Strategy to manage resistance to phosphine in the Australian grain industry

Compiled by Dr Pat Collins

An initiative of the National Working Party on Grain Protection.

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Contents

1 Goal

To ensure the long-term sustainability of phosphine through the strategic adoption and implementation of commercially viable, practical, scientifically-based management strategies.

2 Objectives

- To maintain the biological efficacy of phosphine
- To maintain its cost-effectiveness
- To extend the useful life of phosphine.

3 Core strategy principles

3.1 Reduce selection:

- Limit the number of applications of phosphine
- Use non-chemical methods such as cooling and storage, and equipment hygiene, to minimise insect populations
- At a site, reduce re-colonisation by complete empting of all storages

3.2 Destroy resistant insects:

- Make every fumigation count by ensuring recommended gas concentrations and exposure periods are achieved throughout the storage
- Replace phosphine with alternative fumigants or controlled atmospheres
- Use alternative chemicals and physical treatments to replace fumigation

3.3 Monitor and test:

- Monitor gas concentrations during fumigations
- Test insects for resistance to phosphine and other chemicals

4 Rationale

Phosphine is unique. There is no other insect management tool with the combined attributes of phosphine. This fumigant is cheap, effective when applied correctly, well understood, accepted by domestic and international markets as residue-free, it is effective with most commodities and compatible with grain handling logistics.

Phosphine is central to pest management in the Australian grain industry and its continued effectiveness is essential to the sustainability of grain biosecurity and market access for Australian grains. In the past 10-15 years, resistance to phosphine in target insect pests has increased in both frequency and strength such that it now threatens effective control.

In response, this strategy was developed in consultation with the Australian grain industry to ensure that practical and commercial constraints inherent to this industry were accommodated without loss of the resistance management aim. The strategy applies to all sectors and is consistent with current best practice integrated pest management. However, the strategy is voluntary, and success will depend on industry commitment and widespread compliance. Where successful, these strategies will limit the spread and impact of resistance but not eradicate it.

5 Implementation plan

5.1 Recommended practice for all fumigations with phosphine

- No more than three conventional fumigations per year on undisturbed grain
- Break strategy
  - After the third fumigation, move the grain to break up incipient infestations and eliminate pockets where fumigant may fail to penetrate
  - Re-treat the grain with an alternative disinfestant or protectant.
- Monitor phosphine concentrations in all fumigations. Pay particular attention to locations within the storage where concentrations are likely to be lower than recommended
- Follow label directions for application rates and exposure periods
- Maintain hygiene in area surrounding storage and clean out grain handling equipment
- Sieve grain on monthly basis to check for insects
- Management procedures
  - If control failures occur before the end of four months investigate the cause:
    - Were the concentration and exposure times appropriate?
    - Were the recommended concentrations reached for sufficient time at the points recording lowest concentrations?
      - Monitoring for gas levels should be carried out at the points most likely to have the lowest concentration.
    - Was the sealing standard met, where applicable?
    - Were wet grain patches present?
    - Were cold (<15ºC) or hot (>35ºC) grain patches present?
    - Does this commodity sorb phosphine rapidly?

    - Was fumigation introduced too late, allowing heavy localised infestations?

  - If a load of grain is infested and requires fumigation, this must be done in a gas tight facility utilising industry best practice. This is to avoid illegal fumigation in-transit or fumigation in other non gas-tight storages.

- Response options - addressing the cause of the problem

  - Storage

    - Test and repair seals

    - Move grain to adequately sealed storage

    - Disturb ('fluff') grain to break up microenvironments

    - Apply diligent workplace hygiene to eliminate insect harbourage sites

  - Resistance

    - Submit insect sample for resistance testing

  - Fumigant

    - Match phosphine concentrations and times to levels recommended to control resistant insects. This may require use of a higher dose of phosphine. See product labels for dosage information

  - Use Alternatives

    - Fumigant or controlled atmosphere

    - Chemical disinfestant or protectant

    - Non-chemical, e.g. heat

  - Improved management

    - Monitor insect pest populations and fumigate only when insect numbers reach economic injury levels

5.2 Additional recommendations for specific storage types

5.2.1 Bulkheads, bunkers and pads

- Choose a well drained site

- Ensure that the base of the storage is made impervious to gases

- Ensure an appropriate number of gas monitoring points, particularly at each end of the storage

- Inspect tarpaulin for holes before use and monitor throughout the storage period

- Ensure that the storage is adequately sealed.

