

**Cooperative Research Centre  
for National Plant Biosecurity**

# **Final Report**

**CRC50098**

**Flat grain beetle fumigation protocol**

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# 1. Executive Summary

Flat grain beetle (FGB) is a major emergency plant pest (EPP) of stored grain in Australia. Populations of FGB have recently developed high level resistance to phosphine (the only viable fumigant available for non-quarantine use) resulting in control failures with current dosage regimes.

As there is no practical alternative to phosphine, failure to control FGB with phosphine places at risk market access for Australian grain worth up to \$7 billion in annual trade. Therefore there is an urgent need to develop appropriate phosphine fumigation protocols to eradicate outbreaks of strongly resistant FGB.

CRC50098: 'Flat grain beetle fumigation protocols' addressed the above biosecurity issue by developing phosphine fumigation protocols to cost-effectively eradicate highly resistant FGB from stored grain.

The project has delivered protocols required to manage the strongly phosphine resistant FGB populations in stored grain. The following are the recommendations to industry:

- The two fumigation protocols (phosphine concentration x exposure period) that were developed at 20°C, that is, 0.5 mg/L (360 ppm) for 30 d and 1 mg/L (720 ppm) for 24 d, should be adopted by bulk handlers for disinfestations of strongly resistant FGB populations.
- Industry should continue to use the eradication strategy established through this project for highly phosphine resistant FGB populations. This involves:
  - The use of methyl bromide at ports
  - The use of registered contact insecticide (eg. Reldan) at country storages
  - Undertaking an intensive hygiene program at infested storage sites, and
  - Monitoring of FGB populations for detection of resistance
- Further research should be undertaken to evaluate the efficacy of sulfur dioxide as an alternative fumigant to phosphine to control resistant insects.

A direct implication of the research finding is that by extending the life of the effective use of phosphine, industry will avoid the use of contact pesticides for the time being. This will in turn avoid potential trade issues and save the industry from significant economic loss.

An independent cost-benefit analysis for this project by Grains Research and Development Corporation (GRDC) (Ross McLeod) has suggested that even if there are significant changes to key variables such as costs of fumigation, probability of success and volumes of grain treated with contact insecticide, prolonging the life of phosphine through development of new protocols will still result in substantial economic benefits.

## 2. Aims and objectives

### **Aim:**

To develop phosphine fumigation protocols that will eradicate highly resistant populations of Flat Grain Beetles in stored grain and prevent further development of resistant populations under fumigation.

**Objectives:**

- A fumigation protocol to eradicate the highly phosphine-resistant biotype of flat grain beetle (FGB).
- An eradication strategy for the phosphine-resistant biotype of flat grain beetle.

**Beneficiaries:**

All stakeholders of the grain industry will benefit from this research. The primary end-users will be those storing grain: bulk-handlers, grain growers, grain merchants, and food processors such as flour millers and cereal manufacturers. Chemical companies will also need the information if new label rates are required. Other end-users include extension specialists to deliver information on grain storage management, and scientists and researchers interested in insect resistance to fumigants.

### 3. Key findings

**Occurrence of high resistant FGB populations and the species involved:**

In September 2007, high resistance to phosphine was first detected in a strain of FGB collected from the GrainCorp sites at Moree and Edgeroi.

Fumigation protocols developed to control strong-resistant lesser grain borer (the species/genotype with the highest known resistance to phosphine) failed to control the collected FGB. Further laboratory tests of FGB surviving these fumigations revealed that their level of resistance was greater than seen in any grain infesting species in Australia to date.

