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# PHOSPHINE BEST MANAGEMENT PRACTICES

Disinfesting stored commodities



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Disinfesting stored commodities

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**COVER:** Low-range phosphine meters are used to ensure storages have been vented adequately after fumigation.

**PHOTO:** Jonathon Kerr

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Well designed conveyor attachments will enable individual silos to be sealed for fumigation while the system can continue filling and emptying other silos.  
Photo: Chris Warrick, Primary Business

## GLOSSARY TERMS

<b>AGIRD</b>	Australian Grain Insect Resistance Database
<b>APVMA</b>	Australian Pesticides and Veterinary Medicines Authority
<b>AS</b>	Australian Standard
<b>CO<sub>2</sub></b>	Carbon dioxide
<b>C</b>	Concentration
<b>CT</b>	Concentration x time
<b>CRC</b>	Cooperative Research Centre
<b>DD</b>	Discriminating doses
<b>DE</b>	Diatomaceous earth
<b>EDN</b>	Ethanedinitrile
<b>EF</b>	Ethyl formate
<b>FAO</b>	Food & Agriculture Organization of the United Nations
<b>HCN</b>	Hydrogen cyanide
<b>IRM</b>	Integrated resistance management
<b>LD</b>	Lethal dose
<b>LGB</b>	Lesser grain borer ( <i>Rhyzopertha dominica</i> )
<b>MB</b>	Methyl bromide
<b>MC</b>	Moisture content
<b>NSW DPIRD</b>	New South Wales Department of Primary Industries and Regional Development
<b>O<sub>2</sub></b>	Oxygen
<b>OH<sub>2</sub></b>	In a chemical context; also known as H <sub>2</sub> O, water
<b>OH<sub>3</sub></b>	Hydroxide
<b>Pa</b>	Pascals
<b>ppm</b>	Parts per million
<b>PPE</b>	Personal protective equipment
<b>PH<sub>3</sub></b>	Phosphine (hydrogen phosphide)
<b>QDPI</b>	Queensland Government Department of Primary Industries
<b>RD&amp;E</b>	Research, development and extension
<b>RFB</b>	Red flour beetle ( <i>Tribolium castaneum</i> )
<b>RGB</b>	Rusty grain beetle ( <i>Cryptolestes ferrugineus</i> )
<b>RR</b>	Resistance ratio
<b>RW</b>	Rice weevil ( <i>Sitophilus oryzae</i> )
<b>SR</b>	Strong resistance
<b>SF</b>	Sulfuryl fluoride
<b>TPE</b>	Time to population extinction
<b>TWA</b>	Time-weighted average
<b>TLV-TWA</b>	Threshold limit value – time-weighted average
<b>WA DPIRD</b>	Western Australian Department of Primary Industries and Regional Development
<b>WR</b>	Weak resistance



# 01. INTRODUCTION

There is a wide range of implications from insect infestations in stored commodities. These include economic loss due to physical damage, quality degradation, rejection by consumers leading to loss of markets, impact on workplace health and safety, and costs associated with their management (Nayak and Daglish, 2018). Several species belonging to three insect orders – Coleoptera (beetles), Lepidoptera (moths) and Psocoptera (psocids) – generally infest stored commodities (Rees, 2004). Although storage managers routinely use non-chemical tactics – such as hygiene, cooling, and drying – for pest management of post-harvest commodities, including grain, these are often insufficient to maintain the quality standards required by markets.

Among the available chemical control methods, contact insecticides (grain protectants and structural treatments) and fumigants are at the forefront of providing measurable success in pest management, meeting the logistical requirements set by storage operators and markets. While contact insecticides are used for the provision of long-term protection of commodities from insect attack, fumigants are used to disinfest the commodities when infestations are detected. Over the past two decades, there has been a gradual decline in the use of contact insecticides due to increasing regulatory restrictions, consumer sensitivity towards insecticide residues, development of resistance in major pest species, and high costs associated with the development and registration of new chemistries.

Among several fumigants that have been used by the industry so far, methyl bromide (MB) and phosphine (hydrogen phosphide,  $\text{PH}_3$ ) have the longest history of success. However, MB was declared as an ozone-depleter in the 1990s and recommended for gradual phase-out for non-quarantine uses (Nayak et al., 2020). With the restricted use of MB and the loss of several contact insecticides due to regulatory restrictions and resistance issues,  $\text{PH}_3$  has emerged over the past three decades as the preferred disinfestant for grains and durable commodities globally.

Phosphine's success as a disinfestant over several decades is due to its:

- relatively low cost
- ease of application to a range of commodities
- compatibility with commodity handling logistics across different storage structures
- effectiveness against major pest species
- global acceptance by markets and regulatory authorities as a residue-free treatment (Nayak et al., 2020).

Phosphine tablets placed in the top of silos should be on trays to ensure residual powder can be removed after the exposure period.  
Photo: WA DPIRD



From an Australian perspective, the over-reliance on phosphine has resulted in the development of strong levels of resistance in major insect pests of stored grain and the frequency of resistance continues to increase (Nayak and Jagadeesan, 2024). Of particular concern is the emergence of strong resistance in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), which cannot be controlled effectively at rates that are adequate for other major storage pests (Nayak et al., 2013).

Sulfuryl fluoride (SF) is gaining market share and is being used across all sectors of the industry as a 'phosphine-resistance breaker' to combat the strongly resistant rusty grain beetles. However, it is significantly more expensive than phosphine and, currently, the risks associated with fluoride residues on food are not well understood. Moreover, several other treatments that have been developed globally and trialled as alternatives to  $\text{PH}_3$  for the control of storage pests have failed to match all the positive attributes offered by  $\text{PH}_3$  (Nayak and Jagadeesan, 2024). Notable among these alternatives are nitrogen (low oxygen [ $\text{O}_2$ ]), carbon dioxide ( $\text{CO}_2$ ), propylene oxide, carbonyl sulfide, ethyl formate, ozone, chlorine dioxide, ethanedinitrile (EDN) and nitric oxide. In this scenario, it is expected that  $\text{PH}_3$  will continue to be used by the grain industry in the foreseeable future.

Sustainable and effective use of  $\text{PH}_3$  will be critical in maximising the value, integrity and competitive advantage of Australia's post-harvest grain supply chain, currently valued at \$32 billion. It is noted here that Australia has a legal requirement of 'nil tolerance' for live insects in its grain destined for both domestic and international markets.

Based on global research and development, extensive literature is available on the effectiveness of  $\text{PH}_3$  against a range of stored products pests, development and management of resistance (Nayak et al., 2020). Australia is globally recognised for its significant contribution to the area of post-harvest commodity protection through advanced R&D, particularly in the characterisation of phosphine resistance in major stored product pests, development of new fumigation protocols to manage them, and deployment of a national resistance monitoring program and a resistance management strategy. However, information on the practical application of  $\text{PH}_3$  across different storage systems, including logistical and workplace health and safety challenges related to pest management, is scattered across individual publications.

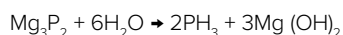
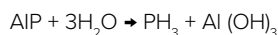
The purpose of this manual is to bring together all the available information on the use of  $\text{PH}_3$  in disinfestation of post-harvest commodities across a range of storage sectors to create a single authoritative source of best management practices. This detailed practical guide will help end-users – including growers, millers, food processors and bulk storage operators – to enhance their capability to supply premium, insect-free products and maximise market access and profitability. Wider industry adoption of these best management practices will help sustain the effectiveness of  $\text{PH}_3$  as a critical fumigant for the foreseeable future. The manual can also be useful for a new generation of students, researchers, extension specialists and industry workers who aim to pursue future careers within the Australian grains industry, particularly in post-harvest commodity protection.

## 02. PHOSPHINE AS A KEY FUMIGANT FOR DISINFESTATION OF STORED COMMODITIES

### 2.1. Key properties

PH<sub>3</sub> was first described chemically by Philippe Gengembre in 1783 as a product of heating elemental phosphorus in a potassium carbonate solution (Gengembre, 1783). As discussed earlier, PH<sub>3</sub> offers a range of positive attributes. These include its residue-free status, relatively low price, ease of application through different forms (e.g. tablets, blankets and cylinderised liquefied gas) making it suitable for all types of storage structures (e.g. silos, bag stacks, bunkers, sheds and shipping containers), excellent ability to penetrate through commodities, and its effectiveness against all life stages of major grain storage pests (Nayak et al., 2020). These critical characteristics have enabled PH<sub>3</sub>'s widespread use through global registration. Its rapid dispersal within grain enclosures also eliminates the need for additional fumigation equipment. Moreover, PH<sub>3</sub> can be safely transported in its original packaging and has no adverse effects on seed germination (Chaudhry, 2000; Nayak et al., 2020).

PH<sub>3</sub> is classed as a pnictogen hydride (non-metal hydrides), and in its gaseous form is colourless, flammable and highly toxic with a very unpleasant odour resembling rotting fish or garlic. The tablet or solid formulations can quickly emit the gas when they encounter air. PH<sub>3</sub> has a low molecular weight (33.99758 grams per mole), a low boiling point (minus 87.7°C), and a density of 1.38 kilograms per cubic metre, making it slightly heavier than air. The low molecular weight and density help it to diffuse rapidly and penetrate deeply into materials, such as large bulks of grain or tightly packed materials (Chaudhry, 1997). The gas is produced from formulations of metallic phosphides (usually aluminium or magnesium phosphide) that contain additional materials (see below) for regulating release of the gas.



PH<sub>3</sub> is highly corrosive and, as discussed under subsection 2.6 (page 10) in the text, its usage is restricted in storage structures that have metal fittings, electrical wiring and appliances made up of gold, silver and copper. Moreover, if large numbers of PH<sub>3</sub> pellets or tablets are stacked in confined spaces, hazardous situations can occur due to the spontaneous ignition of the gas when it reaches high concentrations (Phillips et al., 2012).

### 2.2. Mode of Action

Fumigants, whether gases or highly volatile liquids that vaporise at ambient temperatures, primarily enter exposed organisms through the respiratory system. The uptake of a fumigant is generally proportional to the respiration rate of the exposed animal, meaning that factors increasing respiratory activity also heighten the uptake and toxicity of the fumigant (Chaudhry, 1997). In the case of PH<sub>3</sub>, O<sub>2</sub> plays a pivotal role in its Mode of Action along with temperature, which can exacerbate PH<sub>3</sub> intoxication in insects. This is likely due to higher metabolic rates and increased O<sub>2</sub> consumption (i.e. respiration rate).

PH<sub>3</sub> disrupts vital respiratory functions and affects cellular processes by directly targeting the mitochondria that generate ATP (adenosine triphosphate) through aerobic respiration (Chaudhry, 1997). The enzyme cytochrome c oxidase, also known as complex IV, is central to this process, which facilitates the final step of electron transport, coupling O<sub>2</sub> reduction with water production. PH<sub>3</sub> disrupts this pathway by inhibiting the activity of cytochrome c oxidase, its primary action site, thereby impeding the flow of electrons. Consequently, the cell's ability to utilise O<sub>2</sub> is compromised, triggering a cascade of metabolic dysfunctions (Chaudhry and Price, 1992; Chaudhry, 1997). Furthermore, PH<sub>3</sub> exposure prompts the generation of reactive oxygen species within cells, exacerbating cellular damage and oxidative stress. These highly reactive molecules, such as superoxide radicals and hydrogen peroxide, cause extensive damage to cellular components, contributing to cellular dysfunction and potentially pest mortality (Nath et al., 2011).