- Vacuum pressure test is recommended
- Monitor fumigant concentrations during the fumigation

5.2.2 Siroflo® fumigation

- Siroflo® is a process of adding phosphine continuously under pressure into incompletely sealed storages. The nature of this application method makes it difficult to achieve high concentrations of gas at the surface of the grain bulk.
- This is a technically difficult type of fumigation requiring a high level of skill in operators.
- To be applied by licensed fumigators only
- Cap with diatomaceous earth (amorphous silica)
- Seal base and openings of silo
- Sheet grain faces in sheds to direct gas flow

5.2.3 Small scale (<150 tonne) sealable storages

- Use aeration to reduce and stabilise the grain temperature, preferably to about 20^{o}C, when grain is initially placed into storage
- If fumigation is required, ensure that the silo meets the *Australian Standard* for gas-tightness. This standard requires that the storage can maintain a pressure half-life of five minutes minimum for a fully loaded sealed silo
- Ensure that the correct concentration of phosphine (i.e. number of tablets, bags or chains) is used for the total volume of the silo
- If grain moisture content is <12% and if the grain is not immediately needed, silos should remain sealed after fumigation
- If grain moisture content is >12% the silo should be opened following fumigation and aeration system activated
- To avoid moisture migration in sealed storages, storages need to be monitored weekly and when necessary recommence aeration

Improve management – get smart

- Monitor phosphine concentrations at the weakest point, generally inside the bottom cone. Ensure that fumigant concentrations and fumigation time are consistent with recommended rates. See product labels for dosage information.
- Grain temperature influences time needed for a successful fumigation. At grain temperatures above 25^{o}C, the standard seven days under fumigation is required before ventilation. At 20 to 24^{o}C, fumigate for at least 10 days before ventilating and at 15 to 19^{o}C, fumigate for at least 14 days.

5.2.4 Large scale (>150 tonne) sealable storages

- Recirculation for fumigation is recommended

- Measure phosphine concentrations during fumigation
- Exposure period commences when adequate concentrations reached at weakest point in storage
- Use aeration to reduce and stabilise the grain temperature, preferably to about 20^{o}C, when grain is initially placed into storage
- If fumigation is required, ensure that the silo meets the *Australian Standard* for gas-tightness. This Standard requires that the storage can maintain a pressure half-life of five minutes minimum for a fully loaded sealed silo
- *Australian Standard* for gas tightness needs to be updated for large scale sealable storages
- Ensure that the correct concentration of phosphine (i.e. number of tablets, bags or chains) is used for the total volume of the silo
- If grain moisture content is <12% and if the grain is not immediately needed, silos should remain sealed after fumigation
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5.2.5 Silo (harvest) bags

- These are very temporary harvest storages not currently recognized as suitable for fumigation
- Best management procedures are not yet available

5.2.6 Imported grain

- If grain insects are detected on imported product, these should be tested for resistance to phosphine and the product treated appropriately

5.3 Recommendations for industry wide implementation of phosphine resistance management

- Greater numbers of grower, processors and end-users are installing large-scale (~1000 t) fixed storage and utilising bunker storage. There is a strong need for grain storage best practice training including resistance management.

- Phosphine is a toxic gas. It is believed that fumigation practice and the risk of resistance would improve markedly if all users were required to be appropriately trained.

- Silo bag storage is new to Australia and research is required on best management practice for these storages.

- To ensure that fumigation with toxic gases can be undertaken safely and effectively Australian silo design standard must be adhered to.

6 Supporting discussion

6.1 Background

6.1.1 Introduction

Both domestic and foreign markets demand and expect grain that is both insect free and increasingly, free of chemical residues. The Australian grain industry, at all levels, relies on phosphine to provide access to these markets. This fumigant is relatively easy to use, versatile, cheap, and accepted internationally as a residue-free treatment. Although a number of alternative fumigants are being developed for stored grain none can match the combined properties of phosphine.