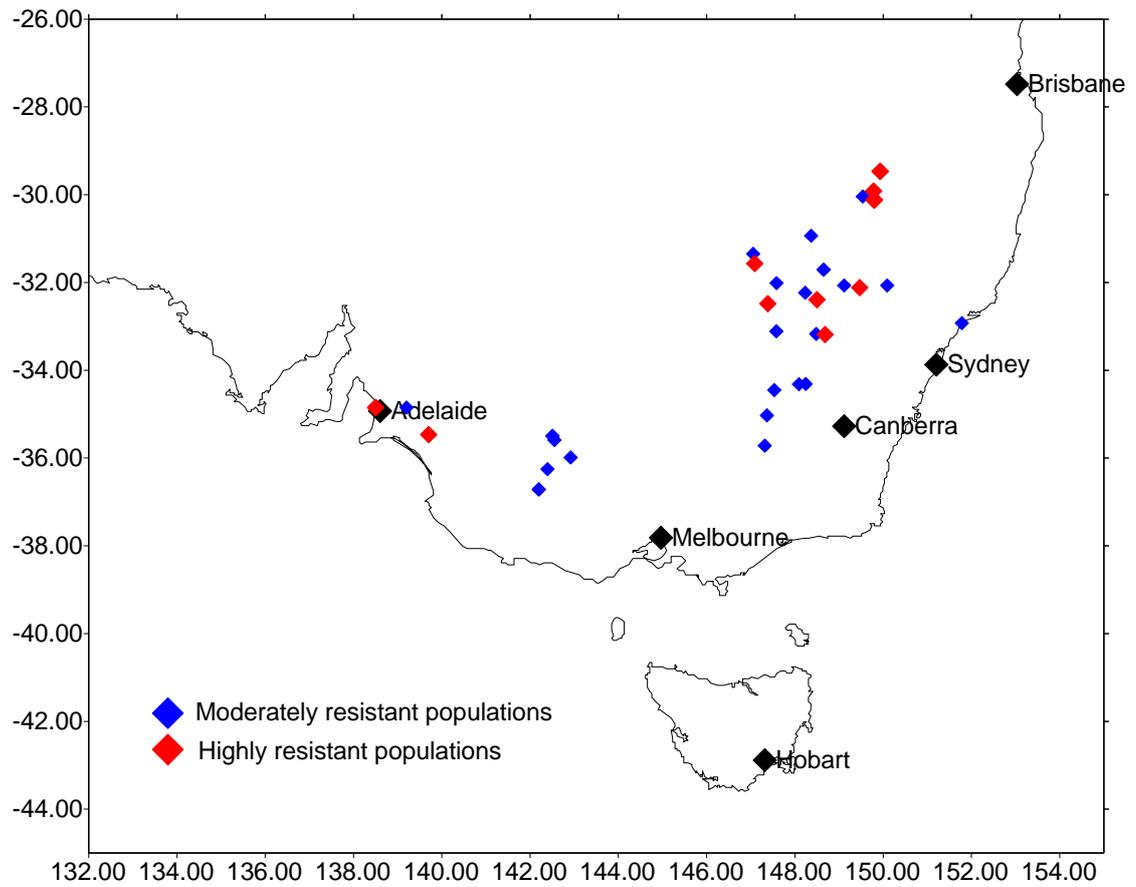
Since this first detection, high level resistance has been detected in 15 population samples from 10 sites in the Southern Region (refer to Figure 1) and 126 samples from 32 sites in the Northern Region (refer to Figure 2).

Samples were sent from bulk storages to the Department of Employment, Economic Development and Innovation (DEEDI) and Industry and Investment New South Wales (I&INSW) laboratories for resistance testing and comprised collections from GrainCorp, Viterro and AWB storages. Currently only two strains from farms in the Southern Region have been detected with this high level resistance (in 2007), but follow-up targeted sampling in these farms in 2008-09 showed no trace of these resistant FGB insects. Examination of the occurrence of resistance outbreaks strongly suggests that repeated fumigations with phosphine in central storages has led to the development of highly resistant FGB populations at individual storage sites rather than their spread through transportation of grain.