QDPI and GrainCorp staff setting up a bunker fumigation. Photo: QDPI

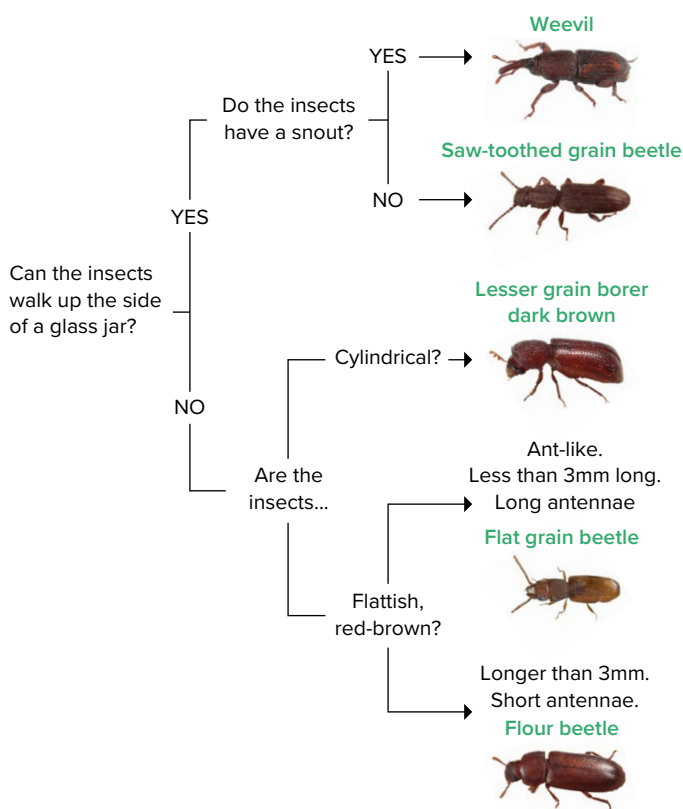
## 2.3. Effectiveness against all life stages of major insect pest species

The effect of several biological, non-biological and environmental factors on the efficacy of  $\text{PH}_3$  against insect pests has been well documented and has helped researchers develop practical approaches to optimise fumigation protocols to manage them. A key biological factor influencing  $\text{PH}_3$  efficacy is the developmental stage of the pest. Generally, eggs and pupae have been found to be the life stages most tolerant of phosphine compared with adults and larvae across most of the storage pest species (Bell, 1992; Bond, 1984; Daglish et al., 2018; Hole et al., 1976; Nayak et al., 2020). Several studies have also reported delayed egg hatching in many pests after exposure to  $\text{PH}_3$ , requiring amendments to traditional fumigation protocols aimed at achieving complete population elimination (Nayak et al., 2003; Rajendran et al., 2004). These studies suggest that extending the recommended exposure period of  $\text{PH}_3$  by a few additional days allows eggs and pupae to develop into more susceptible life stages (larvae and adults), which helps in the complete eradication of the population.

Development of resistance also plays a major role in influencing the effectiveness of  $\text{PH}_3$  (refer to sections 5 (page 23) and 6 (page 25) later in the text), which has been reported in major beetle and psocid pests in Australia (Nayak et al., 2020). These include the lesser grain borer, *Rhyzopertha dominica* (Fabricius); red flour beetle, *Tribolium castaneum* (Herbst); rice weevil, *Sitophilus oryzae* (L.); rusty grain beetle, *C. ferrugineus*; saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); and the psocid, *Liposcelis bostrychophila* (Badonnel).

Two key non-biological factors significantly influencing the efficacy of phosphine are concentration (C) and exposure period (t), both of which have been successfully manipulated over the years to extend the effective life span of phosphine, specifically in controlling strongly phosphine-resistant pest populations (Daglish et al., 2002; Collins et al., 2005; Nayak and Collins, 2008; Kaur and Nayak, 2015) (refer to subsection 2.4, page 9).

Figure 1: Identification of common pests of stored grain.



Source: QDPI

Recent R&D in Australia has considered these biological and non-biological factors to develop practical fumigation protocols effective against all life stages of resistant populations of major pest species under realistic field conditions (Collins et al., 2005; Nayak and Collins, 2008; Kaur and Nayak, 2015).



## 2.4. Relationship between concentration and exposure period

As mentioned above, concentration (**C**) and time (**t**) (exposure period) significantly influence the efficacy of  $\text{PH}_3$  (Bell, 1979; Bell, 1992; Daglish et al., 2002; Winks and Waterford, 1986). The dosage or **Ct** (concentration x time) product, achieved in any given situation, is largely determined by the fumigant loss rate during the decay phase. If there is no fumigant added to the enclosure during this phase, the concentration, **C**, decays exponentially with time, **t**, according to the following equation:

$$C = C_0 e^{-k(t-t_0)}$$

In this equation:

- **C** is the concentration at any time **t**
- **C<sub>0</sub>** is the concentration at time **t<sub>0</sub>**
- **k** is the decay rate constant in units of (time<sup>-1</sup>), (e.g. per day)
- **e** is the base of natural logarithms.

The value of **k** can be found from the slope of the semilogarithmic graph of concentration against time. This equation has been simplified to reflect a more general relationship in the form of **C<sup>n</sup> x t = K**, where **K** is a constant equalling the dosage for a specified level of response, such as the LD<sub>99</sub> (lethal dose for 99% of the pest population present) and **n** is known as the toxicity index, which is a specified value that describes the toxicity relationship between a fumigant and the developmental stage of a species.

The **Ct** relationship has generally been shown to exhibit a linear correlation under constant environmental conditions when plotted logarithmically (Bell, 1979; Bell, 1992; Winks and Waterford, 1986; Daglish et al., 2002). However, recent research has been more focused on developing fumigation protocols effective against all life stages of resistant pest populations under realistic field conditions (Collins et al., 2005; Kaur and Nayak, 2015; Nayak and Collins, 2008).

These studies have demonstrated that increasing either 'C' or 't' enhances  $\text{PH}_3$  efficacy against strongly resistant pests, although sometimes 't' plays a more critical role than 'C'.

## 2.5. Role of fumigation temperature and moisture content of commodity

It is well established that gaseous fumigants vaporise and diffuse more slowly at lower temperatures, and that insect activity and metabolism also decrease as temperatures decline (Bond, 1984). Insects mainly absorb a fumigant through their respiratory systems, and its uptake through respiratory activity is directly influenced by temperature. Hence,  $\text{PH}_3$  exhibits increased toxicity to insects at higher temperatures. Application labels of both solid and liquefied gas formulations of  $\text{PH}_3$  indicate that the minimum ideal conditions for the reaction of phosphide salts to yield  $\text{PH}_3$  gas are 25°C to 32°C and 70% relative humidity of the surrounding air and moisture content (MC) of the grain within 9 to 12% (Phillips et al., 2012). Apart from quality issues, grain with a high MC is proven to be highly sorptive to  $\text{PH}_3$  (Reddy et al., 2007; Daglish and Pavic, 2008a, 2008b) (see below). Thus, a decrease in the respiration rate of insects at low temperatures, a consequent reduction in the uptake of a fumigant and a higher MC of the grain could jeopardise the effectiveness of a fumigation event. As part of preparing highly moist grain for fumigation, efforts should be made to reduce the MC to an acceptable level through blending with dry grain or using aeration drying (Phillips et al., 2012). This principle has been applied in developing practical fumigation protocols for managing  $\text{PH}_3$ -resistant pests across grain storages in Australia (Kaur and Nayak, 2015; Nayak and Collins, 2008).

Optimisation of fumigation also needs to address another critical factor: sorption by commodities. This leads to the loss of gas from the treated space, which can significantly reduce the effectiveness of the fumigant. Sorption and desorption of  $\text{PH}_3$  by a range of stored products has been well documented through laboratory (Reddy et al., 2007; Daglish and Pavic, 2008a, 2008b) and field research (Plumier et al., 2018, 2020; Rajendran and Muralidharan, 2001). These studies have shown that  $\text{PH}_3$  sorption increases with higher grain temperature and MC. An increase in temperature also caused faster rates of sorption of  $\text{PH}_3$  in cereal grains.  $\text{PH}_3$  desorbs slowly because it reacts chemically with the commodity. Not all sorbed gas is desorbed, resulting in a fixed residue. If a significant amount of the sorbed gas does not desorb from the commodity at the end of ventilation, it affects the quality of the commodity through the accumulation of unacceptable levels of residues (Banks, 1986). Therefore, when developing fumigation strategies, it is essential to consider the sorption characteristics of commodities. This factor significantly influences treatment efficacy and affects workplace health and safety considerations by limiting fumigator and bystander exposure.

## 2.6. Limitations

Due to its highly corrosive nature,  $\text{PH}_3$  is restricted from use in certain metal storage structures and structures with metal fittings, including gold, silver and copper (Phillips et al., 2012). Electrical appliances, wiring, lighting and electronic equipment containing integrated circuits, computer chips, copper or other conductive materials are at risk of damage during  $\text{PH}_3$  fumigation. This is the main factor that restricts the use of  $\text{PH}_3$  in buildings such as flour mills, food plants, climate-controlled warehouses and other structures containing extensive electrical wiring, telecommunications equipment and computer-controlled machinery susceptible to damage by  $\text{PH}_3$ .

$\text{PH}_3$  can cause hazardous situations through spontaneous ignition if the gas concentration exceeds 18,000 parts per million (or  $25.7\text{g/m}^3$ ) (Phillips et al., 2012). This may arise if large numbers of  $\text{PH}_3$  pellets or tablets are piled up in a small-volume space.  $\text{PH}_3$  pellets and tablets are also prone to smouldering. Ignition and fires can occur within buildings or grain masses when pellets are piled together in direct contact or if standing water is present. Dangerously high concentrations are more likely when cylinderised pure  $\text{PH}_3$  gas is used without proper dilution. Similar to the risks of spontaneous ignition at high concentrations, fire hazards resulting from piling pellets can be avoided by following proper application procedures as recommended on the product label.

Another limitation of  $\text{PH}_3$  is the development of resistance in major pest species, resulting from industry's over-reliance on this single treatment due to the lack of suitable alternatives (Nayak et al., 2020). The factors responsible for development of resistance and their management are described in sections 5 (page 23) and 6 (page 25).



In addition to wearing a full face mask and elbow length PVC gloves, operators should point phosphine containers away from themselves while opening and ideally with wind at 90 degrees (not from directly in front or behind them).  
Photo: Chris Warrick, Primary Business





Ensure warning signs are placed at all entry points to the area where a silo is under fumigation.  
Photo: Chris Warrick, Primary Business



## 03. CURRENT FUMIGATION PRACTICES

### 3.1. Typical use patterns across different storage structures and registered formulations

This section presents the use patterns of  $\text{PH}_3$  across all sectors of the industry in Australia. We focus on the practical aspects of  $\text{PH}_3$  use across various storage structures and formulations without referencing specific commercial registrants or products.

#### 3.1.1. On-farm use

$\text{PH}_3$  is available in three forms for on-farm use (bag chains, blankets and tablets), with various application methods available for use in gas-tight, sealable storages (see Table 1, page 15). The traditional and most recognised form is tablets, which can be bought in tins of 100 (300g) or 500 (1.5kg). Each 3g tablet liberates 1g of  $\text{PH}_3$ . Bag chains and blankets are the safest form to guarantee no residue spills into the grain during liberation or removal. Bag chains are typically purchased in 340g sizes, and blankets are supplied in tins containing two units, each weighing 1.7kg.

$\text{PH}_3$  application rates are based on the internal volume of the gas-tight, sealable storage to be fumigated (see Table 1, page 15). When purchasing a new silo, it is important to choose a high-quality, gas-tight, sealable unit fitted with aeration cooling. The manufacturer should guarantee that the silo meets the Australian Standard for sealable silos (AS 2628-2010). Tablets, bag chains and blankets are typically used interchangeably depending on the storage size and application technique. Traditionally, fumigants were applied in the head space of silos using tablets, bags or blankets; however, some modern silos are equipped with ground-level fumigation boxes featuring either a recirculation system or a passive thermosiphon, enabling safer application from the ground.

Powered recirculation improves gas distribution through the silo, enabling 7-10 day exposure rather than 20-day exposure when fumigating silos greater than 300t capacity. Photo: Chris Warrick, Primary Business

Bunkers typically store larger volumes of grain, so use of blankets is more common.  $\text{PH}_3$  bag chain and blanket labels specify the dose rate in well-sealed bunkers greater than 1000 tonnes as  $0.6\text{g}/\text{m}^3$ , and the exposure period for 20 days. Grain bags, like bunkers, store larger volumes of grain; however, tablets are more commonly used as they can be inserted into tubes spaced 7m apart. Sheds fall into the same category as a larger storage volume; however, as they are difficult to seal, often licensed fumigators will be employed to tarp up grain for effective fumigation.

#### 3.1.2. Use in bulk storages

Depending on the size of silos and bunkers, bulk handling companies also use tablets, bag chains and blankets in their storages, and the procedures of  $\text{PH}_3$  application are the same as described above for their on-farm use. Additionally, for large storage structures where maintaining effective concentration levels becomes challenging for the required fumigation periods, bulk storage operators prefer the use of cylinderised form of liquefied  $\text{PH}_3$  that provides greater confidence in managing infestations effectively. Two types of cylinderised  $\text{PH}_3$  are commercially available for such use; one comes with a mixture of 2%  $\text{PH}_3$  and 98%  $\text{CO}_2$ , while the other comes in a pure form (100%  $\text{PH}_3$ ).

