Developing strategies to manage highly phosphine resistant populations of flat grain beetles in large bulk storages in Australia

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Abstract

Development of high level resistance to phosphine fumigant in flat grain beetles (*Cryptolestes ferrugineus*) in large bulk storages in Australia poses a serious threat to the biosecurity of Australian grain. The level of resistance in this species is the highest ever detected in any stored grain insect pest in Australia with a resistance factor of 875. Laboratory studies showed that at 0.5 mg/L and at 1 mg/L of phosphine 30 and 24 days are required, respectively, to attain population extinction. These doses are currently being tested in field trials for their validation. Moreover, we have developed an action plan in collaboration with project scientists and the major Australian bulk handling companies aimed at eradicating infestations of phosphine resistant flat grain beetles and preventing their spread. The key components of this plan include the use of grain protectants and sulfuryl fluoride to eliminate phosphine-resistant populations, adoption of an intensive hygiene program and monitoring of insect populations through inspection, sampling and resistance testing.

Keywords: Flat grain beetle, *Cryptolestes ferrugineus*, Phosphine, Resistance, Fumigation protocols

1. Introduction

Both domestic and international markets demand insect-free grain. Due to a range of advantages such as its universal acceptance as a residue-free treatment, cheap price, and versatility in application, phosphine is the fumigant of choice used by the Australian grain industry to maintain its grain free of insects and to mainatin market access. This situation is unlikely to change in the foreseeable future due to the lack of suitable alternatives to phosphine.

However, a major threat to the sustainability of phosphine, is the development of resistance in key stored grain pests. Australia has already witnessed the development of strong resistance in the lesser grain borer, Rh*yzopertha dominica* (F.), (Collins et al., 2005) and the psocid, *Liposcelis bostrychophila* Badonnel (Nayak and Collins, 2008). However, research has characterised these resistances and appropriate fumigation protocols have been developed to successfully manage them (Collins et al., 2005, Nayak and Collins, 2008). Among the range of storage pests that occur in both farms and bulk storage environments in Australia, flat grain beetle (FGB), *Cryptolestes ferrugineus* (Stephens), has always been considered to be a minor pest compared with the lesser grain borer, the rice weevil and the rust red flour beetle (Emery and Nayak, 2007). In 2007, however, this changed with the detection of a much higher level of resistance to phosphine in the FGB than that reported earlier for the lesser grain borer and psocids. Since then, there has been a steady increase in the incidence of strongly resistant populations in the bulk storage environment, and this poses a serious threat to the biosecurity of Australian grain.

Currently, as there is no practical alternative to phosphine, failure to control FGB with this fumigant will jeopadise market access for Australian grain. Our previous experience with the lesser grain borer and psocids has proven that manipulation of concentration and exposure periods can be utilised to manage strong resistant populations. Taking the same approach, we aim to develop appropriate phosphine fumigation doses to eradicate strongly resistant FGB populations.

2. Materials and methods

2.1. Characterisation of resistance in FGB

A population sample of strongly resistant FGB, which was originally collected from Edgeroi, New South Wales, was subjected to mass-selection in the laboratory to establish a purified strongly resistant strain. This process involved exposure of 1000 adult FGB to phosphine at 1 mg/L (720 ppm) for 7 d (168 hours) for six generations to maximise homozygosity of resistance genes. Adult insects were also exposed to phosphine at fixed periods of 48, 72 and 144 h to establish base-line response. Probit analysis was used to estimate the concentrations required to achieve different mortality levels including the LC 50 and LC 99.9 (Genstat, 2008). Mortality levels were compared with that of a laboratory susceptible strain for estimation of resistance factor. Once the purified strain was established, 50 adults were taken and mass cultured in 500 mL glass jars on a diet of 100 g of rolled oats and 5 g yeast powder at constant regimes of 30°C and 60% r.h. Several of these culture jars were organised to generate mixed-age populations (containing all life stages living in the culture medium) over a period of 6-8 wk for the following experiments.

