours, is another method being used. This technique identifies an insect's ability to detect an odour. An insect can only identify an odour if it has the receptors for that odour.

There are several practical ways in which this research might be useful to industry.

Essentially, air sucked off the grain and passed through a biosensor would enable an entire silo to be sampled at once. For example, in an aerated silo, a biosensor might be incorporated in the aeration system, so the air is continually sampled and checked for insects.

A second option would be to sample air from the end of the auger or spear used to collect grain for sampling. If the biosensor detects there might be insects present a grain sample could be gathered from that part of the storage, rather than taking blind, random samples.

Current research to identify and isolate the biological receptors will end in 2012. However, it will probably be a minimum of six or seven years before a working prototype will be available in the grains industry. □

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BIOSENSOR  
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GRDC Research Code NPB00004 (CRCNPB project CRC20081)  
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# IMPROVED GRAIN SAMPLING STRATEGIES

Applying sampling theory to grain aims to improve the probability of detecting pests **By Grant Hamilton**

EXPORT MARKETS HAVE a nil tolerance for live insects, so the possible cost of failing to detect and control live insects is extremely high.

To maximise the probability of detection, appropriate grain sampling strategies must be applied. Inappropriate sampling strategies can result in over or under-treating grain to control insect infestations.

Current sampling strategies are based on the premise that pests are spread evenly through the grain bulk. However, previous research has demonstrated that insect pests tend to form clusters in response to microclimatic factors within the grain stack.

The research team is challenging the appropriateness of current sampling strategies that were developed based on practical constraints rather than considerations of pest ecology. Strategies that take into account both of these factors are required.

By applying statistically based sampling theory to grain containing a heterogeneous rather than homogenous distribution of pests, more appropriate sampling strategies are being developed.

The statistical model developed jointly by Grant Hamilton (a CRC for National Plant Biosecurity (CRCNPB) project leader), Dr Andreas Kiermeier (from the South Australian Research and Development Institute) and Dr David Elmouttie (a CRCNPB Fellow at Queensland University of Technology) is forming the foundation for this research.

Previous sampling models have worked on the need to sample a total weight of grain, based on the size of the grain stack. Indeed, this is the standard recommended by Grain Trade Australia.

Our new model takes into account the ecology of the pest species and their tendency to cluster and create 'hot spots' of infestation. Using this model, we have established that taking more sub-samples while keeping the total amount of sampled grain constant is a more accurate sampling strategy.

This sub-sample-based strategy encourages more samples to be taken from more locations, increasing the possibility of sampling a 'hot spot'.

Dr Kiermeier collaborated on the statistical model and is now working to develop a simulation model to assess the appropriateness of sampling at different points in the supply chain.

In the future, sampling strategies may be modified by regional conditions, size of store and level of risk. Tailoring grain sampling in this way will increase the efficiency of pest detection and limit over-treatment of grain.



A model that takes into account the ecology of pest species and their tendency to cluster and create 'hot spots' of infestation could result in new sampling strategies for grain.

This project will provide the grains industry, including growers, with improved strategies for sampling for the presence of grain pests in a range of storages and environments. While it is early days for this three-year trial, by the end of the project we will be on track to provide recommendations for improved grains sampling. □

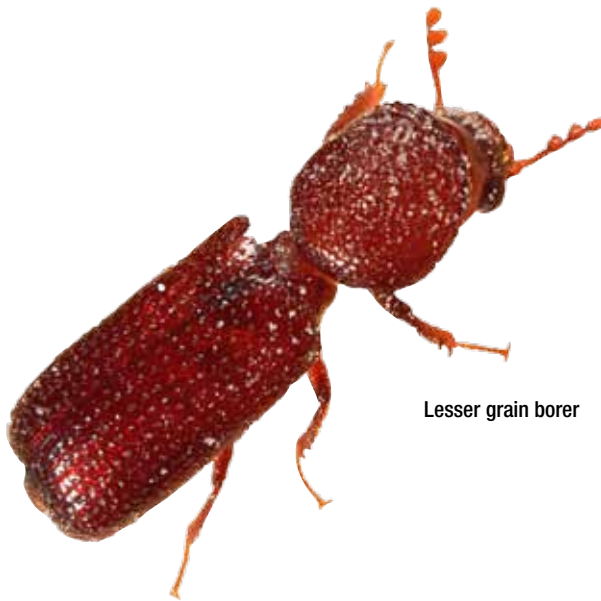
GRDC Research Code NPB00004 (CRCNPB project CRC30086)  
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# Gene knowledge helps model and monitor resistance

## Understanding the genetics behind resistance is helping in the fight against phosphine failure **By Catherine Norwood**

IN AUSTRALIA, MORE than 80 per cent of all stored grain is treated with phosphine, which means it is the chemical most in contact with insects, creating the greatest chance for insects to develop resistance.