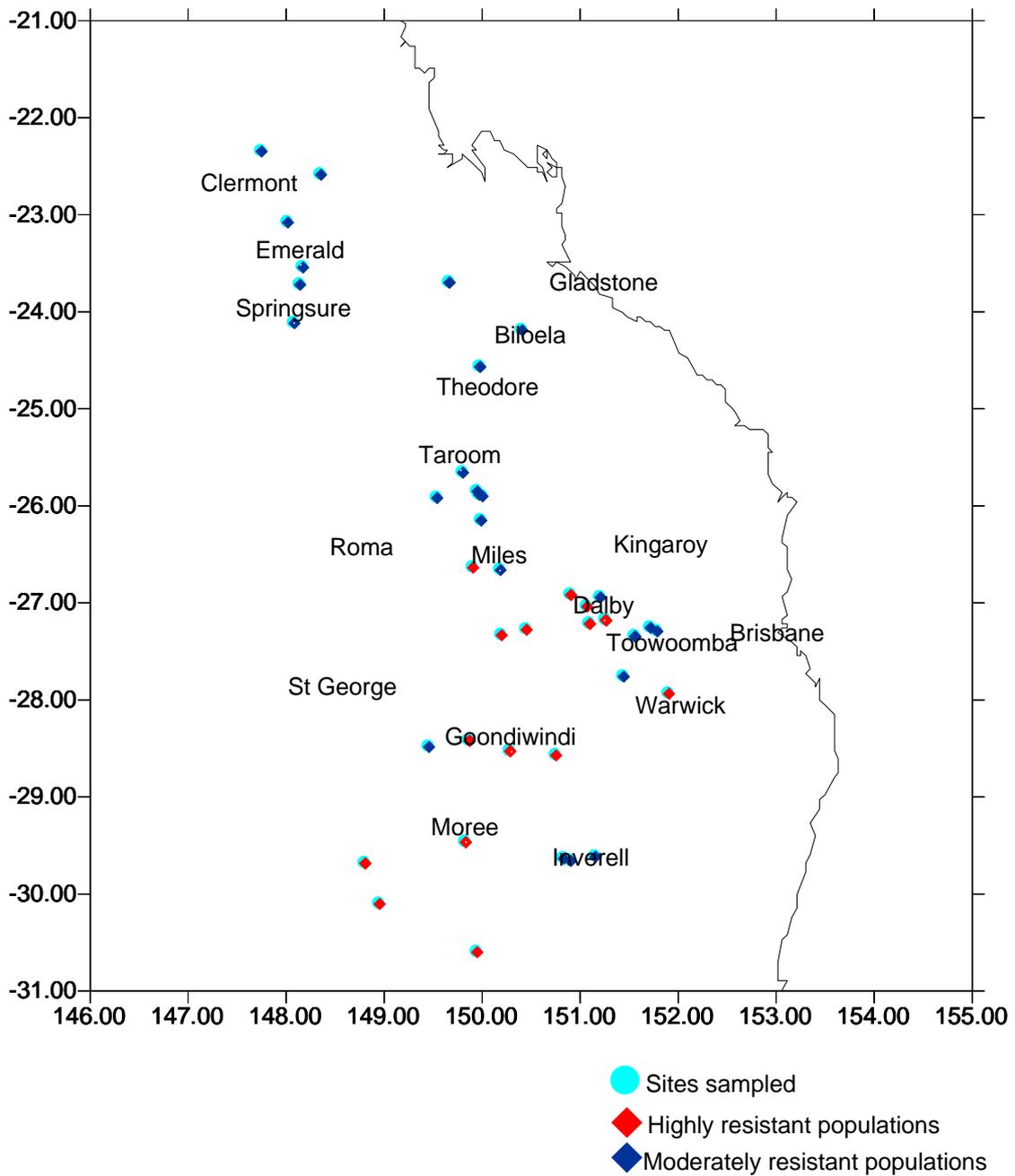
To examine concerns that more than one species may be involved in the FGB resistance, a DEEDI taxonomist undertook a screening of 29 FGB samples. These strains were suspected to have at least one other species (either *C. pusillus* or *C. pusilloides*) in addition to *C. ferrugineus*. Results showed that eleven (11) samples had mixed species and three samples consisted exclusively of a non-*ferrugineus* species.

Similar results were found from a barcoding genetics study undertaken by NSWI&I on a further 23 FGB populations from the Southern and Northern regions. The morphological taxonomic identification process was undertaken while the insects were alive so that they could be cultured. However, as it is difficult to separate *C. pusillus* from *C. pusilloides* when alive, accurate identification requires the material to be killed which jeopardises the establishment of a pure culture of each of these species. To overcome this problem it is planned to run a series of fumigation experiments, a process which has helped in separating

*C. ferrugineus* from the other species in that it is the only species which has shown the high level of resistance to phosphine (non-*ferrugineus* species have shown a low level of resistance).



**Figure 1.** Sites in the southern region where high and moderately resistant flat grain beetle populations were detected during 2006-2009.



