Strategy to Manage Resistance to Grain Protection Chemicals in the Australian Grain Industry

An initiative of the National Working Party on Grain Protection

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Summary

The development of resistance to fumigants and grain protectants is the most significant challenge to the management of insect pests of stored grain. This Strategy outlines a practical approach to combating resistance to both fumigants and grain protectants for the grain industry in Australia.

The core strategy principles are to:

1. Reduce selection. Emphasise use of hygiene and non-chemical methods such as cooling. Limit the number of repeat treatments. Use traps, and apply non-chemical treatments to storage fabric.
2. Destroy resistant insects: Apply protectants and fumigants at correct rates and follow recommended rotations.
3. Monitor and test for resistance. Monitor gas concentrations during fumigations. Regularly submit insects for resistance testing. Undertake industry–wide monitoring.

This document also provides information on best practice application for all fumigations, including responding to control failures, fumigation in specific types of storages and application of protectants.

The status and development of resistance, operational constraints to resistance management, and the range of potential resistance management tactics and their applicability to this industry are reviewed and discussed.

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1. Goal

To ensure the long-term sustainability of grain protection chemicals including the fumigants phosphine and sulfuryl fluoride, and a range of grain protectants, through the strategic adoption and implementation of commercially viable, practical, scientifically-based management strategies.

2. Objective

- To maintain the biological efficacy, cost-effectiveness and useful life of all grain protection chemicals.

3. Core Resistance Management Principles

3.1 Reduce selection:

- Limit the number of re-treatments of the same grain with the same chemical
- Use non-chemical methods such as cooling, and storage and equipment hygiene to minimise insect populations
- At a site, completely empty and clean all storages to reduce re-colonisation by resident populations. Minimise re-colonisation from external populations by trapping and applying non-chemical treatments, such as diatomaceous or silica treatments, to storage fabric.

3.2 Destroy resistant insects:

- **Make every treatment count** by applying protectants and fumigants at correct rates. With fumigants ensure that recommended gas concentrations and exposure periods are achieved throughout the storage. With protectants ensure that the grain receives an even coverage.
- Periodically replace **phosphine** with sulfuryl fluoride, ideally use sulfuryl fluoride after every third phosphine fumigation of the same parcel of grain or where strong resistance to phosphine has been diagnosed.
- Rotate protectant mixtures.
- Where practicable, use protectants, controlled atmosphere and physical treatments (e.g. diatomaceous earth) in place of fumigants. Note resistance management recommendations for protectants.

3.3 Monitor and test:

- Monitor gas concentrations during fumigations
- Monitor resistance to fumigants and protectants
    - Submit insects for testing after fumigation failures
    - Undertake industry-wide monitoring

4. Background and Rationale

4.1 General

Insecticides, including fumigants, grain protectants and storage treatments, are key components of grain insect pest management systems. Although grain storage managers routinely use non-chemical tactics including hygiene, and grain cooling and drying, in many situations these are not effective enough to maintain grain at the standard required by markets.

Health, safety, environmental and economic considerations severely limit the range of chemicals that can be applied to grain, and in recent years we have seen various authorities around the world reduce the number of chemicals available. Chemicals that are suitable to be applied to grain are rare and very costly to develop.

In addition to these pressures, the targets of these chemicals, the insects themselves are developing resistance to the few alternatives we have.

Fumigants registered for treatment of grain include phosphine, sulfuryl fluoride, methyl bromide and ethyl formate. Only phosphine and sulfuryl fluoride are considered in this Strategy as methyl bromide and ethyl formate have limited and specific uses.

Phosphine is by far the most commonly used fumigant. It is relatively cheap, well understood, accepted by domestic and international markets as residue-free, can be applied to most commodities and is 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.

Strong resistance to phosphine has now been detected in all five major insect pests of stored grain and the frequency of resistance is continuing to increase. Of particular concern is the emergence of strong resistance in the rusty grain beetle, *Cryptolestes ferrugineus*, which cannot be controlled with current rates.