Two projects under the CRC for National Plant Biosecurity are looking at the genetics of insect resistance to phosphine to understand the development of resistance and to rapidly identify resistant insects.



Lesser grain borer

in lesser grain borers, each of which produces a relatively weak resistance. Most insects have two copies of each gene, one inherited from their mother and the other from their father. Hybrids that carry single copy of a resistance gene and a single copy of the susceptible gene are known as heterozygotes; they are generally susceptible or very weakly resistant.

But if an insect carries both resistance genes, they are called homozygous for that gene. When the resistance genes are homozygous they produce a higher level of resistance. However, strong resistance is only found when an insect carries both copies of both genes.

When resistance genes first appear in a population they are rare. Resistant individuals carry only one copy, but may have enough resistance to survive low doses of phosphine. These heterozygotes become



Rice weevil

### Resistance model

One of the greatest threats to emerge is the development of resistance to phosphine in the lesser grain borer (*Rhyzopertha dominica*), one of the most common pests of stored grain. Low levels of resistance were first found in Australia in the early 1990s and in 1997 highly resistant borers were discovered in storages in Queensland.

A senior lecturer at the School of Mathematical Sciences at Queensland University of Technology, Dr Glenn Fulford, has been modelling the development of resistance in populations of lesser grain borers as part of a CRC project.

Molecular analysis has found that two genes on separate chromosomes control phosphine resistance

relatively more common and breed with others, producing homozygous resistant insects. Further fumigations continue to purify the insect population, producing almost entirely resistant strains.

Dr Fulford says where insects have one gene they will be 2.5 to 30 times more resistant to phosphine than insects with no resistance genes, depending on which of the two chromosomes the gene occurs.

However, insects homozygous for both resistance genes are at least 250 times, and possibly upwards of 600 times, more resistant than insects with no resistance genes. The double-gene nature of resistance in borers has delayed its development and given the industry some breathing space to develop counter-measures. But it also significantly

increases the level of resistance where it occurs.

In eastern Australia, almost every strain of lesser grain borer tested has at least one of the resistance genes. Insects that inherit both genes from both parents are still relatively rare, occurring in only about five per cent of insect samples.

Dr Fulford says CRC PhD candidate Jason Thorne is building on the current resistance modelling for the lesser grain borer by adding life-cycle factors to genetic influences.

“We already know that lesser grain borer are less susceptible to phosphine during the egg and pupal stages and this greatly affects the success of fumigations. Other research the CRC is doing will help identify exactly how much more resistant they are during this stage, so that we can refine the modelling and help develop more effective fumigation strategies.”

Varying levels of phosphine resistance have been identified in eight other insect species collected from farms and central grain storages

### Genetic markers

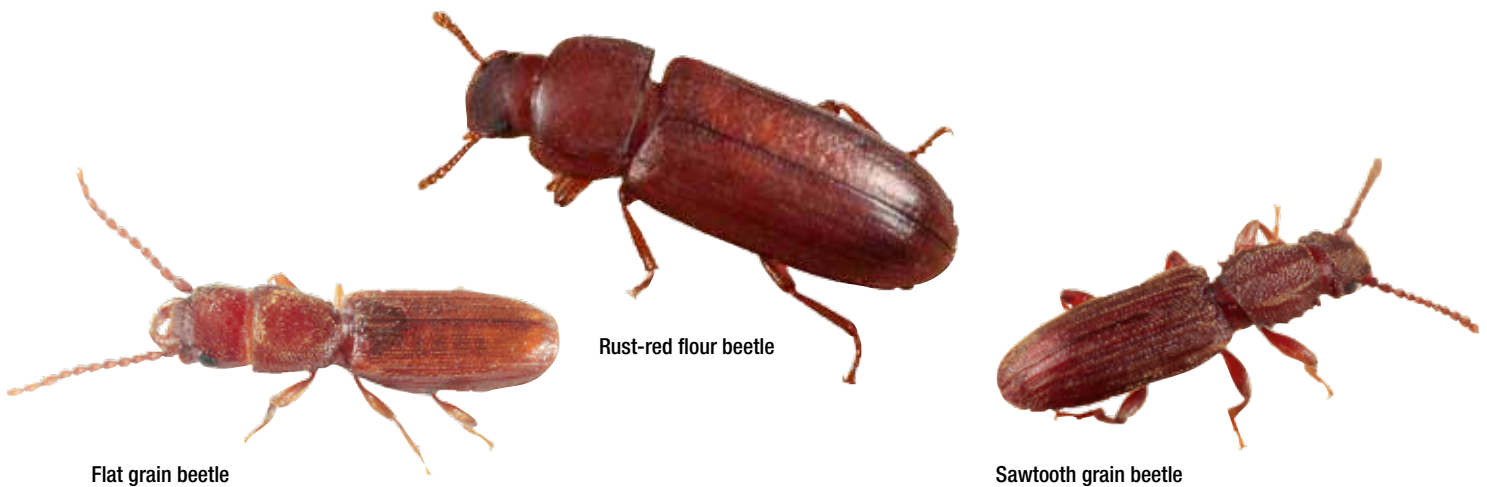
Dr David Schlipalius says this technique does not identify strains with low numbers of resistant insects. Based at DEEDI AgriScience Queensland, Dr Schlipalius is working to identify changes in the genes of the lesser grain borer and in the rust-red flour beetle.