Unlike the solid formulations of  $\text{PH}_3$ , the cylinderised formulations require an accredited person to handle the application. Moreover, the cylinders in use should be in open air or in a force-ventilated fume room. Apart from ensuring the gas-tightness of the storage structures, the cylinderised  $\text{PH}_3$  use involves regular monitoring of concentration during the fumigation period and topping up with additional gas.



## 3.2. Modern storage structures to optimise the effectiveness of each fumigation

### 3.2.1. Storages fitted with a recirculation system

Modern silo designs increasingly incorporate powered and passive recirculation systems to improve gas distribution throughout the storage. For example, silos larger than 150t will benefit from a sealed, powered recirculation system, which helps achieve uniform gas concentration earlier in the fumigation period. Trials have shown that without powered recirculation, phosphine moves through grain at a rate of approximately 6m per day in any direction. In silos larger than 300t without powered recirculation, the phosphine label stipulates an extended exposure period of 20 days, referred to as 'surface only application'. By contrast, powered recirculation enables the fumigation period to be reduced to the standard 7 to 10 days, depending on grain temperature.

### 3.2.2. Storages fitted with a thermosiphon

Thermosiphons (passive recirculation systems) are fitted to some silos as purpose-built chambers for applying phosphine from ground level. This offers a safety advantage, as the operator does not need to climb to the top of the silo to apply the fumigant. However, top lids and vent openings must still be accessed and sealed properly before fumigation, which may require climbing. Research has shown that thermosiphons or passive recirculation systems offer negligible benefit to phosphine distribution. Their main advantage lies in the ability to apply the fumigant from the ground, allowing the gas to enter the storage from both the top and bottom simultaneously. However, in the absence of air movement,  $\text{PH}_3$  can reach explosive levels if allowed to accumulate in a confined space. This risk has been reported in both passive and active recirculation systems where fans were stopped prematurely.



Some silos are built with a chamber and passive (thermosiphon) or powered recirculation system for applying phosphine from the bottom.  
Photo: Ben White, Kondinin Group

### 3.2.3. Gas-monitoring equipment for remote data collection

Various devices are commercially available for monitoring gas concentration during and after a fumigation.  $\text{PH}_3$  can be measured by chemical detector tubes or electronic meters. The more commonly used electronic meters are available in high range (0 to 2000ppm) for measuring concentration during fumigation, and low range (0 to 20ppm) for measuring concentration post-venting for clearance and safety. Meters can be read manually – requiring an operator to connect, sample and record – or read in real time, where readings are logged periodically to the device or to a remote dashboard.

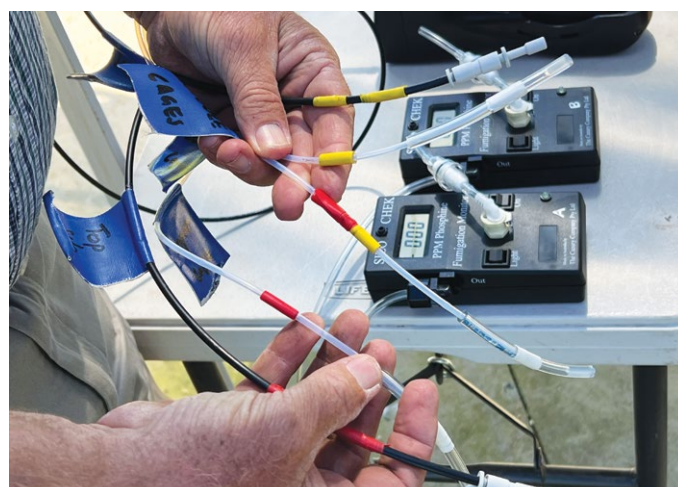
Fumigation meters are an essential part of best practice use of phosphine, especially when fumigating storages that cannot be pressure tested, such as bunkers and bags. The required equipment includes a meter, a gas sampling line (hard nylon tubing with a two-millimetre inner diameter), and a pump (used to evacuate the gas line). Gas lines are set up to sample the atmosphere in the fumigated space. All gas lines should be routed to a safe location where concentrations can be measured. Commercially available remote sensors can also be placed inside fumigated storages to transmit concentration readings to computers or mobile phones.

### 3.3. Maximising the effectiveness of existing older storage structures

#### 3.3.1. Retro-sealing

To improve fumigation outcomes in older silos, some growers have invested in retro-sealing them. Retro-sealing specialists use a range of rubber, specialised rubberised cements and silicone compounds to seal sheet joints, bolts, rivets, lids and openings on older silos. These materials are typically applied using an air-operated gun with coarse flow settings to handle the heavy product viscosity. Special attention should be given to the interface between the pad and the bottom sheet of the silo, and where the top sheet meets the roof, as these are common points of limited seal integrity. Customised sealing plates can be fabricated for doors, vents and openings. Oil-filled pressure-relief valves can also be fitted. The cost of retro-sealing an older-style silo can be significant, often up to 50% of the cost of a new sealed unit. Ensure the retro-sealing contractor provides a guarantee that the completed silo will meet the Australian Standard for sealed silos AS 2628-2010.

Alternatively, growers may choose to invest in pest prevention measures in unsealed silos and buy new sealable silos for batch fumigation when required.



Monitoring of  $\text{PH}_3$  gas concentration is a critical step towards a successful fumigation. Photo: QDPI

#### 3.3.2. Investing in regular maintenance

A well-built, aerated, quality gas-tight sealable silo constructed to meet AS 2628-2010 with a thorough maintenance regime can be expected to provide around 25 years of serviceable life before major repairs are required. Common repair points include latches and seals on openings as well as damaged fill and empty points. On flat-bottom silos, particular vigilance is needed around the storage base where the wall meets the concrete slab. Replacing or cleaning damaged fans and recirculation systems are examples of other maintenance activities that will drastically improve silo performance and fumigation efficacy.

For bunkers, regular maintenance is essential to address any wear and tear that may compromise the seal. This includes inspecting and repairing tarps, welds, joints, bird entry points etc.

### 3.4. Application rates for different formulations

Application rates vary across commercially available forms of  $\text{PH}_3$ , but in all cases they are aligned with the storage capacity, the commodity stored, the target pests, and – most importantly – the commodity temperature. Table 1 (page 15) presents consolidated application rates for both solid and cylinderised forms of  $\text{PH}_3$  based on information retrieved from registered product labels. Some lower application rates have been deliberately omitted from the table due to limited industry use in recent years, primarily because of poor efficacy against target pests and logistical constraints (e.g. extended exposure periods).



**Table 1: Registered PH<sub>3</sub> formulations and their application in different storage conditions for management of stored grain insect pests. Note: This is consolidated information based on labels of each product available on the APVMA website, to be used as a guide only. Always read and follow product labels.**

Formulation and active ingredient	Commodities and storage structures	Target pests	Application method	Commodity temperature	Minimum application rate (g/m <sup>3</sup> ), exposure period (days) and minimum concentration (ppm) (for cylinderised liquefied gas)
<b>Tablets</b> (solid tablets) (56% PH <sub>3</sub> )	<b>Commodities:</b> Raw cereal grains (barley, maize, millets, oats, rice, rye, sorghum, triticale, wheat); other food commodities (milled cereal products, flour, breakfast cereals, dried fruits, pulses, peanuts, oilseeds, cocoa and coffee beans); seeds for propagation, bulk stockfeed  <b>Structures:</b> Well-sealed structures, fumigation enclosures, grain storage sheds, silos and structures, which are suitable for fumigation	Major stored-products pests, including resistant populations of lesser grain borer, red flour beetle, confused flour beetle, saw-toothed grain beetle, flat grain beetles (including the rusty grain beetle, <i>Cryptolestes ferrugineus</i> ), dried fruit beetle, cigarette beetle, warehouse beetle, bean weevil, rice weevil, maize weevil, granary weevil, all storage moth species, and psocids	Place tablets in a single layer, separated from the commodity, e.g. on non-flammable trays/sheets evenly spread on the commodity surface	<b>Above 25°C</b>	<b>Tablets, bag chain or blankets</b> <b>Storage capacity: less than 375m<sup>3</sup></b> (e.g. 300t wheat capacity) 1.5g/m <sup>3</sup> (7 days)  <b>Storage capacity: greater than 375m<sup>3</sup> (&gt;300t)</b> (surface only application) 1.5g/m <sup>3</sup> (20 days) 1.5g/m <sup>3</sup> (10 days) with powered recirculation  <b>Bag chain or blankets</b> <b>Storage capacity: bunker storages greater than 1000t</b> 0.6g/m <sup>3</sup> (20 days)  <b>Important:</b> The above-recommended rates for tablets, bag chains and blankets do not control strongly resistant rusty grain beetle, <i>Cryptolestes ferrugineus</i> . Apply alternative treatments including cylinderised PH <sub>3</sub> , sulfuryl fluoride or grain protectants.
<b>Bag chains</b> (sachets linked as a chain) (57% PH <sub>3</sub> )	<b>Commodities:</b> Same as above  <b>Structures:</b> Well-sealed structures such as sealed enclosures, grain storage sheds, silos and structures, which are suitable for fumigation, well-sealed plastic-covered bunkers not less than 1000t capacity	Same as above	Open the product container in the open air, remove the bag chain and hang from the top of the closed storage structure or lay on the top of the commodity or in space to be fumigated	<b>15-25°C</b>	<b>Tablets, bag chain or blankets</b> <b>Storage capacity: less than 375m<sup>3</sup></b> (e.g. 300t wheat capacity) 1.5g/m <sup>3</sup> (10 days)  <b>Storage capacity: greater than 375m<sup>3</sup> (&gt;300t)</b> (surface only application) 1.5g/m <sup>3</sup> (20 days) 1.5g/m <sup>3</sup> (10 days) with powered recirculation
<b>Blankets</b> (grey-green powder enclosed in a blanket-like package) (57% PH <sub>3</sub> )	<b>Commodities:</b> Same as above  <b>Structures:</b> Well-sealed structures such as fumigation enclosures, grain sheds, silos, well-sealed plastic covered bunkers not less than 1000t capacity	Same as above	Open product container in the open air, remove blanket and roll out on the top of commodity or lay on the floor in space to be fumigated		<b>Bag chain or blankets</b> <b>Storage capacity: bunker storages greater than 1000t</b> 0.6g/m <sup>3</sup> (20 days)  <b>Important:</b> The above-recommended rates for tablets, bag chains and blankets do not control strongly resistant rusty grain beetle, <i>Cryptolestes ferrugineus</i> . Apply alternative treatments including cylinderised PH <sub>3</sub> , sulfuryl fluoride or grain protectants.

continued page 16

Table 1 (continued): Registered PH<sub>3</sub> formulations and their application in different storage conditions for management of stored grain insect pests. Note: This is consolidated information based on labels of each product available on the APVMA website, to be used as a guide only. Always read and follow product labels.

Formulation and active ingredient	Commodities and storage structures	Target pests	Application method	Range of commodity temperatures	10g/m <sup>3</sup> (140ppm)	15g/m <sup>3</sup> (215ppm)	25g/m <sup>3</sup> (360ppm)	35g/m <sup>3</sup> (500ppm)	50g/m <sup>3</sup> (700ppm)
<b>Cylinderised PH<sub>3</sub></b> (2% PH <sub>3</sub> and 98% CO <sub>2</sub> )  <b>Cylinderised PH<sub>3</sub></b> (100% PH <sub>3</sub> )  <b>Note:</b> PH <sub>3</sub> concentration needs to be monitored throughout the fumigation period for top-ups in case concentration falls below the required level.	<b>Commodities:</b> Raw cereal grains (barley, maize, millets, oats, rice, rye, sorghum, triticale, wheat); other food commodities (milled cereal products, flour, breakfast cereals, dried fruits, pulses, peanuts, oilseeds, cocoa and coffee beans); seeds for propagation, bulk stockfeed  <b>Structures:</b> Silos, bins, flat storages, fumigation chambers	Major stored-products pests, including the resistant populations of lesser grain borer, red flour beetle, confused flour beetle, saw-toothed grain beetle, rusty grain beetle, flat grain beetle, dried fruit beetle, cigarette beetle, warehouse beetle, bean weevil, rice weevil, maize weevil, granary weevil, all storage moth species, and psocids  <b>Strongly resistant rusty grain beetle, <i>Cryptolestes ferrugineus</i></b>	Cylinders in use should be in open air or in forced-ventilate fume room  Apply the required amount of gas only with approved high-pressure metering equipment by turning the cylinder valve fully on	15°C to 19°C	16 days	14 days	13 days	12 days	10 days
				20°C to 24°C	15 days	12 days	10 days	10 days	9 days
				25°C to 29°C	16 days	10 days	7 days	6 days	5 days
				30°C or higher	18 days	11 days	7 days	4 days	3 days
				<b>Range of commodity temperature</b>	<b>15g/m<sup>3</sup> (215ppm)</b>	<b>25g/m<sup>3</sup> (360ppm)</b>	<b>35g/m<sup>3</sup> (500ppm)</b>	<b>50g/m<sup>3</sup> (700ppm)</b>	<b>70g/m<sup>3</sup> (1000ppm)</b>
				20°C to 24°C	n/a	30 days	n/a	23 days	n/a
				25°C to 29°C	n/a	27 days	n/a	18 days	12 days
				30°C to 35°C	n/a	n/a	n/a	16 days	10 days
				35°C or higher	n/a	n/a	n/a	15 days	6 days

**Table 2: Current recommended venting periods for phosphine across different storage types.**

Storage type	Aeration system	Minimum ventilation time	Notes
Flat/cone-bottom silo	Without aeration	5+ days (passive) <sup>#</sup>	Larger silos may need longer due to slow desorption from grain
Flat/cone-bottom silo	With aeration	1+ days (with fan operating) <sup>#</sup>	Faster gas desorption when recirculation is used
Well-sealed bunker (>1000t)	Passive (natural airflow)	2–5 days (with wind flow) <sup>#</sup>	May be extended depending on structure tightness
Grain bag (200–300t)	Passive	35+ days*	Fan-assisted venting can reduce this to 2+ days

<sup>#</sup>Label recommendation; \*Guide only, based on research.