Sulfuryl fluoride is gaining market share and is being used across all sectors of the industry. It is significantly more expensive than phosphine. The risk of fluoride residues is not well understood and therefore re-fumigation is not recommended. There is no known resistance to this fumigant.

Grain protectants have been used by the grain industry since the 1960s. Unlike fumigants, protectants are designed to provide up to 9 months protection, and are intended to protect uninfested grain, not to treat infested grain. Currently there are only six registered protectants available in Australia. These include the organophosphates fenitrothion, chlorpyrifos-methyl and pirimiphos-methyl, the pyrethroid deltamethrin, the insect growth regulator 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 because of poor efficacy. Therefore they are applied as mixtures. Resistance occurs in several of the major pest species to one or more protectants. The lesser grain borer, *Rhyzopertha dominica*, is the most difficult to control and several protectants have been specifically developed to manage this pest.

In summary, resistance to the essential and unique fumigant phosphine is increasing in both frequency and strength, and resistance to several protectants is widespread. Given the dependence of the grain industry on these materials, and that replacements are unlikely to become available, it is imperative that the grain industry take action to manage resistance. In addition, we need to do all that is required to delay or prevent the development of resistance in those chemicals are currently resistance-free.

4.2 Updating the strategy

The grain industry has already developed and published a “Strategy to manage resistance to phosphine in the Australian grain industry”. Since that document was written, there have been some significant developments including the detection of very high levels of resistance to phosphine in certain pests species, the introduction of a new fumigant (sulfuryl fluoride) and registration of two new protectants (deltamethrin and spinosad).

The strategy to manage phosphine resistance has been in place for more than 10 years. Strong resistance to phosphine was first detected in 1997, and compared with other regions where resistance has been documented, although increasing, frequencies are still relatively low. For example, compared with a 10% overall incidence of strong resistance in Australia (Collins et al., 2017; Holloway et al., 2016; Nayak et al., 2017), surveys in India (Kaur et al., 2015; Rajendran, 1999) indicate frequencies of greater than 95% incidence of strong resistance in *R. dominica* in some regions and high frequencies in other species. Frequencies of phosphine resistance are also very high in the Philippines (Sayaboc et al., 1996), Morocco (Benhalima et al., 2004), Brazil (Lorini et al., 2007; Pimental et al., 2010), Turkey (Koçak et al., 2015), USA (Opit et al. 2012) and Vietnam (Duong et al. 2016). These comparisons indicate the success of the resistance management strategy in Australia.

It is now timely that this strategy be updated and expanded to include all grain protection chemicals.

As with the previous document, this Strategy has been written in consultation with the Australian grain industry to ensure that practical and commercial constraints inherent to this industry are 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.

Recognition of the importance of this issue is demonstrated by inclusion of the Strategy in the Australian Grain Industry Code of Practice, http://www.graintrade.org.au/grain-industry-codes. Adherence to this Code of Practice is mandatory for all members of Grain Trade Australia.

5. Implementation Plan

5.1 Summary

- ### Phosphine

Following a maximum of three fumigations on the same parcel of grain, change to sulfuryl fluoride or to a grain protectant or a non-chemical treatment, such as controlled atmosphere.

- ### Sulfuryl fluoride

Fumigate each parcel of grain only once, then change to phosphine or to a grain protectant or a non-chemical treatment, such as controlled atmosphere.

Note: A fumigation period of at least 4-6 days, depending on temperature, is required to achieve complete control. Current rates and practices urgently require revision.

As SF is a large molecule, re-circulation is recommended to ensure uniform distribution

- **Protectants**

Use mixtures and rotate every one or two years:

Spinosad + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

OR

Deltamethrin (+ piperonyl butoxide) + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

OR

Deltamethrin (+ piperonyl butoxide) + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

Note: Protectants are not permitted to be admixed with grain in Western Australia except in certain cases by Bulk Handling Authorities.