**Figure 2.** GrainCorp sites in the Northern Region where high and moderately resistant FGB populations have been detected during 2007-09.

## **Characterisation of high resistance to phosphine in FGB**

A purified resistant strain representing a 'worst case' of resistance for *C. ferrugineus* was established in the laboratory. The strain was originally collected from a storage facility at Edgeroi and had undergone a purification process involving a series of laboratory-based fumigation experiments.

Adults of the highly resistant population sample were selected by exposing them to a higher dose of phosphine for fixed exposure periods aiming for a maximum of 30% survival. The surviving adults were cultured and their offspring fumigated at the same and next higher dose and so on until there was no further change in the response of all individuals in the test and all moderately resistant individuals were eliminated from the population. During this selection process several field samples of FGB were also exposed concurrently alongside the Edgeroi strain.

Based on these experiments we successfully distinguished between populations that are highly resistant from those which were moderately resistant to phosphine. For example, we estimated that to achieve 100% mortality of adults at a fumigation period of 72 hours (three days); we need more than 20 mg/L of phosphine for the Edgeroi strain compared with 0.25 mg/L that is required for several other samples, which we classified as moderately resistant. The characterisation of moderately resistant populations is still ongoing to establish a reference purified moderate or low resistance strain.

During the characterisation of resistance in FGB population samples, resistance levels of FGB exceeded those previously recorded in Australia for any stored grain pest. For example, at 20°C and a phosphine dose of 1 mg/L (720 ppm), a fumigation period of 24 days was required to achieve population extinction compared with 17 days, 11 days and 7 days required to control strongly resistant populations of rice weevil, psocid and lesser grain borer, respectively.

## **Development of a 'rapid test' to detect strong resistance to phosphine in FGB**

A 'rapid test' to detect the high-level resistance in FGB was developed. In this test, adults are exposed to a higher dose of phosphine (2 mg/L) for a period of 5 hours and the survivors are classified as 'highly resistant'. The rapid test has enabled the provision of 'same day' advice to industry which allows for the timely deployment of remedial measures against the strongly resistant FGB populations.

### **Protocol development**

On the advice of industry, 20°C was prioritised as the temperature for protocol development. This was based on industry observation that in bulk storages the FGB populations generally show a preference for moist grain and/or the cooler part of the bulk.

Through extensive laboratory experiments, two fumigation protocols (phosphine concentration x exposure period) at 20°C aimed at achieving 'population extinction' (killing of all life stages) were developed for the purified highly resistant FGB strain.

The first protocol was established at 0.5 mg/L (360 ppm) and determined that at least 30 days exposure is required to achieve population extinction; the second protocol was established at 1 mg/L (720 ppm) and requires a 24-day fumigation to achieve population extinction.

## **Validation of the first fumigation protocol through field trials**

A field trial was undertaken in December 2008 to validate the first protocol developed against the purified highly resistant strain of flat grain beetle.

A fumigation of 30 days at 0.5 mg/L (360 ppm) of phosphine was undertaken in a 3450 m<sup>3</sup> silo at Clifton, Queensland. The silo had 2750 t of sorghum, which was fumigated with phosphine on 10 December 2008.

Firstly 6296 g of phosphine was applied and a gas reading of >1000 ppm was recorded after four days, which came down to 200 ppm on 23<sup>rd</sup> day of the fumigation. On the 26<sup>th</sup> day, 2528 g of phosphine was added to compensate for the loss of gas, which resulted in a gas concentration of 480 ppm at the time of ventilation after 30 days of the fumigation. Mixed-age populations of the purified highly resistant strain were kept in four test cages inside the fumigated silo. At the end of the fumigation all insect cages were brought back to the laboratory for assessment. All life stages of insects in all four cages were found dead, which proved that the established fumigation protocol was successful.

Delays in validating the second protocol were experienced due to unavailability of storages in view of the busy harvest season; negotiations are ongoing to undertake a field trial during the early part of 2010.

## **Efficacy of sulfuryl fluoride as an alternative fumigant for control of strongly phosphine-resistant FGB:**

Trials to evaluate sulfuryl fluoride (Profume<sup>®</sup>) as an alternative to phosphine for control of resistant insects were undertaken at the request of CRC industry collaborators. Project staff completed two field trials, in collaboration with GrainCorp Queensland, Dow AgroSciences (sulfuryl fluoride registrant) and South Australia Rural Agencies Pty Ltd (fumigator and distributor for sulfuryl fluoride), to assess the efficacy of this fumigant against highly phosphine resistant strains of FGB and strongly resistant strains of other key pest species.