**Table 3: Current recommended withholding periods for commodities fumigated with phosphine.**

Grain type	Withholding period	Additional notes
All cereal grains, pulses and oilseeds	2 days after ventilation	Required after gas levels fall below 0.3ppm TLV-TWA (threshold limit value – time-weighted average) after the completion of a recommended ventilation period. Human consumption or use of fumigated grain for stockfeed is not allowed without the completion of withholding period.



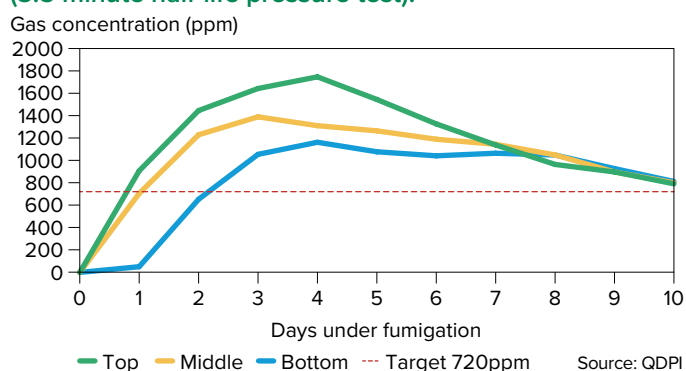
## 04. FUMIGATION PROCESS

Fumigants are gaseous pesticides that penetrate grain masses to eliminate pests. However, their effectiveness depends on maintaining a sealed environment. Therefore, sealing storage facilities is essential to ensure the efficacy of fumigants used for insect control. Key considerations for sealing storages include the following.

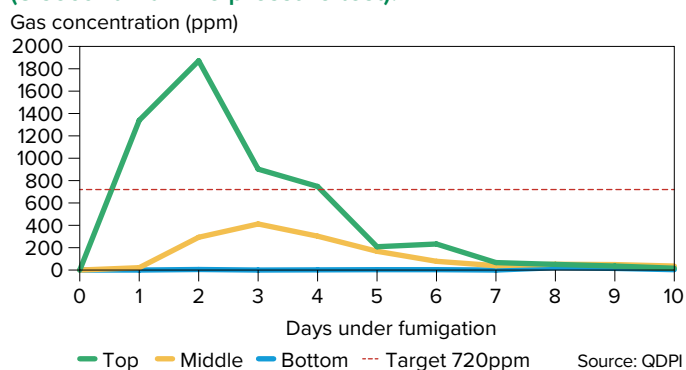
### 4.1. Structural integrity

Ensuring that storage structures are free from cracks, gaps and other openings is vital. Any breach in the structure can lead to the escape of fumigants, reducing their concentration and effectiveness.

**Figure 2: Gas concentration in a gas-tight silo (3.5 minute half-life pressure test).**



**Figure 3: Gas concentration in a non-gas-tight silo (8 second half-life pressure test).**



### 4.1.1. Ensuring 'sealability' of silos through pressure testing

Pressure testing is essential to check the gas-tightness of sealable silos. A gas-tight silo ensures that fumigants remain at the required concentration long enough to kill all life stages of grain pests. Leaks allow gas to escape too quickly, reducing treatment effectiveness and increasing the risk of insect resistance. Australian Standard AS 2628-2010 outlines the minimum pressure retention requirement for sealable silos. According to AS 2628-2010, a silo must maintain a 25mm water gauge pressure half-life (250 to 125 pascals) for no less than five minutes. Passing this test confirms that the silo is gas-tight and suitable for effective fumigation.

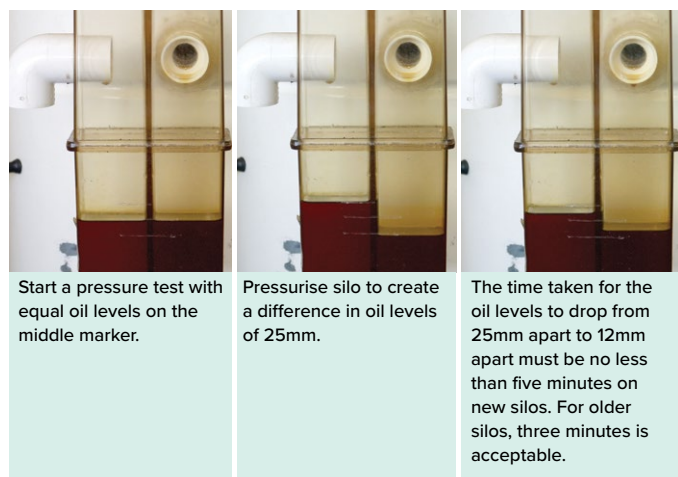
#### 4.1.1.1. When to perform the pressure test

Perform a pressure test:

- before each fumigation
- after any repairs, maintenance, or modifications to the structure
- at least once a year as part of regular storage maintenance.

#### 4.1.1.2. How to perform the pressure test and locate leaks

1. If using the pressure relief valve on the silo, ensure the oil level is at the middle mark, or draw a mark at the level point. A sensitive manometer or pressure gauge can also be used.
2. Conduct the pressure test when ambient conditions are stable, as temperature fluctuations inside the silo affects air pressure and therefore results.
3. Seal all openings and ensure the oil level in the relief valve does not move for several minutes, indicating the temperature is not affecting internal pressure.
4. Use a leaf blower to pressurise the silo until the oil in the relief valve shows a difference of 25mm or 250Pa.
5. With the leaf blower removed and the silo sealed, measure how long it takes for the oil in the relief valve to drop from 25 to 12.5mm apart or from 250 to 125Pa. The time taken should be no less than five minutes.



Pressure testing a gas-tight, sealable silo – required for effective phosphine fumigation. The Australian Standard (AS 2628-2010) states that sealable storage must perform a five-minute, half-life pressure test (photos left to right). Photo: Chris Warrick, Primary Business

## 6. If the silo fails the test:

- inspect common leak points such as roof hatches, access holes, aeration ducts, wall seams and outlets
- repressurise the silo and spray soapy water along seals and joins; bubbles will appear where leaks exist
- repair identified leaks and repeat the test.

## 4.1.2. Bunker sealing

Sealing bunker storages for fumigation involves using tarps to create a gas-tight environment, which is essential for maintaining fumigant concentration. To achieve this, both sewing and welding techniques can be employed to join tarps. Sewing tarps involves stitching them together with durable, weather-resistant thread, ensuring that the seams are tight and secure. To further enhance the seal, it is necessary to apply a sealant to the sewn seams. This sealant fills any small gaps or holes that might be present, ensuring a completely gas-tight seal. Welding tarps, on the other hand, uses heat to fuse the material, creating a seamless and robust bond. It is crucial to join floor tarps with top tarps effectively, as any gaps can compromise the gas-tight seal. Proper sealing not only enhances fumigation efficacy but also helps preserve the quality of the stored grain.



Correct phosphine use will ensure we do not add to the population of resistant grain storage pests. Photo: WA DPIRD

## 4.2. Workplace health and safety considerations

The fundamental approach to grain storage safety is the same as for all other farming activities. The aim is to have a safe workplace for everyone on the farm and at bulk storage facilities, including workers, contractors, families, visitors and the owner/managers. Working around grain storage sites presents several safety risks that must be managed under Australian workplace health and safety regulations. Key hazards include gas exposure during fumigation, dust explosions, falls from height, and equipment-related accidents.

### 4.2.1. Personal protective equipment

Appropriate personal protective equipment (PPE) is essential when working in or around silos and bunkers, especially during fumigation or maintenance. This includes:

- respirators or full-face masks (with appropriate filters) when handling fumigants such as phosphine
- elbow-length PVC gloves and long-sleeved clothing
- harnesses and fall protection when working at heights (e.g. silo roofs or ladders)
- dust masks and eye protection.

### 4.2.2. Gas monitoring and exposure limits

Phosphine is a highly toxic gas and must be carefully monitored.

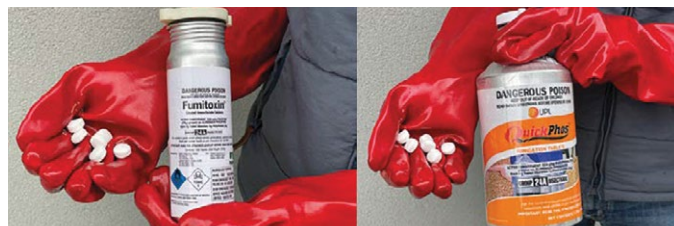
- Phosphine TLV-TWA (threshold limit value – time-weighted average): 0.3ppm.
- Continuous or portable gas monitors should be used during and after fumigation to check for residual gas before entry.
- Ensure warning signs are placed at all entry points to the area where a silo is under fumigation – the sign must contain the words ‘DANGER – POISONOUS GAS, KEEP AWAY’.

### 4.2.3. Electrical safety

- All electrical equipment used in and around silos must be compliant with Australian electrical standards and suitable for use in dusty environments.
- Regular inspection and maintenance of motors, fans, lighting and wiring help prevent short circuits or ignition sources.
- Fumigants are corrosive, so it is important to check all electrical items that come into contact with the gas.

### 4.2.4. Other considerations

- Emergency plans should include first aid procedures, contact information and evacuation routes specific to silo incidents.
- Provide appropriate training for all storage operators and certified training where required, for example, working at heights, working in confined spaces and fumigation procedures.



Phosphine tablet packs. Left: 100-tablet tin (300g) suitable for 50t of wheat storage capacity. Right: 500-tablet tin (1.5kg) suitable for 250t of wheat storage capacity. Source: Ben White, Kondinin Group



Phosphine bag blanket packs. Left: Bag chain (340g) suitable for 60t of wheat storage capacity. Right: Blankets 1.7kg x 2 (total 3.4kg) suitable for 600t (or 2 x 300t) of wheat storage capacity in silos. Or 1500t of wheat capacity in a bunker. Source: Ben White, Kondinin Group

## 4.3. Introduction of fumigant

The effective introduction of  $\text{PH}_3$  depends on the type and configuration of the grain storage. In gas-tight **sealable silos**,  $\text{PH}_3$  can be introduced from the top, the bottom, or both. **Top introduction** involves placing tablets or bag chains in trays in the headspace, ensuring even distribution and good airflow around the fumigant for effective gas dispersal. **Bottom application**, often done through a purpose-built chamber, offers safety advantages by avoiding the need to climb the silo, but must include a passive (thermosiphon) or powered recirculation system to prevent gas build-up and potential explosions. **Powered recirculation systems** are beneficial in silos over 150t and essential for silos above 300t. These systems use a small fan to gently move  $\text{PH}_3$  gas through the grain, ensuring faster and more uniform distribution. Without powered recirculation, gas movement is much slower (about 6m/day), requiring longer exposure periods (up to 20 days).

In **grain bags**,  $\text{PH}_3$  is typically applied using slotted PVC pipes placed at regular intervals (every 7m), ensuring the gas is evenly distributed. These pipes must allow resealing of the  $\text{PH}_3$  and prevent residue contamination. Monitoring with high and low-range  $\text{PH}_3$  meters is critical throughout the fumigation and venting process.