Note: Ensure your markets will accept protectant treated grain before applying

5.2 Recommended best practice for all fumigations

- Ensure that storages are tested and sealable to the Australian Standard for Sealed Grain Storage Silos (Standard AS2628)
- Active re-circulation is recommended to rapidly achieve uniform distribution of fumigant.
- Where re-circulation is not possible or practicable, such as grain stored in bunkers, allow extra time for fumigant to distribute to all parts of the storage. Monitor gas concentrations to ensure correct dosages are achieved.
- Ideally, monitor concentrations in all fumigations. Pay particular attention to locations within the storage where concentrations are likely to be lower than recommended and check for gas leaks.
- Where aeration cooling is available, cool grain as it is in-loaded. Monitor grain for pests and fumigate as required.
- If grain is to be sold within 6 weeks after harvest and there is high risk of insect infestation, fumigate soon after in-loading. Ensure grain moisture content is suitably dry.
- For longer term storage and where aeration is not available, monitor regularly and fumigate when infestations are detected.
- Where grain must be ready for shipment at short notice, schedule fumigation may be required.
- Sieve grain and inspect insect traps on monthly basis.
- Maintain hygiene in area surrounding storage and clean out grain handling equipment.
- Follow label directions for application rates and exposure periods.
- Where there are multiple storages holding grain for extended periods, aim to fumigate all grain at the same time to reduce cross infestation.
- As a general rule, where grain is to be stored for more then 2-3 weeks after completion of fumigation, return the storage to aeration cooling.

- Ventilation post fumigation – follow the label and industry directions to ensure safe grain handling. Note that the current label for aluminium phosphide is in process of being updated.

Break strategy

- No more than three consecutive fumigations with phosphine per parcel of grain. Follow the third phosphine fumigation with sulfuryl fluoride, then return to using phosphine if required.

- After the third phosphine fumigation, consider moving the grain to break up incipient infestations and eliminate pockets where fumigant may fail to penetrate before applying sulfuryl fluoride or protectant.

Management procedures

If control failures occur investigate the cause:

- Was the fumigation monitored?
- 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?
- Were wet grain patches present?
- Was the grain temperature adequate? Were cold (<15ºC) or hot (>35ºC) grain patches present?
- Is the commodity likely to sorb fumigant rapidly?
- Was fumigation introduced too late, allowing heavy localised infestations?

Responding to a control failure

i.e,, Addressing the cause of the problem

- Is the storage suitable for fumigation?
  - Test and repair seals
  - Move grain to adequately sealed storage
  - Disturb/move ('fluff') grain to break up microenvironments and disperse incipient infestations and to eliminate pockets where fumigant may fail to penetrate
  - Apply diligent workplace hygiene to eliminate insect harbourage sites
- Resistance suspected?
  - Submit insect sample for resistance testing (see Appendix for further information)
- Fumigant failure?

    - Ensure that concentrations and times match levels recommended to control resistant insects. See product labels for dosage information

    - If strongly phosphine-resistant rusty grain beetle (*Cryptolestes ferrugineus*) are present, apply sulfuryl fluoride or protectant

    - 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.

- Protectant failure?

    - Check that protectant correctly applied (e.g. that pumps are working and nozzles clear) and at correct dosage

    - Apply fumigant. Do not re-apply protectant

- Improved management

    - During storage, monitor insect pest populations and fumigate only when insects are detected

5.3 Recommendations for phosphine fumigation of specific storage types

5.3.1 Bulkheads, sheds and bunkers

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 to ensure that the storage is adequately sealed

- Negative pressure test using fans to check for leakage points is recommended

- Monitor fumigant concentrations during the fumigation

Sheds

- Where 6 weeks or more storage time is likely, hang tarp curtains on the shed walls before filling the shed with grain. When fumigation is required, tarp the top grain surface and seal this tarp (roll together & clip) to the top of the wall curtains tarp to form a sealed, gas-tight fumigation.