2.2. Development of fumigation protocols

At the time of the initiation of the research, the industry prioritised 20°C as the temperature for the development of fumigation doses as this was regarded as the 'worst case' i. e., lowest temperature that infestation were likely to be detected. This was in view of anecdotal evidence that in bulk storages the FGB populations show a preference for cool grain. Fumigation doses were developed at two phosphine concentrations, 0.5 mg/L (360 ppm) and 1 mg/L (720 ppm) against the purified strongly resistant FGB at 20ºC and 60% r.h.

The experimental set-up was essentially similar to that previously described by Collins et al. (2005) for lesser grain borer. Batches of culture media containing mixed-age of strongly resistant FGB were exposed to constant phosphine concentrations in a continuous flow application of phosphine mixed with air aimed at establishing time to achieve population extinction. Briefly, the continuous flow apparatus which mimics a series of small air-tight silos consisted of six cylindrical stainless steel fumigation chambers that were connected in parallel via stainless steel tubing to cylinders of phosphine and compressed air (BOC, Brisbane Australia). Six cages with the mixed-age cultures (100 g) were inserted into the six fumigation chambers. These cages were sealed at both ends with stainless steel mesh to facilitate gas flow, and to prevent the escape of insects from the mixed-age culture. Controls were organised in the similar fashion, except that they were not fumigated with phosphine.

Fumigation was carried out with the coordination of mass flow controllers and a series of flow monitors, which force the flow of gas in one direction from the cylinders through the tubing and fumigation chambers and vents it to the atmosphere through a fume cupboard. During the fumigation, relative humidity was maintained at around 60% by passing the phosphine-air mixture through chilled water at 19°C inside a camping fridge. Phosphine concentration was monitored on a daily basis to ensure that the required concentration was maintained. Gas samples were taken from both ends of each fumigation chamber and measured using a pulsed-frame photometric detector mounted in a gas chromatograph. Test cages were removed from the fumigation chambers after pre-determined exposure periods and insects were sieved and the numbers of live and dead individuals recorded. After this, the counted insects were discarded and rest of the test material was kept at 30°C and 60% r.h. for 8 wk for an assessment of emergence of live insects. If there were no adults recovered, the grain was stored for a further 8 weeks for a final assessment of live adults. Time to population extinction was defined as the earliest exposure period (in whole days) from which there was no emergence of live insects. For both concentrations (0.5 and 1 mg/L), the experiments were replicated once.

2.3. Field trial

A field trial was undertaken in December 2008 to validate the first dose. A fumigation of 30 d at 0.5 mg/L (360 ppm) of phosphine was undertaken in a 3450 m^3 vertical silo at Clifton, Queensland containing 2750 tonnes of sorghum. Mixed-age populations of the strongly resistant FGB were placed in four test cages, two of which were buried a metre below the surface of the grain and other two in the bottom of the silo. There was provision for monitoring phosphine concentration at two points at the bottom of the silo and one point at the top. Three insect cages representing controls were buried inside sorghum in a bag kept inside an office near the silo. Data loggers (I-buttons, Maxim Integrated Products, Inc., CA, USA) were kept inside each of the test and control cages for hourly monitoring of temperature and humidity. At the end of the fumigation, the test and control cages were brought back to the laboratory where the numbers of live and dead adults were recorded immediately and again after the media had been incubated for 8 wk at 30°C and 60% r.h.

3. Results

3.1. Characterisation of resistance

During the purification process, a series of results were obtained on the response of FGB at fixed exposure periods to phosphine. To calculate resistance factor in the resistant FGB, we used the 50% mortality level (LC_{50}), as recommended in the FAO method (Anonymous, 1975). The LC_{50} for the susceptible strain exposed for 72 h was 0.007 mg/L (95% fiducial limits: (0.0052 -0.00863) compared with 6.12 mg/L (5.24 – 7.24) for the resistant strain (Table 1). Based on this comparison the resistance factor in the resistant FGB was approximately x 875.

Table 1 Probit analysis of results of exposure of flat grain beetle adults to phosphine for 72 h at 20ºC and 60% r.h..