“We will then be able to use markers that will allow us to quickly identify resistant insects and the level of resistance,” he says.

Earlier CRC research had hoped to use the proteins encoded by genes as a shortcut for identifying resistance. But there was no distinctive difference between the proteins from resistant and non-resistant insects, which sent researchers back to the DNA itself to identify changes in the sequence of genes.

Research partners in the US were able to provide a full sequence for the rust-red flour beetle genome, which has helped identify a number of candidate resistance genes, with one in particular a prime candidate for a likely molecular marker for

**INSECTS  
HOMOZYGOUS  
FOR BOTH  
RESISTANCE  
GENES ARE  
AT LEAST 250  
TIMES MORE  
RESISTANT TO  
PHOSPHINE.**



Flat grain beetle

Rust-red flour beetle

Sawtooth grain beetle

around Australia, with the highest levels of resistance emerging in the flat grain beetle (*Cryptolestes ferrugineus*) in recent years.

Other species identified with varying levels of resistance include two additional *Cryptolestes* species, rice weevils (*Sitophilus oryzae*), granary weevil (*Sitophilus granarius*), rust-red flour beetle (*Tribolium castaneum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*) and the psocid *Liposcelis bostrychophila*.

Current techniques to test for resistance are either relatively blunt or time consuming. They rely on identifying survivors at prescribed fumigation doses and breeding further generations of insects from survivors to confirm initial diagnoses, which can take six weeks or more.

resistance. Dr Schlipalius says this has indicated where researchers should look within the genetic sequencing of the lesser grain borer to find the corresponding gene.

He says developing a genetic marker will allow researchers to identify more subtle differences in the level of resistance in insect populations and to track how it develops in populations over time – before and after fumigation and even over several years.

“This will help us to identify the types of storages or practices that tend to select for resistance, so that we can change those practices and prevent a build-up of resistance in the field. At the moment we can only track resistance when fumigation fails.” □

\* Please note that photos are for illustrative purposes only and insects are not sized relative to each other.

GRDC Research Code NPB00004 (CRCNPB projects CRC20080, CRC60129)  
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# AUTOMATION IMPROVES FUMIGATION EFFICIENCY

Telemetry is offering a more efficient and reliable method of monitoring fumigation gas concentrations **By Les Zeller**

THE CBH GROUP has literally hundreds of grain silos and storages located across Western Australia from Esperance to Geraldton. Ensuring the level of fumigation management is equal at all sites is a challenge. A new remote method of monitoring the concentration of phosphine gas being circulated in a store has been developed. This work is a collaboration between bulk handlers and the CRC for National Plant Biosecurity as part of the post-harvest integrity program. This system is currently being tested and should offer improved efficiency and accuracy.

Initially, the research team was approached to set up phosphine monitors that could be accessed directly via Bluetooth to a personal digital assistant (PDA). This step would have removed the need for data from the phosphine monitors to be gathered and recorded manually.

However, almost two years ago, it was proposed to develop a phosphine monitoring and recording system that utilises telemetry to access information about fumigant levels in grain storages via the internet. Using this method, data is automatically transferred to a central database that can be accessed in real-time from any location with internet access.

Engineer Paul Kamel completed the first phosphine monitoring system ahead of schedule, which is now installed in a large commercial storage at Tammin, Western Australia. A further three modules will be installed in Queensland, South Australia and NSW in early 2011.