5.3.2 Siroflo® fumigation – not recommended where phosphine-resistant strains of rusty grain beetle are suspected

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 (cereal grains) with diatomaceous earth (amorphous silica)
- Seal base and openings in walls of silo
- Sheet grain faces in sheds to direct gas flow (see 5.3.1)
- Ensure concentration monitors placed at points where most likely to get lowest gas concentrations

5.3.3 Small scale (<150 tonne) sealable storages

- Use aeration to create uniform grain moisture conditions and to reduce the grain temperature when grain is initially placed into storage after harvest. Aim for grain temperatures of less than 23°C in summer and less than 15°C in winter. Note that fumigation times must be extended for cool grain. Follow label directions.
- If fumigation is required, ensure that the silo meets the *Australian Standard* for gas-tightness (AS2628). This standard requires that the storage can maintain a pressure half-life of at least five minutes for a fully loaded sealed silo.
- Recirculation is not essential in smaller silos as phosphine typically will distribute within 24 hr from point of application
- Ensure that the correct amount of aluminium phosphide (i.e. number of tablets, blankets or bag-chains) is used for the total volume of the silo
- For both preservation of grain quality and insect pest management, grain is best maintained under aeration cooling. In most cases, once fumigation is completed, return to aeration cooling and monitor grain regularly.
- Grain temperature influences time needed for a successful fumigation as aluminium phosphide tablets react more slowly at lower temperature and gas moves through the storage more slowly. Insects are also more tolerant at lower temperatures. At grain temperatures above 25°C, the standard seven days under fumigation is required before ventilation. At 20 to 24°C, fumigate for at least 10 days before ventilating and at 15 to 19°C, fumigate for at least 14 days. Failure to ventilate adequately may result in high concentrations of phosphine gas at delivery and exposure of persons to unsafe levels of fumigant.
- Research is indicating that strongly phosphine resistant rusty grain beetle may not be controlled using the current application rate of aluminium phosphide. New protocols are under development. It is likely that fumigation times will need to be lengthened.

5.3.4 Large scale (>150 tonne) sealable storages

- Use aeration to create uniform grain moisture conditions and to reduce grain temperature when grain is initially placed into storage. Aim for less than 23°C in summer and less than 15°C in Winter. Note that fumigation times must be extended for cool grain. Follow label directions.
- Ensure that the silo meets the *Australian Standard* for gas-tightness (AS2628). This Standard requires that the storage can maintain a pressure half-life of five minutes minimum for a fully loaded sealed silo
- Use the correct amount of aluminium phosphide (i.e. number of tablets, blankets or bag-chains) for the total volume of the silo

- <u>Active Recirculation is recommended to ensure rapid and even distribution of gas</u>

- If recirculation is not available, in most cases, phosphine will eventually distribute throughout the silo over time. The current aluminium phosphide label specifies a 20-day fumigation for surface application in sealed structures greater than 375 cu m.

- Monitor phosphine concentrations at the weakest point, generally the furthest point from phosphine application. Ensure that fumigant concentrations and fumigation time are consistent with recommended rates. See product labels for dosage information.

- Exposure period commences when adequate concentrations are reached at weakest point in storage

5.3.5 Silo (harvest) bags

- Prepare free draining, flat site free of stubble and other objects that may puncture the bottom of the bag. Bags should be sealed at both ends to become a gas tight structure.

- Inspect bags regularly and repair holes.

- Make 10 “gas release tubes” for each standard grain bag (75 m long) so spent tablet dust can be removed from the grain. Cut 20 mm PVC pipe into 1 m lengths and seal with cap at one end. Cut multiple slots across three quarters of the top surface of each tube to allow release of the gas. Attach a cord to the unsealed end so the tubes can be easily removed.

- The fumigation application rate is based on volume of the bag. Grain bulk densities can be used for calculation. For example, a 75 m standard bag containing approximately 250 tonnes of wheat will require 500 tablets (50 tablets in each of the 10 spears).

- Mark bag every 7 m along the length of bag at chest height. Push 10 thumb holes in bag at marks and fully insert tubes horizontally into each hole with only the cord hanging out. Once tubes are inserted, seal holes using silicone sealant.