Phosphine in bag chains removes the risk of residue being spilt, but at least 1% of residue will not evolve until it comes into contact with moisture so a respirator and PPE are also required to remove it from the silo.

Photo: Chris Warrick, Primary Business



For **small bunkers**, tablets should be evenly distributed throughout the grain stack using trays or blankets to ensure even dispersal and circulation of gas. For **large bunkers**, consider using  $\text{PH}_3$  blankets or bag chains for easier application and removal.

## 4.4. Monitoring of gas concentration

High-range  $\text{PH}_3$  meters are used during the fumigation process to ensure the gas reaches and maintains the required concentration throughout the storage structure, especially in unsealed storages such as bunkers or grain bags. Detection range is typically from 0 to 2000ppm or higher and is used with monitoring lines inserted into the silo or structure. Measure gas levels at **multiple points** in the silo (top, middle, bottom) and in bunkers as distribution can vary, especially without recirculation systems. Low-range  $\text{PH}_3$  meters are essential for determining safe re-entry and grain delivery post-fumigation. These are used after ventilation to confirm that the atmosphere has dropped below the maximum allowable limit (0.3ppm for TWA exposure, see subsection below). Detection range is typically 0.01 to 20ppm. These devices are not used to monitor gas concentrations throughout a fumigation event due to their low detection range.

## 4.5. Ventilation after completion of fumigation

While under fumigation, commodities tend to absorb  $\text{PH}_3$  gas (sorption), which they later emit back to the atmosphere (desorption). Some commodities, such as oilseeds, can be highly sorptive compared with wheat, barley and sorghum. The process of sorption/desorption continues during the fumigation period, and desorption can take some time to complete at the end of the fumigation. A critical workplace health and safety requirement for  $\text{PH}_3$  fumigation is to follow the recommended ventilation period after fumigation to allow the fumigated commodity to complete the desorption process to a level where the concentration remains below 0.3ppm. This standard, called the threshold limit value–time weighted average (TLV-TWA), is based on a worker's exposure to an average airborne concentration of  $\text{PH}_3$  over an eight-hour working day, five days a week. Failure to meet this standard will lead to rejection of the fumigated grain at the receival point.

Table 2 (page 17) presents the recommended ventilation periods for different types of storage structures. It highlights the use of fan-forced aeration/recirculation systems in significantly reducing the ventilation period compared with passive ventilation that depends on the natural airflow.

## 4.6. Withholding period

Fumigation is considered complete only after the fumigation period, the ventilation period and the recommended withholding period have all been observed. Currently, a withholding period of two days is recommended for all cereal grains, pulses and oilseeds fumigated with  $\text{PH}_3$  (see Table 3, page 17). Fumigated produce should not be used for human consumption or stockfeed until the two-day withholding period has been completed.



Phosphine tablets. Photo: QDPI



Photo: Paul Jones/GRDC

## 05. CAUSES OF DEVELOPMENT OF RESISTANCE IN KEY INSECT PEST SPECIES

### 5.1. Genetic basis of resistance

PH<sub>3</sub> resistance occurs as two phenotypes: weak (WR) and strong (SR). This resistance is caused by two major genes (*rph1* and *rph2*). These genes are autosomally inherited and incompletely recessive (Collins et al., 2002; Daglish et al., 2014; Nguyen et al., 2015). However, in certain life stages of some species, the genes may be dominant or semi-dominant (Collins et al., 1996; Kaur et al., 2012; Venkidusamy et al., 2018). This means that the genes will be passed down through generations and persist in the population, but the condition can manifest with varying severity. Further, there do not appear to be any fitness costs associated with possession of these resistance genes, with development time, number of progeny and longevity all similar to susceptible populations (Jagadeesan et al., 2012; Daglish et al., 2015; Singarayan et al., 2021).

Briefly, WR is controlled primarily by a single major gene (*rph1*), while SR is controlled by the two major genes (*rph1* and *rph2*) (Nguyen et al., 2015). There is a strong synergistic epistatic reaction when both genes are homozygous for resistance (Schlipalius et al., 2002; Jagadeesan et al., 2012). This means that when all alleles on the genes are coded for resistance, the resulting SR insects have a level of resistance several times above that of WR insects. This can range from resistance factors of 9 to more than 1000, depending on the species (Nayak et al., 2013; Nguyen et al., 2015). Of the five major stored grain beetle pests in Australia, rusty grain beetle (*Cryptolestes ferrugineus*) has been recorded with the highest level of resistance yet detected (Nayak et al., 2013) and current rates prescribed on the Australian PH<sub>3</sub> label are unable to control them (Nayak et al., 2010) (refer to application rates in Table 1, page 15).

WR appears to develop relatively easily resulting in it being common and widespread (Emery et al., 2003, 2011) but is readily controlled with the current PH<sub>3</sub> fumigation protocols registered in Australia. SR is less common, as it takes time to develop – it must be homozygous for both *rph1* and *rph2* (Schlipalius et al., 2002; Jagadeesan et al., 2012). However, it has been stated that once the frequency of WR approaches 80%, SR will soon develop (Emery et al., 2011).

### 5.2. Underdosing

The primary cause of the development of SR to PH<sub>3</sub> in stored grain insects is the use of sublethal doses (Emery et al., 2003; Holloway et al., 2016). This may occur through the use of an inadequate concentration and/or duration of PH<sub>3</sub> during fumigation. By underdosing, susceptible and some WR individuals may be controlled, but SR insects survive. This creates a population with

a higher proportion of resistant genes, increasing the probability of genetic homogeneity leading to the development of strong resistance. Repeated fumigations, particularly if done in the same manner, will exacerbate the problem (Emery et al., 2011).

Underdosing can occur through a variety of methods, which are as follows.

#### 5.2.1. Incorrect application of phosphine formulation

As PH<sub>3</sub> acts as a gas, irrespective of its various available forms, dose calculations must treat the entire volume of the storage, regardless of the amount of commodity it contains. This includes any external features such as thermosiphons.

While concentration time (CT) and exposure periods can be manipulated to some extent (refer to subsection 2.4, page 9), all fumigations must be maintained for the minimum duration stated on the label. This is due to the variation in tolerance levels to PH<sub>3</sub> among different life stages of the insects. Adults and larvae, the most visible stages due to their mobility, are generally the most susceptible. This can lead to the misconception that the fumigation was successful. However, eggs and pupae require a longer duration under gas to be effectively controlled (refer to subsection 2.3, page 8). This is primarily because they are in a dormant or less metabolically active phase (Venkidusamy et al., 2018). Survival of these stages can form a nucleus for reinfestation or dispersal of resistance.

Maintaining PH<sub>3</sub> concentrations for the minimum duration is more challenging in larger storages as it takes time for the gas to penetrate the commodity. This can be alleviated with active recirculation (see Table 1, page 15) using a fan or a thermosiphon that circulates the air through the structure, passively utilising temperature and pressure changes.

#### 5.2.2. Inappropriate infrastructure

PH<sub>3</sub> is a highly mobile gas that is slightly (1.17 times) heavier than air. It flows freely and tends to follow the path of least resistance. Consequently, PH<sub>3</sub> gas will tend to escape through any cracks or holes into the atmosphere rather than flow through the commodity. In unsealed silos, or sealed silos that are not properly maintained, this results in a loss of gas to the atmosphere and concentrations falling below those recommended for the entire duration of the fumigation. It is not surprising, therefore, that most SR insects have been detected in unsealed storages (Emery et al., 2011; Holloway et al., 2016).



SIROFLO® is a system designed to allow fumigation in leaky storages. It is a pressurised system that dispenses a continuous stream of  $\text{PH}_3$  into a calculated airflow fed at the base of a silo (CSIRO, 2014). The aim is to distribute the gas evenly throughout the grain for a sufficient duration to kill even the most resistant insects, including eggs and pupae. Unfortunately, almost all SR insects in eastern Australia were initially detected in unsealed central storages using SIROFLO® (Emery et al., 2011). It appears that the low concentrations recommended for the SIROFLO® system were insufficient to control the target pests, and continued use led to the development of SR insects in these storages.

### 5.2.3. Temperature and moisture

Both temperature and moisture influence a  $\text{PH}_3$  fumigation (Hole et al., 1976; Collins and Daglish, 2001; Nayak and Collins, 2008) (refer to subsection 2.5, page 9).  $\text{PH}_3$  is most effective at higher temperatures and lower humidity.

At low temperatures, fumigants vaporise and diffuse more slowly (Bond, 1984). There is also a reduction in the killing action of the gas, as insects are less active with lower metabolic rates, resulting in a reduction in the uptake of the gas. Consequently, fumigations at cooler temperatures require higher label rates and/or longer exposures to be effective.

As moisture affects fumigant penetration, commodities and storages with a higher moisture content require higher doses of  $\text{PH}_3$ . Higher moisture content generally leads to greater sorption of the gas into the grain, thus reducing the overall concentration of  $\text{PH}_3$  within the storage. However, solid formulations of  $\text{PH}_3$  require humidity to generate gas. If the air is too dry or commodity moisture content too low, gas generation is slowed and the peak concentration delayed resulting in a potential to underdose.

The ideal temperature range for a  $\text{PH}_3$  fumigation is 25°C to 35°C. Fumigations may still be conducted between 15°C and 25°C, but the exposure period must be extended to counteract the factors listed in Table 1 (page 15). While fumigations are possible at temperatures <15°C, it is not practical as the insects are too hard to kill. For solid formulations of  $\text{PH}_3$ , commodity moisture content must be above 9%, or the storage must have a relative humidity of >25%.

## 5.3. Multiple fumigations of the same parcel of commodity

One problem with  $\text{PH}_3$  fumigations is the illusion of success. The visual, mobile life stages of the insect are generally easily killed. However, inconspicuous eggs and pupae may survive. Depending on storage conditions, in a few days or weeks, it appears that the storage has been reinfested and requires another fumigation. Due to the lack of inexpensive alternatives,  $\text{PH}_3$  is often used multiple times on the same commodity. Likewise, central storages that maintain grain ready for delivery may have a calendar-operation system in place to fumigate every three months or so.

Multiple fumigations on the same parcel of grain select for resistance, particularly if the fumigation is not 100% effective. Surviving insects are more likely to carry resistance genes, making them more tolerant to  $\text{PH}_3$ . By eliminating the susceptible genes, there is a higher proportion of resistant genes in the population. This results in a higher probability of the insect progeny being homozygous for the resistant genes. Fumigating again will eliminate the weaker, more tolerant insects, which again increases the proportion of resistant genes. Eventually, no susceptible genes are present, resulting in 100% SR insects.

Furthermore, multiple doses of fumigants may increase the chances of gene mutations occurring. This may lead to insects with a much higher tolerance for the fumigant.

## 5.4. Inadequate hygiene providing refuges for pest populations and reinfestation

Insects do not require much food to survive long periods and can survive in cracks and crevices. Inadequate hygiene within a grain storage can lead to multiple fumigations on the same insect, resulting in selection for resistance (see previous subsections). Stored grain insects can also survive within unclean machinery and small grain spills.

Stored grain insects are highly mobile and disperse over distances of several kilometres (Holloway et al., 2018, 2020; Ridley et al., 2011, 2016). Despite conditions appearing suitable with plenty of food, stored grain insects are still driven to disperse. Ridley et al. (2011) found that 88% of female red flour beetles (*Tribolium castaneum*) emerging from a farm silo had already mated. This means that colonisation of populations in storages was ready to begin immediately. Furthermore, any resistance genes developed in the original storage are spread throughout the environment.

## 06. DEVELOPMENT AND IMPLEMENTATION OF A RESISTANCE MANAGEMENT STRATEGY

### 6.1. Early detection of resistance development through monitoring

A critical step in managing resistance is monitoring pest susceptibility to chemical treatments. This helps to identify early failures of control measures and the onset of resistance in pests. It also shows trends in the frequency of existing resistances. The key factors that enable assessment of the susceptibility status of a pest population to a specific treatment are:

- a carefully designed insect sampling strategy that targets focal points across all storage sectors
- diagnosis of resistance in collected samples  
(Collins et al., 2017; Holloway et al., 2016; Nayak et al., 2013; Nayak et al., 2017; Nayak et al., 2021).