The first trial was undertaken on wheat in sealed silos at Fisherman Island export terminal (Brisbane) (1,750 tonne) and the second trial was undertaken on sorghum in a bunker storage at Goondiwindi, Qld (~40,000 tonne). In each trial, mixed age cultures of resistant insects were placed in various locations within the grain being fumigated.

In most trials, all life stages of lesser grain borer (LGB), flat grain beetle (FGB), sawtoothed grain beetle (SGB), rust-red flour beetle (RFB) and rice weevil (RW) were controlled. However, there were a number of issues with these trials which reduced their reliability and exposed several problems with this technology.

In both trials, the fumigator applied very high concentrations for a short exposure period of 24 hours. This resulted in the concentration x time profiles achieved being at least three times greater than the current registered rates. While the rates showed that sulfuryl fluoride could be effective against grain insects they would not be feasible for use by the grain industry.

Therefore further research is needed to define the concentrations and fumigation times required for insect control. It is important to note that sulfuryl fluoride has been developed for the fumigation of mills and other structures in the USA which are very different situations to the requirements of the Australian industry.

In addition, we found that the presence of carbon dioxide interfered with the accurate measurement of sulfuryl fluoride concentrations using the instrument supplied by Dow AgroSciences (DOW). This instrument needs to be better calibrated or replaced by

another type of detector. There were also apparent problems with the even distribution of the gas within the storage allowing some test insects to survive.

Although the trials were not conclusive, they did demonstrate the potential problems and research gaps associated with this technology.

### **Future prospects of sulfuryl fluoride as an alternative to phosphine**

On a trial basis, a bunker underwent sulfuryl fluoride fumigation prior to Christmas 2009 and to date, no insect activity has been reported in this bunker.

Until suitable protocols (concentration x exposure periods) are established through further research, some industry members have indicated a wish to manage FGB resistance by using the current registered rates of sulfuryl fluoride.

This plan is based on a single fumigation of sulfuryl fluoride per year and continued fumigation with phosphine during the rest of the year as required. It is hoped that this will delay the resistance development in target pests towards sulfuryl fluoride.

### **Development of an eradication strategy for highly resistant flat grain beetle populations at central storages**

An action plan has been developed collaboratively by GrainCorp, I&INSW and DEEDI aimed at eradicating infestations of resistant flat grain beetle and preventing their spread. This plan involves the following components:

- a. Infestations detected at port, treat with Methyl Bromide
- b. Infestations in country depots, where permitted, use registered contact insecticide (eg. Reldan)
- c. In storages with a history of re-infestation, freshly harvested grain should be treated with a contact insecticide (to provide at least 6 months protection).
- d. All storages to undergo an intensive hygiene program that includes detailed cleaning and structural treatments
- e. Monitoring of insect populations should be continued through inspection, sampling and trapping. Insects surviving treatments should be forwarded for resistance testing.

### **Impact of the Eradication Plan**

A trial deployment of the above plan was undertaken.

Fenitrothion and chlorpyrifos-methyl (Reldan) were successfully used to achieve complete control of highly resistant populations of FGB in bulk grain stored in several storages, where fumigations failed to control the infestations. The industry partners involved in turn had to suffer losses due to restricted marketing of this treated grain.

Targeted sampling was undertaken by DEEDI staff at a storage at Moree to evaluate the effect of the key resistant management tactic of hygiene on controlling pest populations outside the main storages. It is important to note here that this site recorded one of the first detections of the strongly resistant FGB populations. The sampling points included grain spills outside the

bunker and stacker; in and around machinery such as hopper, rail outlet, tunnel area, conveyer and grain dump etc.

Thirteen samples were collected from these points and screened in the laboratory. Small numbers of live FGB, LGB, RFB, RW and psocids were found in four samples collected from untreated wheat spills and spills sprayed with Reldan (chlorpyrifos-methyl) as a surface treatment. This result suggests that the hygiene program needs to be intensified in these storage sites, specifically the grain residues which are left untreated. Industry was advised of this finding and the live insects are now in culture for further resistance testing. It will be interesting to see whether the FGB populations found outside the main storage are highly resistant to phosphine and whether these insects moved into the fresh grain that has been stored in the two bunkers mentioned above.