The storage where this automated monitoring system is being tested consists of five silos. In addition a fumigant recirculation system is being trialled.

The fumigant gas within each silo is recirculated by switching valves and the fumigant is pumped from the bottom to the top of each silo. The recirculation system controls the operation of the fan and solenoid valves to recirculate the fumigant for each silo in turn.

The automated phosphine monitoring system is designed to gather information including temperature, humidity and the concentration of phosphine being recirculated and also references this data with the silo being recirculated. Between each sample the phosphine sensor is purged with clean air to ensure the integrity of each sample.

The single monitoring and recording system has been designed to be able to sample from up to 12 points, making the system cost effective even for large grain storages. Equally, a simplified system could be developed for on-farm grain stores.

Normally this data would be gathered manually every week, but with this system fumigation data can be gathered and recorded as often as every 10 minutes. If phosphine concentrations fall below a critical level the storage manager can identify this very rapidly.

Now the research needs to establish the expected useful life of the phosphine sensor currently being used and determine if it is sufficiently robust to carry out this intensity of reading. Options for further modifications to improve the efficiency of the system are also being investigated. □

GRDC Research Code NPB00004 (CRCPB project CRC70085)  
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Paul Kamel (left) and Les Zeller on-site at Tammin, WA, with the telemetry equipment installed to relay data on phosphine concentrations directly to a central database.

PHOTO: DEEDI



# QUICK TEST FOR PHOSPHINE SURVIVORS

**A new test is helping grain handlers to rapidly identify and respond to the presence of insects with phosphine resistance** By Manoj Nayak

KNOWING THERE ARE phosphine resistant pests in the store is one thing; rapidly identifying the level of resistance so that modified fumigation practice can be applied is another.

A simple 'knock-down' assay that exposes grain insects to a high dose of phosphine can provide results within five hours of insects being delivered to laboratories for testing.

Developed through a CRC for National Plant Biosecurity project, the new test has been specially designed to determine whether flat grain beetles (*Cryptolestes ferrugineus*) found in stored grain are resistant to the fumigant phosphine.

This testing method will be evaluated in the near future for other key pest species including the rice weevil (*Sitophilus oryzae*) and rust-red flour beetle (*Tribolium castaneum*).

The flat grain beetle test applies phosphine at a rate of two milligrams per litre or 1440 parts per million (ppm). This kills non-resistant insects in only 30 minutes; insects with moderate resistance survive 30 minutes, but fail to survive 24 hours.

Any insects that survive five hours are suspected of 'strong resistance' and grain storage managers are advised immediately so that they can take remedial actions.

These strongly resistant insects are observed over a 48 to 72 hour period at the same dose and if they survive it confirms the 'strong resistance' diagnosis. Either way the storage manager is informed.

In the past three years 74 samples of flat grain beetle with strong resistance have been identified through this test. These beetles have then survived a more comprehensive test of seven days at 720ppm, validating the resistance findings.

This research work has been run in collaboration with GrainCorp. Storages are checked every month for signs of insect problems. If insects are found after fumigation or appear within three months of fumigation samples are immediately sent for testing. Receiving results within 24 hours dramatically improves store managers ability to respond to resistant insects.

In conjunction with the rapid resistance testing, new fumigation protocols are being developed as well as an eradication plan that can be used once resistance is confirmed.

Protocols to control all life stages of the strongly resistant lesser grain borer (*Rhyzopertha dominica*) require phosphine at a rate of 720ppm for five



PHOTO: DEEDI

days. This is a current label rate for phosphine against the strongly resistant lesser grain borers.

For flat grain beetles new protocols require phosphine at 720ppm over 24 days, or 360ppm over 30 days, to effectively kill all insects. These are not yet registered.

Through the CRC for National Plant Biosecurity, GrainCorp has worked with the research team to develop an eradication plan for the flat grain beetle where strongly resistant populations have been identified.

This plan includes the use of registered contact grain protectants and other fumigants at ports, use of registered contact grain protectants at country storages, an intensive hygiene program at infested storage sites and continuous monitoring of insect populations for resistance.

So far, 82 different strains of the original flat grain beetle species *C. ferrugineus* have been diagnosed with strong resistance. Samples have been collected from 71 central grain storages and two farm storages during the past three years.

Of the three different *Cryptolestes* species that can infest Australian grain storages, two species have shown low-level of resistance to phosphine and only *C. ferrugineus* has shown strong resistance.