- Fumigate for an additional 5 days above label directions to allow for gas distribution between release tubes. Remove tubes at the end of the fumigation and reseal holes before venting.

- Active venting using a small aeration fan to suck air along the full length of the bag from the open finish end.

5.4 Recommended practice for application of sulfuryl fluoride

Follow best practice recommendations listed in sections 5.2 and 5.3 including use of aeration cooling and sealable silos. In addition note the following:

- <u>Recirculation is recommended to achieve even distribution of fumigant</u>

- Sulfuryl fluoride efficacy is temperature dependent. Do not fumigate at grain temperatures less than 20^{o}C

- A minimum of 5-6 days is required for complete control of insect populations. Avoid 24-48 hr fumigations as these allow some survival and may select for resistance.

- Do not re-fumigate the same parcel of grain with SF as this increases the risk of leaving fluoride residue above acceptable limits

- Sulfuryl fluoride has several advantages as a grain fumigant, however, further research is need to develop best practice use of this material

5.5 Recommended practice for application of protectants

- Protectants are liquid insecticides applied directly to the grain stream.
- Check with your buyer that the chemical residue is acceptable. A Commodity Vendor Declaration may be required.
- Protectants are designed to provide 3 to 9 months protection of grain from insect infestation. They are intended to prevent infestations and should be applied to clean, insect-free grain at harvest time. They are not to be used to disinfest grain.
- Use protectants where gas-tight sealable storage is not available
- For best results apply to aerated grain and follow meticulous storage hygiene
- To get an even coating of spray over the grain, apply using one or two flat fan nozzles mounted to spray into the augur (or in-loading belt) as the grain is loaded into storage.
- Avoid spraying onto the surface of grain in a tube-conveyor as this will result in poor distribution of protectant
- Read the label to ensure that the protectant is permitted to be applied to the commodity

Note: Protectants are not permitted to be admixed with grain in Western Australia except in certain cases by Bulk Handling Authorities.

5.5.1 Managing resistance to protectants

The following mixtures are recommended for complete control of all major pest species. These should be alternated every one or two years.

Spinosad + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

OR

Deltamethrin (+ piperonyl butoxide) + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

OR

Deltamethrin (+ piperonyl butoxide) + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

Note: Fenitrothion has a 90-day withholding period at the high rate.

Note: Before applying any of the grain protectants, check with your grain traders or buyers for acceptability for various markets

Note: Protectants are not permitted to be admixed with grain in Western Australia except in certain cases by Bulk Handling Authorities.

6. Supporting discussion

6.1 Background

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

Resistance to phosphine has been detected in all major pest species, that is, the lesser grain borer, *Rhyzopertha dominica*, the rice weevil, *Sitophilus oryzae*, the sawtoothed grain beetle, *Oryzaephilus surinamensis*, the red flour beetle, *Tribolium castaneum* and the rusty grain beetle, *Cryptolestes ferrugineus*.

Research has confirmed that insect movement has the potential to move resistance genes from place to place. Trapping and molecular studies have demonstrated that flight dispersal is important in key pests including *T. castaneum* and *R. dominica* (Ridley et al., 2011, 2016; Daglish et al., 2017).

In all species two phenotypes, expressing two levels of resistance can be recognised: weak-resistant and strong-resistant. The genetics of resistance is complex and varies in detail with species but to generalise we can say that weak-resistance is mediated by one major gene (r1), while strong-resistant insects possess the same gene plus an extra major gene (r1 + r2). Both genes are incompletely recessive in expression so that full resistance is only expressed in insects homozygous for both genes.

Strong resistance was first detected in *R. dominica* (1997), followed by *O. surinamensis* and *T. castaneum*. Resistance in *R. dominica* was highest but research showed that no change was required to aluminium phosphide (solid formulations) application rates as long as the fumigation was undertaken using best practice including sealing silos to the Australian Standard (AS2628). Changes to exposure period x concentration protocols were required for full control of resistant insects using cylinder formulations and these changes were added to registered labels (Collins et al., 2005).