## **4. Implications for stakeholders**

### **Biosecurity Problem**

Flat grain beetle (FGB) is a major EPP of stored grain in Australia. Populations of FGB have recently developed high level resistance to phosphine (the only viable fumigant available for non-quarantine use) resulting in control failures with current dosage regimes.

As there is no practical alternative to phosphine, failure to control FGB with phosphine places at risk market access for Australian grain worth up to \$7billion in annual trade. Therefore there is an urgent need to develop appropriate phosphine fumigation protocols to eradicate outbreaks of strongly resistant FGB.

### **Industry Need:**

To be able to cost-effectively eradicate highly resistant FGB from stored grain to ensure market access.

### **Implications for the industry:**

The success of Australia's \$7 billion grain industry depends on the maintenance of high standards in its post-harvest produce through effective pest management. Australia maintains this 'nil tolerance' by adopting a chemical strategy to disinfest the stored grain.

At present, the industry relies on a single fumigant, phosphine, because of an increasing sensitivity of grain markets to pesticide residues, the development of insecticide resistance to other chemical alternatives and the lack of practical alternative fumigants. Phosphine has the advantage over other chemical treatments of being relatively cheap, accepted as a residue-free treatment internationally and having flexibility in its application. Although resistance to phosphine has been developed in key stored grain pests, manipulation of concentration and exposure periods has successfully been used control resistant populations. In this scenario, the Australian grain industry will need to rely on phosphine in the foreseeable future for disinfestations of its stored commodities for market access.

The CRCNPB-supported FGB fumigation protocol development project has delivered two new fumigation protocols that can control highly resistant FGB populations. In addition, a strategy has now been put in place to eradicate infestations of phosphine-resistant FGB and prevent or delay further selection for resistance to phosphine and restrict their spread.

A direct implication of the research finding is that by extending the life of the effective use of phosphine, industry will avoid the use of contact pesticides for the time being. This will in turn avoid potential trade issues and save the industry from significant economic loss.

An independent cost-benefit analysis for this project by GRDC (Ross McLeod) has suggested that even if there are significant changes to key variables such as costs of fumigation, probability of success and volumes of grain treated with contact insecticide, prolonging the life of phosphine through development of new protocols will still result in substantial economic benefits.

## 5. Recommendations

The project has delivered protocols required to manage the strongly phosphine resistant FGB populations in bulk storages. The following are the recommendations to the industry:

- That one of the following phosphine fumigation protocols be used to eradicate highly resistant populations of FGB:
  - 0.5 mg/L (360 ppm) for 30 d
  - 1 mg/L (720 ppm) for 24 d
- Industry should continue the use of the eradication strategy for highly phosphine resistant FGB populations that were developed through this project. This involves use of methyl bromide at ports, use of registered contact insecticide (eg. Reldan) at country storages, undertaking intensive hygiene programs at infested storage sites and monitoring of FGB populations for detection of resistance.
- Further research should be undertaken to evaluate the efficacy of sulfuryl fluoride for its future use as an alternative fumigant to phosphine.

## 6. Abbreviations/glossary

Insert list of abbreviations of acronyms (for example)

<b>ABBREVIATION</b>	<b>FULL TITLE</b>
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
EPP	Emergency plant pest
FGB	Flat Grain Beetle