At this stage we suggest the industry should focus on managing strongly resistant *C. ferrugineus* as the current registered rates of phosphine will control the other two species. □

**Manoj Nayak (right) discusses newly developed fumigation protocols with fumigators that were to be tested at a wheat bunker in the Darling Downs, Queensland, heavily infested with flat grain beetles.**

**GROWERS WHO FIND LIVE INSECTS FOLLOWING FUMIGATION WITH PHOSPHINE CAN CONTACT DR NAYAK REGARDING RAPID RESISTANCE TESTING.**

GRDC Research Code NPB00004 (CRCNPB project CRC50098)  
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# Alternatives explored for fumigation

**Phosphine remains the most important grain fumigant but better methods of administration and recycling might be possible and other chemicals might soon be an option** By James Newman and Yong Lin Ren

The black pipe on the silos above is the visible part of the thermosiphon being trialled on large retro-sealed on-farm grain stores.

RETRO-SEALING EXISTING GRAIN stores, developing and testing alternative fumigants and improving fumigant distribution and maintenance inside grain stores are all topics under investigation by the team at the post-harvest biosecurity laboratory at Murdoch University. The facility, opened in November 2009, is a joint investment of the Department of Agriculture and Food, Western Australia, and the Cooperative Research Centre for National Plant Biosecurity (CRCNPB).

If fumigation is to work properly grain stores need to be basically gas-tight. Rather than investing in new infrastructure, a project is investigating several aspects of retro-sealing large stores.

## Retro-sealing

While retro-sealing of existing large storages is not a new concept, it has been established that the compounds currently used to seal between tin walls and the concrete pad allow the ingress of water, resulting in corrosion. New types of commercially available sealing compounds are being tested. Also being tested are new and old bunker tarpaulins for their resistance to phosphine permeation.

Recent developments in farm silos have included top-lids that can be locked from the ground and the seal is sufficient for fumigation. The team is assessing how this design, coupled with ground-level application systems for loading phosphine into the silo, can improve fumigation practice and reduce the number of times and risks associated with climbing silos. This structural component of the research aims to develop new industry standards for the construction of new storages in relation to sealing, fumigant application systems, recirculation of fumigation gases and aeration.

## Recirculation

Adding recirculation systems to a storage means fumigants, such as phosphine gas, can move through the grain stack more efficiently than relying on internal thermal currents alone. The ability to hold and distribute

lethal gas concentration levels not only improves fumigation effectiveness but will reduce the selective pressure of phosphine resistance and help extend the life of phosphine usage in the grains industry.

Traditional systems of recirculation, such as small fans require a power source, limiting location of the silos to sites near available power. Thermosiphon systems use the heating energy of the sun to recirculate gas within a silo. A thermosiphon refers to the installation of atmospherically controlled pipes to a sealed silo. These pipes are positioned on the outside of the silo, enabling gas to be moved and evenly distributed throughout the silo. When used in conjunction with ground-level fumigant application systems the thermosiphon provides an effective way of distributing the fumigant gas throughout the grain stack.

Thermosiphon pipes attached to the side of the silo are heated by the sun during the day, causing the air in the pipe to move up or down depending on the temperature difference between ambient and commodity. When the ambient temperature is higher than the commodity the gas is drawn up the pipe and into the headspace. At night when the ambient temperature is often lower than the commodity temperature the air reverses, moving down the pipe into the base of the silo.

The thermosiphon has been tested on commercial 75-tonne sealed silos. Results so far have been very promising with phosphine gas levels reaching above threshold lethal concentration throughout the grain within a day during summer and winter fumigations, compared with two to three days with a standard top-loaded sealed silo fumigation.

Experiments are now underway on 1200-tonne silos in WA and South Australia. For large storages such as these, evaluations will determine the number, size and colour of the pipes used. The same principle applies with the pipes attached to the roof and continued to ground level, entering the silo at the aeration transition or the unloading auger casing. As phosphine is a dangerous explosive gas, to avoid

accumulation of phosphine in the reaction chamber for ground level application the size of the chamber for different sizes of silos will also be investigated.

### Alternative fumigants

The third area of research relates to developing alternative fumigants and much of this work is focused on creating low-oxygen environments over a sustained period. In such conditions insects, irrespective of life stage, cannot breathe and eventually die. Incidences of phosphine resistance and markets' growing desire for non-chemical-treated grain has driven the development of cost-effective nitrogen application for on-farm and bulk handler storage.