Reliance on phosphine by the grain industry has increased markedly since about 1992 as a response to market requirements. Although resistance was present in several species in the early 1990s, it was not at level that caused concern. However, in 1997, a new higher level resistance was detected in the lesser grain borer and since that time, strong resistance to phosphine has been detected in most major pest species in many locations in eastern Australia.

6.1.2 Status of resistance to phosphine in insect pests of stored grain

Resistance to phosphine has been detected in all major pest species. In most species two phenotypes, expressing two levels of resistance can be recognised: weak-resistant and strong-resistant. In *Rhyzopertha dominica*, weak-resistance is mediated by one major gene (R1), while strong-resistant insects possess the same gene plus an extra major gene (R1 + R2).

On behalf of the grain industry, the CRC for National Plant Biosecurity sponsors a national resistance monitoring program. Reports for 2004-05 show that, depending on species, weak-resistance is detected in 70 – 100% of insect samples in the northern region (nth NSW and Qld), 53 – 83% in the southern region (SA, Vic, NSW) and 12 - 53% of samples in the Western Region (WA). Strong resistance was first detected in *Rhyzopertha dominica* in the Northern region in 1997. Since that time, it has also been detected in three other major pest species (*Tribolium castaneum*, *Cryptolestes ferrugineus* and *Oryzaephilus surinamensis*) in both the northern and southern regions but not in the west. In the northern region, strong resistance is regularly diagnosed in 2 – 5% of insect test samples and similar results have been reported for the southern region, except for *C. ferrugineus*, in which detections are as high as 17% of strains.

Resistance to phosphine has been characterised extensively in *R. dominca*, *S. oryzae* and *C. ferrugineus.* Characterisation of strains homozygous for strong resistance in this species has shown that some lower application rates (long exposure period x low concentration) registered for use with cylinder formulations of phosphine were inadequate for control of resistant insects. However, rates involving shorter exposure periods and higher concentrations were still effective. Label rates for solid formulations were also effective as long as the fumigation was conducted according to registered rates and in an adequately sealed silo. A range of exposure period x concentration protocols has been recommended for control of known resistance in Australia using cylinder formulations.

Research on resistant *S. oryzae* from China and reports from Chinese scientists in relation to control failures with *C. ferrugineus* and psocids show that resistance has the potential to increase to levels significantly higher than already occurring in Australia.

6.1.3 How has phosphine resistance developed?

There are two major causes of the development of phosphine resistance:

1. Under-dosing. This refers to the failure to achieve either gas concentrations or fumigation exposure periods or both appropriate for control of resistant insects.

Under-dosing allows the survival of resistant genotypes and the destruction of susceptibles resulting in a rapid increase in frequency of resistance in the population. A process called selection.

Under-dosing can be caused by many factors including insufficient application rate, poor or uneven distribution of fumigant within storages, external environmental conditions, poor sealing of the structure, insufficient fumigation period or grain temperatures that are either too high or too low for the dose to be effective.

2. Multiple fumigations. The lack of viable chemical alternatives coupled with the need to maintain grain in a market-ready condition, has resulted in repeated fumigation with phosphine of the same parcel of grain.

The outcome of this practice is the repeated exposure of the same insect population to phosphine. Every application, in particular where there is a risk of under-dosing, can potentially select for resistance.

6.2 Constraints to implementing resistance management in the grain industry

While this strategy has been developed using sound scientific principles and knowledge, we recognise that to be effectively implemented, the strategy must also comply with industry practice. The following is a summary of industry issues, risks and constraints that impact on this strategy.

6.2.1 Marketing

- 'Just in time' grain marketing environment dictates that the commodity must be 'Market ready', i.e. in marketable condition at all times.
- Requirement for residue-free or 'green' product resulting in a reluctance to use any alternatives that may leave residues such as protectants
- Lack of commercial incentives or market signals for improving practices

- Detection based on adult insects. Poor fumigation practice can go undetected as surviving immature stages cannot be seen at inspection

- Repeat fumigation is driven by the need to minimise insect infestations to reduce quality deterioration e.g. bin burnt grain

- Special customer requirements, for example, ship board or extra fumigations

- Deregulation of wheat export resulting in many new players entering the industry with little or no understanding of correct fumigation procedure or resistance management principles

6.2.2 Operational / management

- Length of time required for phosphine fumigation and ventilation conflicts with shipping and transport demands

- Cost of implementing pre-harvest hygiene etc.