Resistance has more recently been detected in *S. oryzae* and *C. ferrugineus* with levels higher than shown in *R. dominica*. In particular, resistance in *C. ferrugineus* is higher than any other species. This resistance is challenging both cylinder and aluminium phosphide rates. Complete control of resistant insects will require longer fumigation times and higher concentrations.

Strong resistance has been mainly detected in eastern Australia with only a few detections in Western Australia. In eastern Australia, strong resistance occurs in 7-10% of populations.

6.1.2 Efficacy of sulfuryl fluoride

There is no evidence of resistance to sulfuryl fluoride and no cross-resistance between sulfuryl fluoride and phosphine (Jagadeesan and Nayak, 2016).

A fumigation period of 4-6 days, depending on temperature, is required to achieve complete control. <u>Current rates and practices urgently require revision</u>.

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 typically occurs in poorly sealed silos (Collins et al., 1996).

Under-dosing allows the survival of resistant genotypes and the destruction of susceptible insects resulting in a rapid increase in frequency of resistance in the population (Shi et al., 2013). 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, grain too dry or over-moist, 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.

6.1.4 Status of resistance to protectants in insect pests of stored grain

A limited range of protectant insecticides is registered and available for use on grain in eastern Australia. Note: Protectants are not permitted to be admixed with grain in Western Australia, except in some cases by Bulk Handling Authorities.

The first protectant, malathion, came into widespread use in the mid-1960's and was responsible for ensuring that the grain industry could meet the Australian government mandated 'nil tolerance' for live insects at export and retain overseas markets. However, by the early 1970's resistance to malathion in *T. castaneum* and *R. dominica* had become a serious problem (Bengston, 1981) and it was replaced in the late 1970's with three organophosphates (OP): pirimiphos-methyl, chlorpyrifos-methyl and fenitrothion. These chemicals were effective against all major species except *R. dominica*, which required addition of the pyrethroid bioresmethrin. Since that time, effective grain protection has required the admixture of at least two protectants in most regions.

Resistance to fenitrothion and pirimiphos-methyl was detected in the early 1980's in *O. surinamensis* (Collins and Wilson, 1987) but chlorpyrifos-methyl was still effective for a number of years until resistance to this protectant also emerged. Resistance to bioresmethrin in *R. dominica* was first identified in 1990 (Collins et al., 1993) and increased in frequency until its registration was removed due to a lack of MRLs in certain markets. Pyrethroid resistance was also detected in *T. castaneum* (Collins, 1990). An alternative protectant for control of *R. dominica*, the juvenile hormone analogue methoprene, was introduced in about 1994. This material had the advantage of also being effective against *T. castaneum* and *O. surinamensis*. However, resistance to methoprene was detected only two years later (Collins, 1998) and its frequency has been steadily increasing since and now appears to be common in eastern Australia (Daglish et al., 2013). Resistance to pyrethroids is prevalent in *S. oryzae*, possibly due to the use of pyrethroid-based seed dressings. Spinosad was registered in 2013 for control of *R. dominica* and has limited effectiveness against other species.

Of the currently available protectants, the organophosphates: pirimiphos-methyl, chlorpyrifos-methyl and fenitrothion remain effective against *T. castaneum*, *S. oryzae* and *C. ferrugineus*. Methoprene is effective against OP-resistant *O. surinamensis* and against *C. ferrugineus*, and the synergised pyrethroid deltamethrin is effective against all species except *S. oryzae* (with the threat of resistance emerging in *T. castaneum* and *R. dominica*). Spinosad is effective against *R. dominica*.