## 7. Plain English website summary

CRC project no:	CRC50098
Project title:	Flat grain beetle fumigation protocol
Project leader:	Dr Manoj Nayak
Project team:	<p><b>DEEDI, Brisbane, Queensland:</b>            Dr Manoj Nayak            Ms Hervoika Pavic            Mr Lawrence Smith            Ms Linda Bond</p> <p><b>NSWI&amp;I, Wagga Wagga, NSW:</b>            Dr Joanne Holloway            Ms Rachel Wood            Ms Julie Clark</p>
Research outcomes:	<ul style="list-style-type: none"> <li>• Characterisation of highly resistance to phosphine in flat grain beetles (FGB) for the first time internationally</li> <li>• Establishment of fumigation protocols and an eradication strategy that will enable industry to eradicate infestations of phosphine-resistant flat grain beetle and prevent or delay further selection for resistance to phosphine.</li> <li>• Development of a rapid test to detect highly resistant FGB</li> <li>• Facilitate continued market access of Australian grain</li> </ul>
Research implications:	<p><b>Biosecurity Problem:</b>            Flat grain beetle (FGB) is a major emergency plant pest (EPP) of stored grain in Australia. Populations of FGB have recently developed high level resistance to phosphine (the only viable fumigant available for non-quarantine use) resulting in control failures with current dosage regimes.</p> <p>As there is no practical alternative to phosphine, failure to control FGB with phosphine places at risk market access for Australian grain worth up to \$7billion in annual trade. Therefore there is an urgent need to develop appropriate phosphine fumigation protocols to eradicate outbreaks of strongly resistant FGB.</p> <p><b>Industry Need:</b>            To be able to cost-effectively eradicate highly resistant FGB from stored grain to ensure market access.</p> <p><b>Implications for the industry:</b>            The success of Australia's \$7 billion grain industry depends on the maintenance of high standard in its post-harvest produce through effective pest management. The absence of detectable levels of insect infestation is such an important issue to world grain markets that to maintain its competitiveness in premium markets, Australia guarantees supply of an insect-free product. This strategy is enforced by Australian Quarantine and Inspection Service (AQIS) through the Exports (Grain) Regulation that specifies a 'nil tolerance for live insects' on all grain leaving the country. Nil tolerance for live insects is</p>

	<p>also the standard generally adopted by domestic buyers of grain.</p> <p>The most cost-effective method to meet the Exports (Grain) Regulation is the application of chemicals to grain. Currently, the industry relies on a single fumigant, phosphine, because of an increasing sensitivity of grain markets to the presence of pesticide residues, the development of insecticide resistance to other chemical alternatives and the lack of practical alternatives. Phosphine has the enviable reputation for being relatively cheap, accepted as a residue-free treatment internationally and having flexibility in its application. Traditionally, the grain industry has managed resistance essentially by replacing redundant chemicals with new materials. Chemical treatments are favoured because available non-chemical methods, on the whole, are either significantly more expensive, less versatile, do not easily match grain-handling logistics, are less effective or require significant capital investment. Therefore, the chemical replacement strategy is no longer viable and at least for the medium term, the Australian grain industry will need to rely on phosphine for disinfestations of its stored commodities to meet the market demands.</p> <p>The CRCNPB-supported FGB fumigation protocol development project has delivered two new fumigation protocols that can control highly resistant FGB populations. In addition, an eradication strategy has now been deployed that will eradicate infestations of phosphine-resistant FGB and prevent or delay further selection for resistance to phosphine and restrict their spread.</p> <p>A direct implication of the research finding is that by extending the life of the effective use of phosphine, industry will avoid the use of contact pesticides for the time being. This will in turn avoid potential trade issues and save the industry from significant economic loss.</p> <p>An independent cost-benefit analysis for this project by GRDC (Ross McLeod) has suggested that even if there are significant changes to key variables such as costs of fumigation, probability of success and volumes of grain treated with contact insecticide, prolonging the life of phosphine through development of new protocols will still result in substantial economic benefits.</p>
<p>Research publications:</p>	<ol style="list-style-type: none"> <li>1. Keynote presentation at International Conference: 'The threat of insect resistance to phosphine in bulk grain storages in Australia', July 2008, International Congress of Entomology (ICE), Durban, South Africa.</li> <li>2. Article in GRDC Ground Cover magazine, July-August. 'Pest paradise raises grain storage stakes'</li> <li>3. Presentation at National Working Party on Grain Protection, August 2009: 'High level resistance to phosphine in flat grain beetles - an update'.</li> <li>4. Presentation at Science Exchange, CRCNPB, September 2009: 'Resistance monitoring and protocol development: key components in ensuring the biosecurity of post-harvest grain'</li> </ol>
<p>Acknowledgements:</p>	<p>The research team expresses sincere thanks to farmers and the</p>

	managers and field staff of GrainCorp for their support and help for accessing storage sites for collection of insect samples and undertaking field trials.
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