This approach also helps in understanding potential risk factors involved in resistance developments and the geographic spread of existing resistances. Australia has a well-established national resistance monitoring program to detect resistance to  $\text{PH}_3$  and contact insecticides in major storage pests and support their management. This program enables us to detect resistance early, characterise its strength, and restrict pests' spread to other geographic regions through their timely eradication (Daglish et al., 2002; Collins et al., 2005; Nayak et al., 2013). This national program covers three grain-growing regions (southern, northern and western) demarcated by GRDC, and monitors research undertaken by a laboratory located in each region.

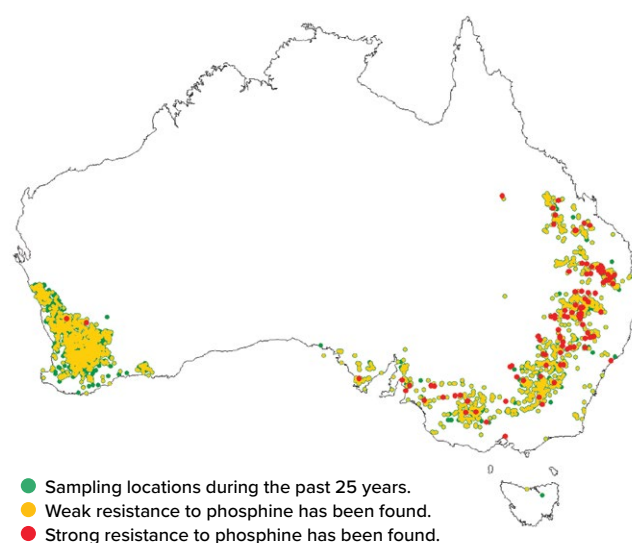
#### 6.1.1. Types of monitoring and their purpose

Australia has the longest history (more than three decades) of monitoring resistance to phosphine and key grain protectants, coordinated nationally through the collaborative efforts of researchers, growers, bulk storage operators and other stakeholders who handle and store grain (Emery et al., 2011; Holloway et al., 2016; Nayak et al., 2020). A statistically robust, nationally agreed protocol is followed for this monitoring program, which involves three principal monitoring activities: systematic, targeted and tactical.

#### 6.1.2. Systematic monitoring

Systematic monitoring involves the collection of insect samples from all sectors of the industry in a pre-determined random survey of storage facilities. At least 100 farms are visited each year in each of the three GRDC regions for this survey and insect samples are subjected to established discriminating doses of  $\text{PH}_3$  and other grain

Figure 4: Phosphine resistance – national situation.



Source: WA DPIRD

treatments (Emery et al., 2011). This activity provides data on overall trends in the frequency of existing resistances in key pest species both regionally and nationally, and it also helps identify emerging resistances.

#### 6.1.3. Targeted monitoring

Targeted monitoring is undertaken at storage sites with suspected resistance problems, as well as sites where a follow-up visit is necessary to evaluate the success of an intervention strategy that has been implemented to manage a known resistance problem.

#### 6.1.4. Tactical monitoring

The third approach is tactical monitoring, where insect samples are sent directly by bulk storage operators, millers and grain processors – mostly from storages where insects survive a treatment leading to control failure. The insect samples are diagnosed within 24-hours of arrival in the laboratory using a 'rapid/quick test' (see subsection 6.3.1.2, page 27) and results are provided to the industry for making timely resistance management decisions in the field (Emery et al., 2011; Holloway et al., 2016; Nayak et al., 2020).

## 6.2. Understanding the ecological implications of resistance development

Molecular tools to detect phosphine resistance have been well utilised in recent years (Schlipalius et al., 2019), as they provide additional value beyond simply detecting resistant insects. Collecting insect populations from a range of storage types across a wide geographical region over time and subjecting them to molecular diagnostics plays a critical role in estimating the accurate resistance allele frequency (including the proportion of heterozygote-resistant insects) in field populations.

Studies also proposed frequency thresholds for heterozygotes, which can effectively be used to predict the outbreak of highly resistant homozygote populations (homozygotes). Additionally, the high-throughput monitoring approach allows researchers to screen for novel resistance variants (i.e. the incursion of new resistances) in field populations, and so the spread of incursions to other regions can also be thwarted in time (Schlipalius et al., 2019; Nayak et al., 2021).

Neutral mitochondrial DNA markers were also deployed in the monitoring program to better understand the gene flow and population structures of major grain insect pests over a large geographical area, addressing a key question in managing resistance: insect movement (Ridley et al., 2011; Ridley et al., 2016). These population genetic studies provided vital ecological information such as the active dispersal (flight) of grain insect pests in the presence and absence of commodities in storage (Toon et al., 2018), and the spread of resistant insects over the broader geographical landscapes, both potentially contributing to the development of resistance. This information, along with key ecological investigations – such as paternity analysis and mating behaviour (polyandry) of resistant/susceptible insects (Malekpour et al., 2018; Rafter et al., 2018; Toon et al., 2018) – complement molecular resistance diagnostics and could provide a new perspective to model the rate and spread of resistance development and to guide management tactics accordingly.

## 6.3. Establishing a discriminating dose for detection of resistance

A global survey on  $\text{PH}_3$  resistance was carried out in the 1970s by the Food and Agriculture Organization of the United Nations (FAO, 1975) to establish phenotypic discriminating dosages (DD) for  $\text{PH}_3$  used in post-harvest grain storage, during which multiple representative insect populations were collected from 82 countries for diagnosis. The DDs developed in this study were based on the response of adult insects from susceptible populations (that had not been exposed to  $\text{PH}_3$ ) to a range of doses leading up to a lethal dose that yielded

99.9% mortality in the exposed adult insects ( $\text{LD}_{99.9}$ ) (see section 6.3.1.1, page 26). This study revealed a worldwide increase in the frequency of resistance to  $\text{PH}_3$  and proposed species-specific DDs for monitoring insect resistance globally. While the DDs established through the FAO are still used across the globe to detect resistance in major pest species by discriminating between susceptible and resistant populations, over the years these DDs have gone through several modifications due to the significant elevation in the strength of resistance to  $\text{PH}_3$ . In Australia, the researchers have established DDs for two levels of resistance: 'weak' and 'strong' (Collins et al., 2005; Daglish et al., 2002; Nayak et al., 2013). As discussed earlier in the text (refer to section 5.1, page 23), the two levels of resistance have been explained through the discovery of two genes (*rph1* and *rph2*) that are responsible for the development of two levels of resistance in key stored-product pest species (Schlipalius et al., 2012).

To establish DDs, randomly selected insect cohorts from field populations of each species are exposed to a set of known concentrations of fumigants based on two criteria: results of range-finding tests (Robertson et al., 2007) and the relevance of selected concentrations to the field application rates (Gautam et al., 2016; Nayak et al., 2013). Results of these dose-mortality assays are used to compare the relative susceptibility of species over the study region (spatial) and time (temporal) (Holloway et al., 2016; Collins et al., 2017; Nayak et al., 2017) and support researchers in establishing comprehensive baseline toxicity information.

### 6.3.1. Different resistance detection methods and their applications

Early detection of resistance and its characterisation form the basis for diagnosing control failures, assessing their likely impact, evaluating the success of intervention strategies, and developing integrated management strategies. Several methods are available to detect phosphine resistance in storage pests collected from the field; these are briefly described below.

#### 6.3.1.1. FAO test

The earliest method to detect  $\text{PH}_3$  resistance in several stored product pests was introduced through a global survey conducted under the auspices of the FAO (1975). This bioassay method, popularly known as the 'FAO test', recommends a diagnostic dose based on a gas concentration equivalent to the  $\text{LD}_{99.9}$  for adult beetles from a susceptible population when exposed for 20 hours at 25°C, followed by confirmatory mortality assessment 14 days after fumigation (FAO, 1975). Examples of diagnostic doses



recommended for  $\text{PH}_3$  resistance in common storage pests include 20ppm (milligrams per litre) for the lesser grain borer (*Rhyzopertha dominica*) and up to 50ppm (mg/L) for the wheat weevil (*Sitophilus granaries*) (Linnaeus). This method was later modified by Australian researchers to accommodate two resistance levels: weak and strong. For example, the discriminating doses to detect weak and strong resistance in *R. dominica* are 20ppm for 20 hours and 180ppm for 48 hours, respectively (Collins et al., 2017); for the rice weevil (*S. oryzae*) 30ppm and 180ppm for 20 hours, respectively (Holloway et al., 2016); and for red flour beetle (*T. castaneum*) 20ppm and 180ppm for 20 hours, respectively (Nayak et al., 2017).

### 6.3.1.2. Rapid knockdown tests

Although the FAO test is valuable and discrete in determining resistance phenotypes in field-collected populations, it is time-consuming and requires significant numbers to perform the test. There has been a significant interest in developing 'rapid' or 'quick' indicative tests that can reveal results on the same day. These assays rely on the proportion of insects knocked down (the inability of insects to move in a coordinated manner) to a known phosphine concentration and exposure time, instead of assessing mortality (% dead insects), as in FAO tests.

Although the rapid test for detection of  $\text{PH}_3$  resistance was first developed by Reichmuth (1991), the concept had gone through major changes over the past three decades. Now, well-developed tests are available to detect strong levels of resistance in field populations of major pest species. These include *T. castaneum* (Herbst) (Cato et al., 2019), *R. dominica* (Afful et al., 2021), *S. oryzae* (Nayak et al., 2019), and *C. ferrugineus* (Nayak et al., 2013). These tests are applied by researchers for the provision of same-day tactical advice to industry and used as a decision-making tool to implement resistance-intervention strategies. Two methodologies are followed:

1. performing discriminating bioassays over time in small gas-tight glass vials (Nayak et al., 2013, 2019)
2. using a commercial field test kit (Degesch) with a standard phosphine concentration of 3000ppm (Steuerwald et al. 2006; Cato et al., 2019; Afful et al., 2021).

The Degesch test diagnoses resistant insects after 8, 11, 12 and 13 minutes in *T. castaneum*, *O. surinamensis*, *S. granarius* and *C. ferrugineus*, respectively, whereas the glass vial assays rely on two concentrations, 1440 and 3600ppm, and discriminate the weak and strong resistant phenotypes over three to five hours in *C. ferrugineus* (Nayak et al., 2013) and *S. oryzae* (Nayak et al., 2019).

### 6.3.1.3. Molecular diagnostics

The identification of the strong resistance gene *rph2* has contributed significantly towards the development of a diagnostic molecular assay to accurately determine strong levels of  $\text{PH}_3$  resistance in major storage pests (Kaur et al., 2013, 2015; Schlipalius et al., 2012, 2018, 2019; Nayak et al., 2021). Apart from its accuracy, a distinct advantage of molecular diagnostics over phenotypic assays (FAO assays and rapid tests) is its ability to identify heterozygotes (carriers of resistance) in both live and dead insects. Researchers use simple low-throughput DNA marker assays as well as high-throughput strategies that involve tagged primers, multiplexing exon sequencing and demultiplexing (Schlipalius et al., 2019). The former is preferred in regional-specific studies in which a single variant is prevalent, and the latter can be adopted to gather more specific insight over larger landscapes.

## 6.4. Characterising the strength and frequency of resistance and modifying fumigation protocols

The process usually involves exposing a group of resistant adults to a range of low to high concentrations of  $\text{PH}_3$ , and the dose-mortality data from respective concentrations are then subjected to probit regression analyses (Finney, 1971). Any individuals surviving higher concentrations are reared over six to eight generations with a series of discriminatory selections promoting the homozygosity of the resistance trait (Collins et al., 2005). This homozygous strain represents the typical field selection and the 'worst case' for the resistance development scenario for that species. Key toxicity parameters such as resistance ratio (RR) and time-to-population-extinction (TPE) curves (against mixed-age populations of eggs, larvae, pupae and adults) are established for this strain. They are then used as a reference for the development of practical fumigation strategies in the laboratory. Once established in the laboratory, these protocols are validated through industry-scale field trials and finally adopted by industry through modification to the registered label (Collins et al., 2005; Lorini et al., 2007; Kaur and Nayak, 2015).

### 6.4.1. Reported cases of resistance in Australia

The national resistance monitoring program in Australia has helped the grains industry by providing early warning of resistance developments in key pest species and analysis of temporal trends and geographic spread over more than three decades. Resistance data stored in a central database called the Australian Grain Insect Resistance Database (AGIRD) revealed several key factors influencing the development of  $\text{PH}_3$  resistance (Emery et al., 2011; Daglish et al., 2014, 2015; Falk et al., 2015; Holloway et al., 2016; Collins et al., 2017; Nayak et al., 2013, 2017). Recent results of monitoring in Australia show that weakly resistant populations are quite common in on-farm as well as bulk storages and contribute to the relative increase in the frequency of strongly resistant populations across the value chain. Such findings highlight the importance of monitoring resistance and how it assists in combating resistance issues on time. Recent AGIRD data analysed suggests that, although the frequency of the common weak resistance has increased significantly over the past two decades and is recorded between 60 and 80% (depending on species), the frequency of strong resistance has remained below 10% for *R. dominica* (Collins et al., 2017), *S. oryzae* (Holloway et al., 2016), and *T. castaneum* (Nayak et al., 2017). The recent emergence of a very strong level of  $\text{PH}_3$  resistance in *C. ferrugineus* has become a major industry issue that requires the application of cylinderised  $\text{PH}_3$  or use of alternative treatments such as sulfuryl fluoride and grain protectants to manage.