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
- Limited 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 resulting quality deterioration
- 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 fumigation and ventilation conflicts with shipping and transport demands
- Lack of acceptance and cost of implementing pre-harvest hygiene etc.
- Lack of infrastructure meeting sealing standards
- 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
    - New grain placed on top/in front of old grain leads to some grain remaining on site for long periods and reduces ability to clean and maintain these storages
- Resistance to protectants has greatly reduced options for controlling phosphine-resistant insects

6.2.3 Insect detection

- Australian government export regulations, and domestic (Grain Trade Australia) and international standards require that grain must meet ‘nil insect detection’.
- Because of the high frequency and mobility of insect pests, and difficulty of detection, all grain is regarded as potentially infested
- 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 fumigant or protectant
- 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 (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 – destroying resistant insects
- Manipulating chemicals – rotating in time or separating geographically

7.2 Detailed discussion of tactics

7.2.1 Reducing selection

1. *Minimise applications of insecticide*

Theory: The more often a protectant or fumigant is used, the more insects are exposed to selection and consequently the more likely that resistance will evolve. Reduce use of the insecticide 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.

Do not retreat grain with protectants if a control failure occurs, this may exacerbate a resistance problem and increase the risk of violating Maximum Residue Limits. Fumigate the grain, then if a protectant is needed, re-treat with different chemical following the resistance management rotation.

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.

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.

Waste grain should be disposed of promptly, either used, burnt, buried or spread to less than 10 mm deep. Insects are easily capable of flying 1-2 km to infest stored grain.

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 in some years or 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 bunkers and bulkheads which are difficult to reliably cool to insect control temperatures.

Reducing grain temperature enhances the effective life of protectants.

4. *Provision of untreated refuges*

Theory: refuges or areas of untreated habitat (grain etc.) serve as sources of large numbers of insects, both susceptible and resistant. If resistant insects have a lower fitness relative to susceptible genotypes 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 resulting in a reduction of 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 significant differences in fitness between resistant and susceptible genotypes.

7.2.2 Destroying resistant insects

5. *High dosages – making resistance recessive*

Theory: Application of dosages 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:

Resistance to phosphine

Insects homozygous for strong phosphine resistance already occur in all major pest species. Thus dosages must be applied that will control these fully-resistant homozygotes. It is too late to target heterozygotes. Phosphine dosages have been revised to ensure destruction of homozygous strong phosphine-resistant *R. dominica* and other species, and they are in the process of being reviewed again with the aim of controlling strongly phosphine-resistant *C. ferrugineus*.

To be effective, the high dose tactic requires best practice optimal application of phosphine including use of sealed silos, and the avoidance of under-dosing.

Resistance to protectants

There is no opportunity to manipulate the rates of protectants admixed with grain. The best approach is to follow best practice and ensure even coverage at the correct rate.

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. Rotations

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: Rotating chemicals in the traditional sense, as described above, is not practicable in the grain industry as most of the conditions described for success of this strategy cannot be met. For example, evidence to date suggests that resistance to phosphine does not decline between applications. Frequency of weak resistance is already very high in insect populations and strong resistance genes are present in most regions so that large populations of susceptibles are not available. Resistance to one or more protectants is also present in many insect populations.

Fumigants

A viable alternative to phosphine, sulfuryl fluoride, is now available. There is no evidence of resistance to sulfuryl fluoride and no cross-resistance between sulfuryl fluoride and phosphine.

Sulfuryl fluoride can be used to control undetected incipient phosphine-resistant populations and to destroy known resistance outbreaks. In the former, the sulfuryl fluoride would be part of a pre-determined rotation whereas in the latter, it would be used when resistance has been diagnosed.

Taking into account the cost of fumigations and the potential risk of residues, it is recommended that sulfuryl fluoride be applied after every third phosphine fumigation. However, a significant problem is that research has shown that the practice of using a fumigation period of 48 hr is not sufficient to fully control pest insect populations. A fumigation period of at least 5 days is required to achieve complete control. <u>These rates urgently require revision</u>.

Protectants

As described in Section 6.1.4 of this document, the pattern of resistance to protectants in insect pests is complex. Mixtures of protectants are required to provide control of all species. There are, however, some overlapping efficacies and several chemicals that elicit different and separate resistance mechanisms, generally according to their chemical group.

The strategy, therefore, is to rotate mixtures consisting, as much as possible, of chemicals from different groups. The following mixtures are recommended for complete control of all major pest species. These should be alternated every one or two years.