- Storage management challenges:

    - Continual 'top-up' (intake) bringing a continual threat of reinfestation leading to multiple fumigation

    - Long term storage leads to repeat fumigation of the same parcel of grain

- Lack of acceptance of pre-harvest hygiene

- Resistance to protectants has greatly reduced options for controlling phosphine-resistant insects

6.2.3 Insect detection

- Domestic (Grain Trade Australia) and international standards require that grain must meet 'zero insect detection'. This includes tolerances for dead insects

- Because of the high frequency and mobility of insect pests, and difficulty of detection, all grain is regarded as infested

- Lack of rapid resistance tests

- Lack of practical insect detection system for all life stages

- Success based on absence of adults

6.2.4 Increasing QA requirements

Certification, and traceability requirements result in repeated fumigation

6.2.5 Regulatory

- Strict OH&S requirements limits chemical options

- Slow and very expensive process for development and registration of alternatives

6.2.6 Business constraints

- Pressure to adopt least cost pathway at the expense of best resistance management practice
- High cost of infrastructure (capital and maintenance)
    - Sealed stores
    - Infrastructure for non-chemical alternatives including controlled atmosphere
    - Monitoring
- High cost of alternatives to phosphine/lack of cost-effective alternatives

7 Resistance management tactics

7.1 Resistance management principles

A practical resistance management strategy relies on three major components:

1. *Information about the system*

Information is required on the state and condition of grain and grain storages in the system and on the occurrence of insect infestation. In addition, we must have information on strengths and frequencies of resistance in insect pest populations. The latter provides early warning of the emergence of new resistances and the occurrence of known resistance. This then allows researchers and industry time to assess the situation, avoid control failures and implement remedial action. Accurate, detailed information permits effective planning and provides feedback on the success of resistance management tactics.

2. *Tactics that reduce the rate of selection*

Tactics that reduce the rate of selection are likely to be the most successful in the long term. This can be achieved by reducing the frequency of use of the selecting agent, by reducing the numbers of insects exposed to the selecting agent, and by maintaining sources of susceptible genes.

For example, cooling grain reduces insect population growth thereby reducing the need to fumigate. Chemical and physical hygiene treatments reduce population numbers and therefore the number of insects potentially exposed to selecting agent.

In summary:

- Minimise number of applications of phosphine
- Storage hygiene – reducing number of insects exposed to selection
- Cooling grain – reducing the number of selection events and the number of insects exposed
- Provision of untreated refuges

3. *Tactics that destroy resistant insects*

In a situation where resistance has already evolved tactics that destroy resistant insects are essential for practical resistance management. These can be either alternative chemicals, such as protectants or fumigants, or physical methods such as heat disinfestation. These tactics are used to eliminate resistance foci, that is, instances where resistance has been detected (resistant homozygotes present), and destroy undetected incipient resistance (heterozygotes present).

In summary:

- Higher doses – making resistance recessive

- Manipulating chemicals – rotating in time or separating geographically

7.2 Detailed discussion of tactics

7.2.1 Reducing selection

1. Minimise applications of phosphine

Theory: The more often a pesticide is used, the more insects are exposed to selection and consequently the more likely that resistance will evolve. Reduce use of the pesticide and this will reduce rate of selection.

Practice: Phosphine is used widely in the grain industry exposing a potentially very large population of insects to selection. In addition, it is often used repeatedly on the same parcel of grain, or in stores where insect populations are maintained in harbourages, so that the same population is serially exposed to selection. Therefore the aim should be to reduce the overall dependence on phosphine and limit repeat phosphine fumigations. This will require the use of alternative disinfestants (chemical and non-chemical, such as heat), more effective disinfestation systems, expanded use of non-chemical controls or expanded use of protectants. To avoid calendar based fumigation, the industry requires better insect detection systems that allow monitoring of whole bulks.