Nitrogen can be used on all grain types without leaving residues, has no withholding period and greatly reduces occupational health and safety concerns. It is proven effective in controlling red flour beetles (*Triboleum castaneum*) and trials are continuing into its use to control a variety of other grain pests. Although nitrogen gas is used in controlled atmosphere storages, producing enough pure nitrogen gas has been a limiting factor.

The researchers are looking at and evaluating new and cheaper methods for the separation of nitrogen from air generators. A method of nitrogen generation of particular interest is a membrane-style generator. Recent developments in this type of generator indicate the ability to produce a concentration of 99.5 per cent pure nitrogen.

Working with Lake Grace grower Doug Clarke, the application and technology of nitrogen generation using the pressure swing absorption (PSA) technique is being trialled to show how this needs to be set-up in on-farm silos. This trial is generating data that will help optimise the concentration of gas and the time period of fumigation required for effective and efficient nitrogen fumigation.

Most of these trials are only just entering the second year of research, but it is hoped this suite of projects will be able to deliver practical fumigation solutions to the industry in a relatively short time period. □

GRDC Research Code NPB00004 (CRCNPB projects CRC50152, CRC50147)  
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**A THERMOSIPHON PROVIDES AN EFFECTIVE WAY OF DISTRIBUTING THE FUMIGANT GAS THROUGHOUT THE GRAIN STACK.**

## Phosphine diffusion monitored in bulk stores

### Patterns of phosphine movement and concentration in bulk grain storages are being measured to help extend the life of phosphine

Phosphine remains the fumigant of choice for bulk storage operators and grain growers because of its effectiveness and environmental suitability. However, with its efficacy being compromised by an increasing number of phosphine-resistant stored grain pests, the industry needs to ensure its long-term use through a better understanding of how it works.

Part of the CRC for National Plant Biosecurity research on ensuring effective phosphine application involves monitoring the movement of phosphine in large-scale storages. The work is led by Viterra and run in collaboration with GrainCorp and the CBH Group.

The scientific team is led by CSIRO's Dr James Darby and includes researchers and modellers from the University of WA and Queensland University of Technology. The project's main aim is to identify gas movement by experimenting with different storage facilities and phosphine types.

A 12,000-tonne, steel-sided, tarpaulin-covered bunker of wheat at Roseworthy in South Australia was the location of the first trial. This trial was completed in February 2010. A repeat trial is now taking place to verify the findings.

While it is too early to report on findings, there is a suggestion that phosphine does not move as effectively longitudinally through a bunker as had been expected.

Monitoring and mapping the movement and concentration of phosphine through the 120-metre long, eight-metre high and 30-metre wide bulk store has required 120 monitoring lines running across its floor and snaking down from the top. Data from these lines is channelled to five monitoring boxes,

each with 24 monitoring points and recorded in real time.

Climatic conditions are being monitored and assessed to determine any influence on phosphine gas movement inside the trial bunker. A weather station is recording wind direction, ambient temperature, atmospheric pressure, ambient relative humidity and precipitation. Wind velocity and wind turbulence is also considered an important influence and is being monitored with three ultrasonic wind sensors.

Using actual data, the project team aims to build and validate a model developed as part of the project that would allow industry to better predict phosphine movements and efficacy through grain bulks. This knowledge could be used to minimise areas of lower concentrations and to help identify whether additional fumigant needs to be introduced given other parameters such as temperature or wind speed and direction.

To date aluminium phosphide tablets, as used by the majority of the industry, and Vapour3phos are the two products that have been tested. Vapour3phos delivers pure phosphine gas without any residues, which offers important advantages and flexibility in its use.

The results of the recent Vapour3phos trial are still being analysed by the project team, with a repeat trial scheduled for late September 2010. At the completion of this trial further data will be available for review to determine how the fumigant disperses through the grain mass.

This type of project clearly illustrates the benefits of the cooperative research structure that brings together government and academic institutions with industry to provide solutions to real issues. □

– GREG HOPKINS

GRDC Research Code NPB00004 (CRCNPB project CRC50091)  
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# **DANGER**

## **POISON GAS KEEP AWAY**

### **PHOSPHINE FUMIGATION IN PROGRESS**



<b>Fumigation:</b>	<input type="text"/>	<b>Start date</b>	<input type="text"/>	<b>End date</b>	<input type="text"/>
<b>Ventilation:</b>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

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**Do not access this storage during fumigation and ventilation**

**Ventilation period:** One day with aeration fan or five days without aeration fan

**Withholding period:** Two days

Place warning signs at all storage access points during fumigation

**Warning sign only - see label for use**