Mixture 1: Spinosad + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

Mixture 2: Deltamethrin (+ piperonyl butoxide) + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

Mixture 3: Deltamethrin (+ piperonyl butoxide) + S-methoprene + EITHER chlorpyrifos-methyl OR fenitrothion OR pirimiphos-methyl

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: Research has shown that insects move across large distances in the landscape and that gene flow is very extensive. Insects can move freely or be carried between farms, central storages and grain merchant premises in grain growing areas. 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.

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 fumigants and grain protectants.

However, using the options available, a practical resistance management strategy would include:

1. Limiting the number of repeat fumigations on the same parcel of grain.
2. Periodically replace one fumigant with the alternative
3. Rotate protectant treatments
4. Do not re-treat grain with the same protectant
5. Ensuring highest standards of fumigation using sealed silos so that recommended concentrations and exposure periods are met to avoid under-dosing.

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

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

9 Further information

AS2628-2010. Australian Standard for sealed grain storage silos - Sealing requirements for insect control.

Australian Grain Industry Code of Practice, http://www.graintrade.org.au/grain-industry-codes.

Burrill, PR. Grower storage and handling facilities – Best Practice checklist.

http://storedgrain.com.au/wp-content/uploads/2015/07/Checklist-Grower-Storage-Handling-PB-Jun15.pdf

GRDC Stored Grain Information Hub www.storedgrain.com.au. This website provides an extensive resource of information on best practice grain protection on farm.

Grain Trade Australia. http://www.graintrade.org.au/. This sites provides comprehensive information on all aspects of grain trade including international Maximum Residue Limits for chemicals and Resistance Management.

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11 APPENDIX

11.1 Resistance Monitoring

11.1.1 Addresses for submitting samples for resistance testing

Note: there may be a charge for testing

Western Australia

Mr Dave Cousins
Department of Agriculture and Food, Western Australia
Locked Bag 4
Bentley Delivery Centre WA 6983
T: 08 9368 3920

Eastern Australia

Dr Manoj Nayak
Department of Agriculture and Fisheries
Ecosciences Precinct
Basement 3 Loading Dock off Joe Baker Street
Dutton Park QLD 4102
T: 07 3708 8539

11.1.2 Notes on collecting and submitting insects for resistance testing

A sample is drawn from the chute at the base of the silo into the sieve. Insects are usually collected using a grain sieve (oats or barley screen size is suitable). The grain or material to be sampled is shaken vigorously a few times before being inspected in the collection tray. Any insects in the sample will have fallen through the sieve along with other fine residue.

As the number of insects of each species collected from each sample site increases, the reliability of the results increases. At least 100 insects (or as many as possible) of each species should be collected from each site. There is no requirement to separate insects by species.

It can be helpful to label the sample vials before taking the sample and using a funnel also helps in getting the insects from the collection tray into the vial. Place some fresh grain etc. in the vial with the insects.

On the other hand, placing too many insects in the sample container may result in oxygen depletion and death of insects in the container. Depending on the number of insects in a collection, it may be advisable to spread a sample over two vials in order for the insects to have the best chance of survival.

If low numbers of insects are present, collect as many as possible to allow for rearing in the laboratory.

11.2 List of Trade Names

Active ingredient	Trade names
Chlorpyrifos-methyl	Reldan
Deltamethrin + piperonyl butoxide	K-OBiol Combi
Fenitrothion	Various - Fenitrothion

Pirimiphos-methyl	Actellic
Phosphine (aluminium phosphide)	Various, e.g. Fumitoxin, Pestex, Phostoxin, Gastion, QuickPhos
Phosphine (gas in cylinder)	Eco_2Fume, $Vaporph_3$os
S-methoprene	IGR, Methoprene, Rizacon S
Spinosad + S-methoprene	Conserve Plus
Sulfuryl fluoride	ProFume

Note: *All pesticide applications must accord with the currently registered label for that particular pesticide, crop, pest and region.*