2. Storage hygiene – reducing number of insects exposed to selection

Theory: Storage hygiene refers to the removal and disposal of all residues of grain, grain dust, dockage etc from storages and associated equipment. Grain insect pests can survive for long periods and even multiply on only a small amount of this material. If high levels of cleanliness are maintained inside storages then the likelihood of insects that carry resistance genes surviving from one storage season to the next is greatly reduced. In addition, it is believed that if grain residues are removed from the outside of storages and storage equipment, then the risk of infestation from these sources by insects carrying resistance genes is greatly reduced. Maintaining strict hygiene standards reduces the risk of insect populations becoming resident in a silo and therefore from being repeatedly subject to selection with phosphine.

Practice: Good hygiene reduces general infestation pressure and is the basis for effective integrated pest management. High standards of hygiene require an investment in time, training, equipment and the determination to do a thorough job.

The practice of applying an insecticidal spray to storage fabric will increase the likelihood of effectively controlling insects and provides some residual effect but risks selection for resistance to that protectant. Diatomaceous earth (DE) treatments should be used instead of chemical protectants wherever practicable. However, DEs are not effective where significant numbers of insects are already present in the grain or in high humidity situations, such as ports.

3. Cooling grain – reducing the number of selection events

Theory: Low temperatures can significantly slow insect development and reproductive rates and hence population growth. Reducing insect population growth rate should reduce the number of fumigations required on any parcel of grain and may permit nil use of phosphine in some cases.

Practice: Cooling alone will not ensure insect-free grain but may be sufficient for some segregations such as feed. However, in practice, feed can come out of any storage and is a potential source of infestation in a common grain path. With effective monitoring, cooling should reduce the number of fumigations required on any parcel of grain. Note that cooler grain may require longer fumigation times or higher fumigant concentrations for effective control. In addition, over half the grain in the central system is stored in pads (bunkers, bulkheads) which are difficult to reliably cool to insect control temperatures.

4. Provision of untreated refuges

Theory: refuges or areas of untreated habitat (grain etc) serve as sources of large numbers of susceptible insects, both susceptible and resistant. If resistant insects have a lower fitness relative to susceptibles then in the absence of selection with phosphine, the presence of refuges will result in an increase in the relative frequency of susceptible genes. Early in a resistance episode, susceptible individuals greatly outnumber resistant insects. Refuges, therefore, also function as a reservoir from which susceptible genes may flow through insect movement and interbreeding into insect populations that are under selection to reduce the frequency of resistance genes in the populations.

Practice: This tactic is often a key part of resistance management strategies for field crops. However, this tactic is difficult to implement in the grain industry as it contradicts storage hygiene and the market requirements for insect-free grain. However, refuges may exist in other parts of the environment.

The potential advantages to be gained because of differences in fitness between resistant and susceptible insects are unlikely to be realised in the grain storage system because we have not been able to detect any differences in fitness between phosphine-resistant genotypes. In addition, one resistance gene is already at a very high frequency in most major pests.

A possible variation of this tactic would be to reduce use of phosphine in certain sectors of the industry thus creating ‘refuges’ from selection. For example, growers could be encouraged to use non-chemical control technologies including hygiene, cooling, controlled atmospheres, diatomaceous earths, and alternative chemicals such as protectants. However, markets are reluctant to accept chemical residue and use of diatomaceous earth is not accepted by the majority of markets.

7.3 Destroying resistant insects

5. High doses – making resistance recessive

Theory: Application of doses high enough to control resistant heterozygotes (insects carrying one copy of the resistance gene(s)) will delay the evolution of resistance because these insects do not survive to reproduce. This tactic requires reliable distribution of adequate concentrations in a closed system. However, if resistant homozygotes (insects carrying two copies of the resistance gene(s)) survive such treatments, resistance will rapidly increase in frequency.

Practice: As homozygous strong-resistant insects are already present in eastern Australia, phosphine dosages have been recently revised to provide for their control. Aiming doses at a level that would control only heterozygotes would result in rapid selection of insect populations where strong-resistant homozygotes may already be present. Fumigation in a silo proven to be sealed will minimise the opportunity for insects to escape the toxicant. In eastern Australia, it is essential that doses are applied that will control strong-resistant homozygotes. In Western Australia, where strong-resistant insects have not been detected, the tactic of using lower doses aimed at controlling resistant heterozygotes could be considered.