Strain	Status	LC_{50} (mg/L) (95% fiducial limits)	$LC_{99.9\,9}$ (mg/L) (95% fiducial limits)
QCF31	Susceptible	0.007 (0.0052 -0.00863)	0.012 (0.00905-0.09124)
QNCR73	Resistant	6.12 (5.24 – 7.24)	26.24 (18.09-50.81)

3.2. Fumigation protocols

Both fumigation doses were developed at 20°C. At 0.5 mg/L (360 ppm), time to population extinction of the strongly resistant FGB required a 30 d fumigation at this concentration to achieve population extinction. At 1 mg/L (720 ppm) 24 d was required fumigation for population extinction of the strongly resistant FGB.

3.3. Validation of the first fumigation protocol through field trial

The first dose of 30 d at 0.5 mg/L (360 ppm) was trialled in a 3450 m^3 vertical silo at Clifton, Queensland. On the first day of fumigation, 6296 g of phosphine was applied from cylinders and a gas reading of >1000 ppm was recorded after 4 d (Fig. 1).

The concentration declined to 200 ppm on 23rd day of the fumigation. To compensate this loss of gas, 2528 g of phosphine was added on the 26th day, which resulted in a rapid increase in gas concentration inside the silo.

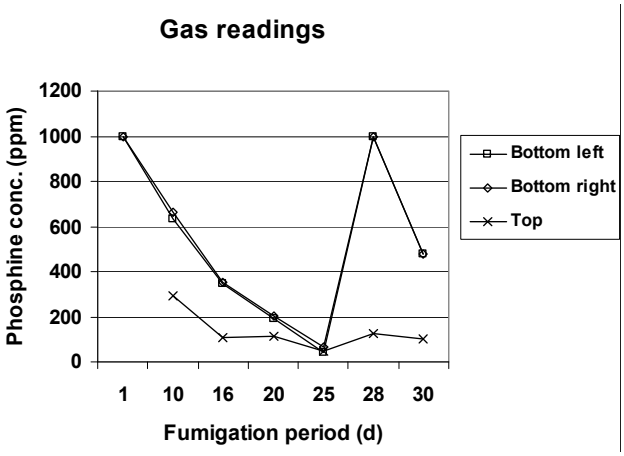

Figure 1 Phosphine concentrations in the silo under fumigation at Clifton for validation of the fumigation protocol of 360 ppm for 30 days.

A final concentration of 480 ppm was recorded at the time of ventilation after the day 30 of the fumigation (Fig. 1). Laboratory assessment of insect cages revealed no live FGB in the test cages compared with an average of 250 adult FGB in the control cages. Similarly, the assessment for progeny after 8 wk revealed no live progeny in the test cages compared with an average of 50 adult progeny in the controls. Data from the temperature-humidity loggers indicated that the temperature at the top of the silo ranged from 23.7°C to 43.2°C, with an average of 42.1°C. The temperature at the bottom of the silo varied between 23.2 to 26.2°C, with an average of 24.2°C. Temperatures inside the control cages ranged from 19.1 to 33.6°C with an average of 24.5°C.

4. Discussion

The main aim of the current research was to develop fumigation doses in the laboratory for control of strongly resistant FGB, which has recently been detected with the highest ever resistance to phosphine in any stored-grain pests in Australia, and to validate them through field trials. We have established protocols at two phosphine concentrations (360 ppm and 720 ppm) at 20°C and one of them (360 ppm for 30 days) has been successfully validated through a large scale field trial in a vertical silo with sorghum. Our results from this research suggest that to control strongly resistant FGB populations, we need much longer fumigation periods than that currently registered for phosphine to manage the previously established strongest resistant pests in Australia such as the lesser grain borer and psocids.

For example, at 20°C and a phosphine dose of 1 mg/L (720 ppm), we require a fumigation period of 24 d to achieve population extinction of strongly resistant populations of FGB compared with current recommended periods of 10 and 12 d for the control of strongly resistant populations of lesser grain borer and psocids, respectively (Fig. 2).