### 6.4.2. Frequency of resistance in Australia compared with overseas countries

Data on periodic monitoring of  $\text{PH}_3$  resistance is also available from several countries across the globe. For example, in the US, recent resistance surveys have reported strongly resistant populations of major storage pest species (Afful et al., 2021; Cato et al., 2019; Gautam et al., 2016; Opit et al., 2012). Reports from Asia include strongly resistant populations of *R. dominica* from India (Kaur et al., 2015); *S. oryzae* from Vietnam (Nguyen et al., 2015), and *C. ferrugineus* from China (Chen et al., 2021). Lorini et al. (2007) reported a strong resistance frequency of 74% in *R. dominica* populations in bulk storage in Brazil, whereas very limited resistance data are available from Europe. A recent report on strong resistance in *T. castaneum* from Turkey (Kocak et al., 2015) reported resistance in *S. granarius* and *T. castaneum* from the Czech Republic (Aulicky et al., 2019) and, more recently, an extensive survey undertaken in Greece recorded resistance in populations of several species (Agrafioti et al., 2019). The resistance frequencies reported in these overseas

countries are much higher than that recorded in Australia, highlighting the importance of our existing monitoring and management strategies.

### 6.4.3. Changes in application rates (label) to manage strongly resistant pest populations

As covered in sections 2.3 (page 8) and 6.4 (page 27), it is important that  $\text{PH}_3$  fumigation protocols be developed against all life stages of resistant populations that mimic the fumigation in the field situation (Collins et al., 2005; Nayak and Collins, 2008; Kaur and Nayak, 2015). Manipulation of the key factors – concentration (C) and exposure period (t) – that influence the efficacy of  $\text{PH}_3$  significantly have been undertaken successfully over the years to extend the effective life span of  $\text{PH}_3$ , specifically in controlling strongly phosphine-resistant pests (Daglish et al., 2002; Collins et al., 2005; Nayak and Collins, 2008; Kaur and Nayak, 2015).

Utilising these research-based data, the  $\text{PH}_3$  label has been modified over the past two decades to accommodate new fumigation protocols (C x t) that have been developed against strongly  $\text{PH}_3$ -resistant populations (see Table 1, page 15). As Table 1 suggests, the modified protocols to control strongly resistant *C. ferrugineus* are only available in the cylinderised form of  $\text{PH}_3$  and are yet to be incorporated into the labels of solid formulations (see Table 1, page 15).

## 6.5. Alternative strategies to break the 'resistance cycle'

The phasing-out of methyl bromide has created opportunities for several alternative fumigants that have been utilised to manage the widespread  $\text{PH}_3$  resistance problems. These include sulfuryl fluoride (SF), ethyl formate (EF) and hydrogen cyanide (HCN). However, both EF and HCN have practical impediments when it comes to field applications (Nayak and Jagadeesan, 2024). Moreover, EF is highly sorptive and holds flammability risks, and therefore requires a suitable carrier gas such as  $\text{CO}_2$  for efficient gas penetration and distribution. For HCN, no detailed information on its safety and efficacy is available (Nayak and Jagadeesan, 2024). Apart from these, the evaluations of some newer fumigants including carbonyl sulfide, ethane dinitrile, chlorine dioxide, ozone, nitric oxide, propylene oxide and allyl isothiocyanate are in the preliminary stage (Nayak and Jagadeesan, 2024). This leaves SF as the only registered fumigant that has been trialled successfully over the past decade by the industry in Australia. It is currently being used as a 'resistance breaker' to mitigate strongly  $\text{PH}_3$ -resistant *C. ferrugineus* (Nayak and Jagadeesan, 2024).

### 6.5.1. Role of sulfuryl fluoride as 'phosphine resistance breaker'

Unlike other treatments, complete withdrawal of  $\text{PH}_3$  due to serious resistance problems across a broad pest spectrum has never been a viable option for the industry globally, considering its versatility and the range of benefits it offers (Nayak et al., 2020). Instead, developing and deploying an effective integrated resistance management (IRM) strategy with viable alternative tools, such as SF, will prolong the usefulness of  $\text{PH}_3$  and ensure the sustainability of chemical treatments in the IRM program.

Although significantly more expensive than  $\text{PH}_3$ , SF – which was predominantly considered as a flour-mill treatment – has successfully made the transition to a bulk grain fumigant in Australia in the wake of the development of strong  $\text{PH}_3$  resistance in *C. ferrugineus*. The biggest advantage SF offers is the lack of cross-resistance in  $\text{PH}_3$ -resistant pests to SF (Jagadeesan et al., 2015). Comprehensive laboratory studies have shown that this lack of cross-resistance to SF remained consistent, irrespective of inherent differences reported among the species, strains and life stages (Jagadeesan and Nayak, 2017).

In Australia, low-moderate concentrations (400 to 800g  $\text{hm}^{-3}$ ) over a range of exposure periods (6 to 10 days) were proven to be most ideal for treating bulk grain storages to eradicate strongly  $\text{PH}_3$ -resistant *C. ferrugineus* populations (Nayak et al., 2016). However, fumigations over short exposure periods (<72 hours) hold significant risks for not controlling the eggs (Nayak et al., 2016) and eventually lead to failure of SF to achieve complete control of pest populations. Utilising SF within the IRM umbrella as a break fumigant would augment other chemical treatments, including  $\text{PH}_3$ , and ensure the long-term viability of both. It is recommended to use SF judiciously to break the  $\text{PH}_3$  resistance cycle in storages with a history of long-term  $\text{PH}_3$  resistance problems. This approach will reduce the number of selection events in insects and thereby avoid the subsequent genetic (resistance response) or environmental consequences (residues).

### 6.5.2. Co-fumigation strategy

Recent studies explored several approaches to enhance the toxicity of  $\text{PH}_3$  and SF, primarily with a focus on overcoming genetic resistance and increased tolerance in eggs, respectively (Constantin et al., 2020; Jagadeesan et al., 2021). These studies have clearly demonstrated that the co-fumigation approach holds excellent potential in achieving complete control of  $\text{PH}_3$ -resistant insect pests with minimal environmental impacts. For instance, co-fumigation of  $\text{PH}_3$  with SF reduced the required concentration of both the fumigants

at least by half in controlling strongly  $\text{PH}_3$ -resistant *C. ferrugineus*. The half-dose rate of  $\text{PH}_3$  (84g  $\text{hm}^{-3}$ ) and one-fourth label rate of SF (375g  $\text{hm}^{-3}$ ) was sufficient over 168 hours to eliminate all insect life stages of strongly  $\text{PH}_3$ -resistant *C. ferrugineus* at 25°C (Jagadeesan et al., 2021). The efficacy relationship was 'additive' and remained consistent, irrespective of whether the combined treatment was applied simultaneously or sequentially (Jagadeesan et al., 2018).

Recent research also examined the potential of co-fumigating  $\text{PH}_3$  with atmospheric gases such as  $\text{CO}_2$ , resulting in an enhanced toxic effect of  $\text{PH}_3$ . The combination of a moderate concentration of  $\text{CO}_2$  (25 to 30%) with  $\text{PH}_3$  caused the synergistic increase in toxicity of both gases (Constantin et al., 2020). However, further research is required before this strategy can be adopted commercially, as it currently relies on the use of cylinderised  $\text{PH}_3$  only.

### 6.5.3. Use of hermetic storage conditions and freezing technology

Maintaining hermetic conditions (hypercapnic and/or hypoxic) in selected storage structures, particularly for industries relying on non-chemical control measures, was also proposed as viable alternatives to  $\text{PH}_3$ . Three different  $\text{CO}_2$  regimes – 40% over 17 days, 60% over 11 days, and 80% over 8.5 days – were proposed to control insect pests of stored products (Annis, 1987). Hypoxia (low oxygen), achieved by purging storage structures with pure nitrogen ( $\text{N}_2$ ), was also evaluated as one of the non-chemical alternatives for controlling stored product pests (Annis, 1987). With no cross-resistance reported to hypercapnia or hypoxia (Annis, 1987, Constantin et al., 2020), these two hermetic treatments can also be used to break the  $\text{PH}_3$  resistance cycle. However, achieving and maintaining higher  $\text{CO}_2$  or lower  $\text{O}_2$  concentrations over a lengthy treatment period poses logistical challenges for the industry. This is highlighted by their limited adoption.

Very recently, GrainCorp and the Queensland Government Department of Primary Industries (QDPI) completed collaborative industrial-scale research to evaluate the potential of refrigeration technology in conjunction with  $\text{PH}_3$  fumigation. The commercial-scale field validation was conducted using two large chiller units connected to two concrete silos at a GrainCorp storage site. Both chiller units successfully reduced the grain temperatures by 5°C within 10 to 15 days – a significant benefit compared with the one to three months taken traditionally by an aeration-cooling system to achieve the same result. The trial results proved the high efficiency of grain chillers in cooling the grain to required low levels of 18°C to 21°C from an initial grain temperature of 25°C or higher within a period of 11 days. This process involved an approximate cost of



\$744 for 1890t of sorghum (at \$0.17 kilowatt per hour over 11 days, approximately \$0.43/t). Two successive trials with  $\text{PH}_3$  achieved a concentration of 444 to 644ppm within the 18°C to 21°C target temperature range, both successfully controlled all life stages of strongly resistant rice weevils (*S. oryzae*) over a 21-day fumigation. However, mortality in strongly resistant rusty grain beetles (*C. ferrugineus*) ranged from only 70% to 99%.

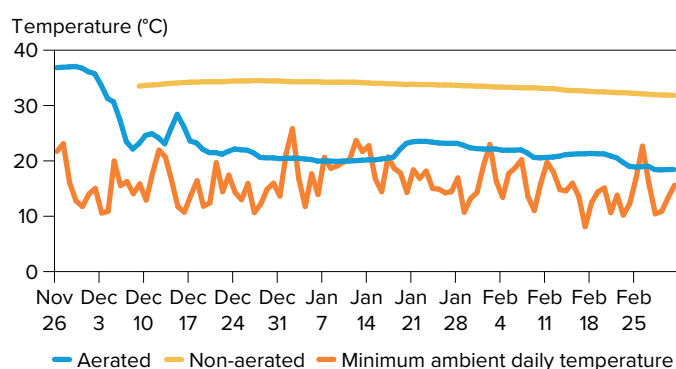
This was the first such research trial conducted in Australia and demonstrated the high potential for using refrigeration in grain storage systems – not only to support pest control but also to maintain the quality of oilseeds such as canola and sesame and to ensure higher germination rates in malting barley during storage.

#### 6.5.4. Integrated strategy utilising grain protectants, structural treatments, aeration cooling and hygiene

Other non-fumigant options can be integrated into the overall management of  $\text{PH}_3$  resistance. Among these, **grain protectants** have been used by the grains industry since the 1960s. Unlike fumigants, protectants are contact insecticides applied directly to freshly harvested grain prior to their storage and are designed to provide up to nine months of protection. These are intended to protect uninfested grain, not to treat infested grain. As there is no cross-resistance in  $\text{PH}_3$ -resistant pests to grain protectants, they can be used to provide protection from  $\text{PH}_3$ -resistant pest species. Currently there are only six registered protectants available in Australia. These include the organophosphates fenitrothion, chlorpyrifos-methyl and pirimiphos-methyl; the pyrethroid deltamethrin; and the insect growth regulators methoprene and spinosad, which is based on bacterial toxins. None of these materials can control the full spectrum of major pests at registered rates either because of resistance or poor efficacy. Therefore, they are applied as mixtures. It is recommended that a triple combination of spinosad, S-methoprene and chlorpyrifos-methyl be applied on grain to provide protection from resistant pest populations belonging to all major species.

Applying contact insecticides to storage structures is also recommended for another form of protection from insect invasion. Not all contact insecticides registered as grain protectants are registered for use as **structural treatments**. Both grain protectants and structural treatments should be used according to their recommended label rates adhering to internationally set standards for maximum residue levels. Diatomaceous earths may also be applied as non-chemical structural treatments.