To be effective, the high dose tactic requires optimal application of phosphine and the avoidance of under-dosing.

A risk with this tactic is the possible selection for even higher level of resistance in target species.

6. Manipulating chemicals – rotating in time or separating geographically

These tactics require two preconditions to be met to be successful. Firstly, the mechanisms of resistance that develop to each of the components should be different and independent (i.e. no cross-resistance). Secondly, the frequency of resistance genes in the target populations must be low and they should not occur together in the same individual. In addition, each tactic relies on its own set of assumptions.

a. Rotation of phosphine with other chemicals

Theory: This tactic involves the rotation of two or more pesticides which do not have cross-resistance. Rotations assume, at least at the beginning of the resistance episode, firstly, that individuals which are resistant to one pesticide have substantially lower fitness than susceptibles, so that their frequency declines between applications of that chemical and secondly, that there is a large gene pool of susceptible insects that will readily mate with resistant insects and dilute the resistance gene frequency, or both. The latter relies on the presence of large areas of untreated habitat. Decisions on when to rotate ideally should be made on the basis of the length of insect generations so that the period of selection of any pesticide does not extend beyond one generation. Rotations also need to be co-ordinated over a large area so that insects functionally belonging to the same gene pool are not simultaneously selected for resistance to the different pesticides used in the alternation.

Practice: Currently, alternative chemicals are limited to grain protectants and ethyl formate (Vapormate®). The former are incompatible with a nil residue marketing system and potential for use of ethyl formate is very limited as it is expensive, must be applied by a licensed fumigator, requires fan-forced distribution and is restricted to small silos. The new fumigant carbonyl sulphide is currently under review for registration by the Australian Pest and Veterinary Medical Association (APVMA) and promises to be a genuine alternative to phosphine in the central system. In addition, Dow Chemical has recently registered the fumigant sulfuryl fluoride (ProFume®) in Australia. Thus, it is hoped that alternatives will be available within the next few years. There is no evidence of cross-resistance between any of these alternatives and phosphine.

Most of the conditions described for success of this strategy cannot be met in the grain industry. Evidence to date suggests that resistance to phosphine does not decline between applications. Frequency of weak resistance is already high in insect populations and strong resistance genes are present in most regions so that large populations of susceptibles are not available. Further research is needed on these aspects

Alternative fumigants have value in that they can be used to control undetected incipient resistant populations and to control known resistance outbreaks. In the former, the alternative would be part of a pre-determined rotation whereas in the latter, the alternative would be used when resistance to phosphine has been diagnosed.

b. *Geographic separation of chemicals*

Theory: The aim of this tactic is to avoid selection for the same resistance mechanism in all regions so that insects that have not been killed in their sector of origin will be killed when they move to another region. The same assumptions apply to this tactic as apply to rotations in time. The success of geographical separation depends on limited cross-mating of insects between regions and the presence of refuges – areas of untreated habitat where resistance gene frequencies can decline due to dilution by mating with susceptibles accelerated by fitness differences.

Practice: Insects can move freely or be carried between farms, central storages and grain merchant premises in grain growing areas. The actual extent of movement of insects requires further research. Ports and some other storage facilities, however, are often located in geographically distinct areas. Phosphine could be limited to use in grain growing regions and alternatives used at port. To a limited extent, this strategy is currently in place where methyl bromide is used for quarantine pre-shipment treatments. The tactic could be expanded when either carbonyl sulphide or sulfuryl fluoride become available.

8 Conclusion

The previous discussion of feasible resistance management tactics reveals that the grain industry has only a limited number of options that can be implemented to manage resistance to phosphine. We are restricted, in particular, by the lack of alternative fumigants.

However, a practical resistance management strategy that could be implemented immediately would include:

1. Limiting the number of repeat fumigations on the same parcel of grain

2. Ensuring highest standards of fumigation using sealed silos so that recommended minimum concentrations and exposure periods are met to avoid under-dosing.

3. Strong emphasis on use of non-chemical control technologies including hygiene, cooling, controlled atmospheres and diatomaceous earths to minimise the use of phosphine across the grain industry.

4. Use of protectants and structural treatments (including diatomaceous earths) where acceptable and effective.

5. Expedite adoption of alternative fumigants.