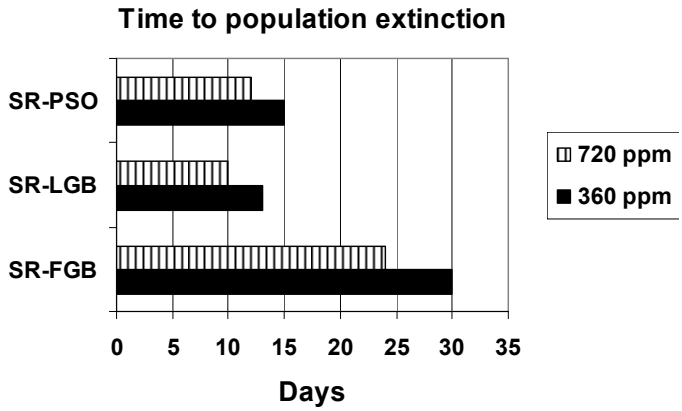

Figure 2 Time to population extinction of key phosphine resistant pests at two phosphine concentrations at 20°C. (SR-FGB: strong resistant flat grain beetle, SR-PSO: strong resistant psocid, source: Nayak and Collins (2008) and SR-LGB: strong resistant lesser grain borer, source: Collins et al., (2005).

Similarly, at 20°C and 0.5 mg/L (360 ppm), a fumigation period of 30 d will be required to control strongly resistant FGB compared with the current recommendations of 13 and 15 d for control of strongly resistant lesser grain borer and psocids, respectively (Fig. 2). This comparative analysis means that appropriate changes to the current label rates of phosphine may be required to accommodate the newly emerged strongly resistant FGB.

Strong resistance to phosphine in FGB was first reported in the 1980s in a population collected from Bangladesh (Mills, 1986). According to this report adults of the strong resistant FGB strain from Bangladesh were successfully controlled in 7 d at 0.66 mg/L (475 ppm) at 25°C, but a higher rate of 2.5 mg/L (1800 ppm) was needed at 15°C. From a more recent study in China, Wang et al. (2008) reported that to control phosphine resistant FGB populations in warehouses, the protocol should aim at achieving an initial concentration of 1 mg/L (720 ppm) and to maintain a concentration above 300-500 ppm for 16-25 d. From another study in China, Li and Yan (2008) recommended a dose of 200 ppm for more than 28 d to control strongly phosphine resistant FGB populations in warehouses. Both these studies concluded that once phosphine concentration drops below 200 ppm there was a possibility of control failure. Our recommendation of 360 ppm phosphine for 30 d falls in between the findings of these studies from China.

While research was in progress on development of new phosphine recommendations, other treatments were also considered for management of FGB. Laboratory evaluation of two currently registered grain protectants, chlorpyrifos-methyl (5 mg/kg) and fenitrothion (6 mg/kg) against 10 field collected FGB populations confirmed that these treatments can effectively control both adults and progeny. This information has been passed onto industry and grain at several bulk storages has been treated with either one of these chemicals. Personal communication from a major bulk storage operator suggests that this treatment has been successfully controlling the phosphine resistant FGB populations (Robin Reid, GrainCorp Operations Ltd., Queensland, Australia, personal communication). Moreover, several bulk storage operators have recently started using sulfuryl fluoride as an alternative fumigant to phosphine and claiming successful control of phosphine resistant FGB populations.

An action plan has been developed collaboratively by the bulk handling companies and the researchers aimed at eradicating infestations of phosphine resistant FGB and preventing their spread. The key components of this plan include use of an alternative fumigant such as sulfuryl fluoride, strategic application of grain protectants (chlorpyrifos-methyl, fenitrothion) and adoption of an intensive hygiene program and monitoring of insect populations through inspection, sampling and resistance testing.

Although the new fumigation rates and the eradication strategy developed through this research have practical application for the management of strongly phosphine resistant FGB populations, there are several research gaps that need to be addressed. These include identifying the causes for the development

of strong resistance and investigating whether the strong resistance is developing independently at different sites or resistant populations are spreading through transport.

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