Figure 5: Aeration cooling vs no aeration cooling.



Source: NSW DPIRD

**Aeration cooling** is a non-chemical method that can be used to significantly reduce insect populations through slowing down their growth and development. It may also deter colonisation of the stored grain by dispersing insects. Although not a substitute for chemical treatment, it can be used in conjunction with fumigation to enhance  $\text{PH}_3$  efficacy (refer to section 6.5.3, page 29), with the additional advantage of maintaining grain quality. While cooling already adequately dried grain can begin immediately, this technology also helps in drying of high-moisture grain to levels meeting the required market standards.

**Hygiene** is a fundamental aspect of grain storage that directly influences the effectiveness of insect control measures. Proper hygiene practices help prevent the introduction and proliferation of pests, reducing the need for chemical interventions. Key hygiene practices include the thorough cleaning of storage facilities before storing new grain to remove any residual grain, dust and debris. These remnants can harbour insects and provide a breeding ground for pests and future reinfestations. Cleaning and sanitising equipment used in grain handling and storage prevents the transfer of pests from one batch of grain to another. This includes conveyors, augers and transport vehicles.



Clean out harvesters and grain-handling equipment thoroughly with pressurised air. Photo: Chris Warrick, Primary Business



A concrete slab under silos makes cleaning easier. Photo: Chris Warrick, Primary Business

## 6.6. Implementation of key intervention strategies

The information generated from live insect bioassays (phenotypes) and DNA analyses based on gene-specific resistance mutations (genotypes) (Schlipalius et al., 2018; Schlipalius et al., 2019; Nayak et al., 2020) allow researchers to develop and deploy key  $\text{PH}_3$  resistance management strategies that can be implemented stepwise in a sequence or according to a systematic pattern. In general, the management approach includes the following four systematic approaches.

1. Rank or prioritise problematic or economically important pest species for the region (Nayak and Daghli, 2018).
2. Categorise geographical regions into zones (e.g. grain growing, pest and resistance categories) (Collins et al., 2017; Holloway et al., 2016; Nayak et al., 2017).
3. Identify or develop appropriate pest intervention strategies suitable for specific storage types (Collins et al., 2017; Schlipalius et al., 2019; Nayak et al., 2021) within the grain value chain (e.g. silos, bunker sheds, open sheds).
4. Implement intervention strategies selectively, emphasising rotating treatments spatially and temporally (Collins, 2009).

This fourfold smart-grid management approach, in combination with a decision-making tree (Figure 6, page 33), is a preferred and more reliable choice for the industry rather than relying on routine calendar-based treatments. Such an information-based interactive management approach will also avoid indiscriminate dosing. Therefore, the

proposed steps aim to eliminate key contributing factors in the genetic development of resistance to  $\text{PH}_3$  and other treatments in stored grain insect pests.

### 6.6.1. Assessment of the overall infestation situation through post-treatment monitoring

One of the important strategies in IRM is post-treatment monitoring, which helps assess long-term effectiveness. As in any pest management program, evaluating the effect of control measures is vital in post-harvest commodity protection as it provides ongoing feedback on the success of intervention strategies. This enables the industry to plan early, schedule and select specific chemical or non-chemical treatments – whether supportive or follow-up – as needed. In general, the following three standard monitoring approaches are widely used.

1. Repetitive sampling of treated commodities (Nayak et al., 2016).
2. Trapping using pheromones or baits at predetermined intervals (Nayak et al., 2021).
3. Diagnosing subsamples for validation (Kaur et al., 2013).

Long-term field studies evaluating the effectiveness of post- $\text{PH}_3$  and SF fumigations have provided critical insights into pest population management. These include changes in resistance frequency (Kaur et al., 2013) and patterns of insect movement and dispersal within storage structures and the surrounding environment (Holloway et al., 2018; Ridley et al., 2011, 2016; Rafter et al., 2021).

### 6.6.2. Tactics to reduce the rate of selection

It is now well understood that underdosing a parcel of grain or leakage during fumigation not only leads to control failures but also plays a critical role in selecting for resistance (Nayak et al., 2020). Therefore, ensuring the storage structure is airtight, that the correct dose is applied at the appropriate temperature in accordance with the registered label and that gas concentration is monitored during the fumigation period to achieve the target dose are critical factors for making fumigation successful (Collins, 2009). For example, in Australia, silos are pressure tested to ensure their sealability/ airtightness prior to fumigation following AS 2628-2010 sealed grain-storage silos. Active recirculation is also recommended for rapid and uniform distribution of gas throughout the commodities, particularly when  $\text{PH}_3$  tablets are used in large silos (see Table 1, page 15) (Collins, 2009).

Repeated fumigation of the same parcel of grain to control surviving populations in leaky storage is a typical example of selection for resistance (Nayak et al., 2020). To avoid selecting for resistance, no more than three consecutive  $\text{PH}_3$  fumigations are recommended for the same parcel of grain (Collins, 2009). If insects are not controlled with these fumigations, the use of a break-fumigant such as SF or a contact insecticide (if appropriate) is recommended. Emphasis should also be placed on storage hygiene along with structural treatments (diatomaceous earth) to reduce residual pest populations thriving on spilled grain, grain dust and dockage (Collins, 2009).

### 6.6.3. Tactics to destroy resistant pest populations

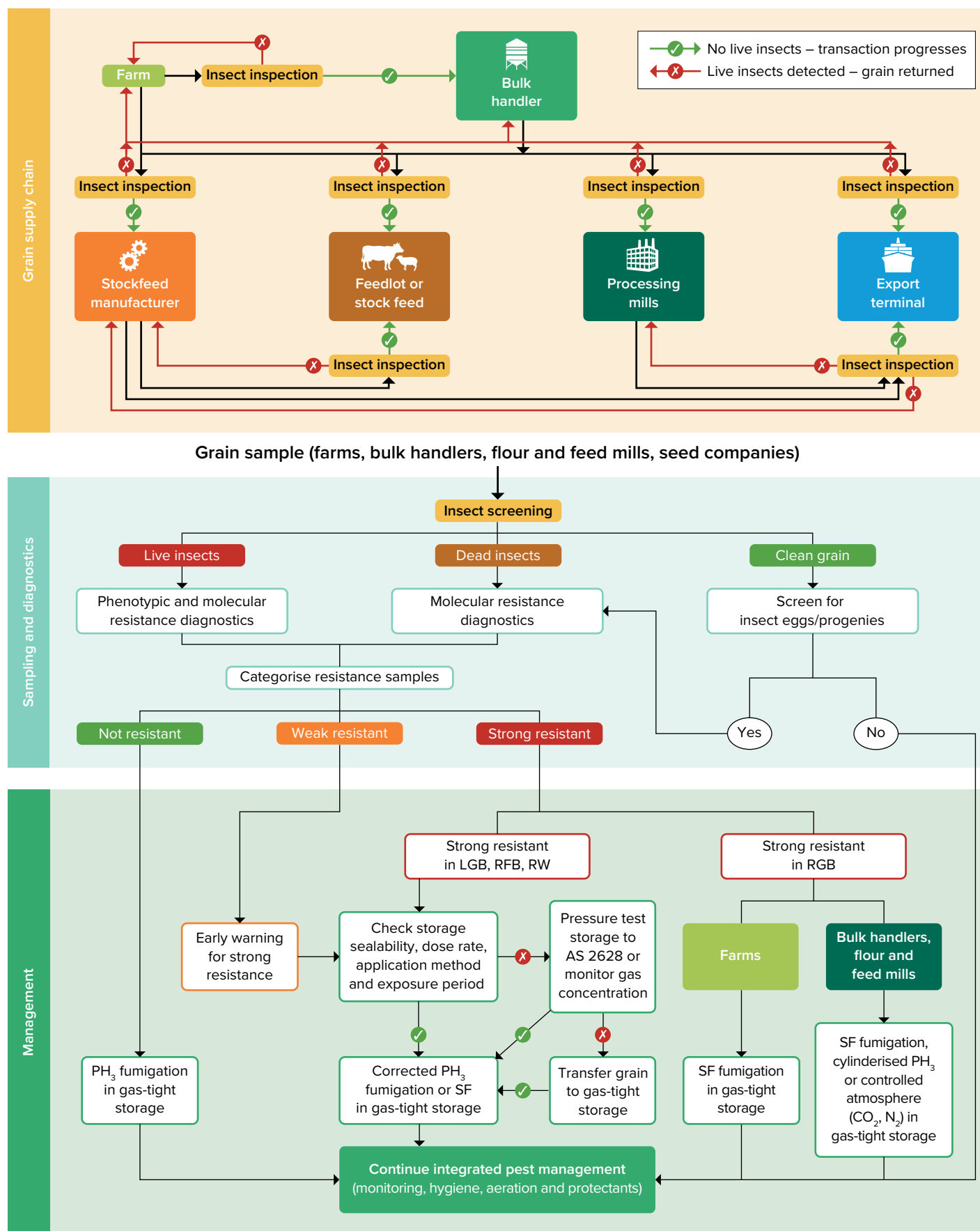
In storages where  $\text{PH}_3$ -resistant populations have already developed and are well established, it is important that they are isolated and completely eradicated to restrict their spread to other storage sites/ commodities through transportation and other means (Collins, 2009; Nayak et al., 2020). Rotation of chemicals and alternative treatments such as fumigants and contact insecticides can be used in such situations. For example, in Australia, *C. ferrugineus* has developed the highest level of resistance recorded in any storage pest. Although  $\text{PH}_3$  protocols have been developed to control this resistance, they are not practically feasible due to their lengthy exposure periods (e.g. 21 days) at very high concentrations (e.g. 720ppm) (Nayak et al., 2013; Kaur and Nayak, 2015). In this scenario, SF had proven successful in managing this strong  $\text{PH}_3$  resistance, with the advantage of having no cross-resistance between both fumigants (Jagadeesan et al., 2015; Nayak et al., 2016; Jagadeesan and Nayak, 2017).

### 6.6.4. Deployment of a 'decision-making tree'

Deploying a 'decision-making tree' as explained in section 6.6 (page 31) and illustrated in Figure 6 (page 33), will help storage operators detect pest and resistance issues early on and guide their successful management.



Figure 6: A decision-making system for pest and resistance management along post-harvest grain value chain.



## 07. OVERCOMING OPERATIONAL AND REGULATORY CONSTRAINTS

Managing pests and resistance to chemical treatments in any post-harvest commodity storage system can be challenging for storage operators – whether on-farm, in the food processing sector, in mills or in bulk handling systems. Commodity storage is a complex system that involves several operational, logistical and regulatory aspects. For appropriate use of  $\text{PH}_3$ , it is important that storage operators receive regular updates on treatment efficacy, alternative protocols, emerging pest and resistance issues, updated regulatory and safety requirements, and regular accreditation in the safe use of chemical treatments. Investing in modern storage structures, maintaining existing infrastructure and adopting good hygiene practices are long-term strategies that improve profitability and help mitigate pest and resistance problems. Failure to follow  $\text{PH}_3$  label directions – such as taking shortcuts in application, underdosing, outloading grain too soon after fumigation, or transporting it to delivery sites before the ventilation period is complete – can lead to serious economic consequences. These include reduced commodity value due to quality degradation, additional costs for retreatment and transportation, and loss of reputation through rejected loads.

## 08. CONCLUSION

Due to a lack of suitable alternatives, storage sectors in Australia and around the world will likely continue to depend heavily on  $\text{PH}_3$  to disinfest stored commodities for the foreseeable future. Although SF has made significant progress over the past decade as an alternative – particularly in managing strongly  $\text{PH}_3$ -resistant *C. ferrugineus* populations – its use is limited by cost and unresolved residue-related issues in international markets. In Australia, collaboration between researchers and industry end-users of  $\text{PH}_3$  has been productive, particularly in providing early warnings of emerging resistance through a national resistance monitoring and management program. This is reflected in the relatively low frequency of  $\text{PH}_3$  resistance in key pest species in Australia compared with other countries. Information provided in this manual will be updated as new data becomes available from research and development.

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### Further resources

*GrowNotes™ – Grain Storage*

A practical guide to on-farm grain storage infrastructure and integrated pest management, [grdc.com.au/resources-and-publications/grownotes/technical-manuals/grain-storage](http://grdc.com.au/resources-and-publications/grownotes/technical-manuals/grain-storage)



QDPI researchers setting up  
a silo-scale fumigation trial  
in Warwick, Queensland.  
Photo: QDPI





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