Plant Biosecurity Cooperative Research Centre

Final Report

Delivering a collaborative monitoring program with industry to manage and facilitate trade.

PBCRC 3160

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AUTHORS

Manoj Nayak, Joanne Holloway and Rob Emery

Project Leader contact details

Name: Manoj Nayak
Address: Department of Agriculture and Fisheries, EcoSciences Precinct, Level 3C West, GPO Box 267, Brisbane, QLD 4001.
P: 07-32554439
E: manoj.nayak@daf.qld.gov.au

PBCRC contact details

Plant Biosecurity Cooperative Research Centre
LPO Box 5012
Bruce ACT 2617

P: +61 (0)2 6201 2882
F: +61 (0)2 6201 5067
E: info@pbcrc.com.au
www.pbcrc.com.au

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1. Executive Summary

The current project represents national resistance monitoring for a period of 12 months with the purpose of transitioning this important program to a long term, self-sustainable model and establishing an enduring legacy. The findings from this research are addendum to the previously submitted final report for project PBCRC3035. While new data on resistance spread and frequency for the period 2015-16 are presented here, the methodology remains the same and the response from the end-user advocate is quite similar to the previous one.
The following are the highlights from the 2015-16 season:

- The project has been proven to be a highly successful program as the outputs from this form the basis for development and deployment of major pest and resistance management strategies. It is clearly evidenced by the fact that the frequency of strong resistance to phosphine in key pests have been managed at a much lower level in Australia (approximately 7-8%) till the 2015-16 period than that recorded in overseas countries such as Brazil, India and China (approximately more than 90%).

- For the first time, the frequency of strong resistance has gone past the 10% level, currently sitting at 12% at the national level, a major factor that may have contributed towards this increase in frequency was the lack of resistance monitoring in the states of Victoria and South Australia in the southern region for two consecutive years (2012-14). Without monitoring, the early detection of strong resistant populations and their timely eradication would have not been possible.

- A dramatic increase in frequency of strong resistance in multiple species in the southern region over the last 12 months has altered the national statistics.

- Although several strongly resistant populations of RGB have either been eradicated completely or being managed by using sulfuryl fluoride as an alternative to phosphine in central storages, we are experiencing a major problem in this sector through new strong resistant populations being detected in several new sites.

- While we have just seen the early incidences of strong resistance in RFB and SW (remained at 1% level only) in Western Australia, the state with the enviable of record of not harbouring strong resistance for several decades should be more vigilant now on.

- Industry across Australia also experiencing the emergence of SGB as a new pest problem, particularly, on farm in the southern and western region, although there are only few cases of strong resistance.

Results from this round of monitoring clearly demonstrates that the selection pressure on phosphine is continuing and there have been instances of strong resistance in saw toothed grain beetles, which is a new resistance for the industry. It is imperative that judicial use of alternative strategies including the use of sulfuryl fluoride be adopted by industry to reduce this ongoing pressure on phosphine. The research and development information generated by this project emphasises the critical need for industry to adopt the nationally agreed 'Phosphine resistance management strategy', last modified in 2009. It is timely that this strategy be modified further to incorporate new information currently available on use of sulfuryl fluoride as an alternative treatment and several ecological aspects of stored grain pests influencing resistance development.

The ongoing detections of strong resistances in the rice weevil and rust red flour beetle requires that industry should confirm and ensure their control through the currently developed new fumigation protocols targeted for strongly resistant rusty grain beetles. If needed further adjustments in these high dosages would be required.

As highlighted by the end user advocate, the future resistance monitoring program should include the monitoring of resistance to spinosad and sulfuryl fluoride, discriminating dosages for both of which, were established through this project. It is also important to note that while we utilise the ‘quick tests’ to provide industry same day advice on resistance status of a pest population; with the development of the molecular diagnostics, industry is keen on molecular screening of individual insects to get a much comprehensive picture of the frequency of resistance alleles in a pest population.

Looking at the future, the research team is currently in negotiation with key end users (GRDC, Bulk grain handling authorities) for funding for the purpose of transitioning the resistance monitoring program to a long term, self-sustainable model and establishing an enduring legacy.

2. Introduction

The development of resistance to the key fumigant phosphine is a critical issue for the Australian grains industry and represents a major threat to industry’s ability to trade grain under the required ‘nil tolerance’ conditions. A program to monitor the status of resistance across grain growing regions has been operating for over 10 years. With support of the CRC there have been a number of technical improvements in recent years including development of a world-first molecular assay and a re-structuring of the survey based on diagnostic and biometric support. This work has placed Australia as world leaders in understanding the genetic basis of resistance. The current program includes deployment of 'quick tests' that provide 'same day advice' to industry for decision making. Industry has acted on this knowledge, in a number of instances pro-actively eradicating populations of storage pests with high levels of resistance. Industry strongly supports the service and their advice is that it is a key component of decision making at grain aggregation sites. In addition, findings from the monitoring program have driven demand for other PBCRC investments such as the recent deployment of new phosphine fumigation protocols and alternatives to phosphine such as nitrogen technology and sulfuryl fluoride. The current project represents a final request for funding for the purpose of transitioning the resistance monitoring program to a long term, self-sustainable model and establishing an enduring legacy.

The development of resistance to the key fumigant phosphine is a critical issue for the Australian grains industry and represents a major threat to industry’s ability to trade grain under the required ‘nil tolerance’ conditions. A program to monitor the status of resistance across grain growing regions has been operating for over 10 years. With support of the CRC there have been a number of technical improvements in recent years including development of a world-first molecular assay and a re-structuring of the survey based on diagnostic and biometric support. This work has placed Australia as world leaders in understanding the genetic basis of resistance. The current program includes deployment of 'quick tests' that provide 'same day advice' to industry for decision making. Industry has acted on this knowledge, in a number of instances pro-actively eradicating populations of storage pests with high levels of resistance. Industry strongly supports the service and their advice is that it is a key component of decision making at grain aggregation sites. In addition, findings from the monitoring program have driven demand for other PBCRC investments such as the recent deployment of new phosphine fumigation protocols and alternatives to phosphine such as nitrogen technology and sulfuryl fluoride. The current project represents a final request for funding for the purpose of transitioning the resistance monitoring program to a long term, self-sustainable model and establishing an enduring legacy.

3. Materials and Methods

The project team comprised key researchers from QDAF, DAFWA, NSWDPI as well as industry collaborators from GrainCorp, CBH, Viterra, and grain growers. This team has a history of delivering tangible outputs to industry in the area of Phosphine Resistance Management. Project leaders at each of these laboratories agreed that due to the urgency in providing immediate responses to industry (sometimes same day advice) on resistance status of the pest samples sent directly to the laboratories, it was imperative that each laboratory had the access to a Technical Officer proficient with fumigant bioassays. A part-time (0.5 FTE) Technical Officer was also required at QDAF to maintain the unique collection of resistant and susceptible pest populations for all current and future resistance related research. Each laboratory aimed at processing at least 500 samples collected from approximately 80-100 farms and 30-50 bulk storages within the associated region. It is noted that a sample collected from a particular storage may contain more than one pest population; therefore, the number of populations tested is far greater than the number of samples collected. Here we presented resistance test results on only five key pest species, lesser grain borer (Rhyzopertha dominica), red flour beetle (Tribolium castaneum), rice weevil (Sitophilus oryzae), rusty grain beetle (Cryptolestes ferrugineus) and sawtoothed grain beetle (Oryzaephilus surinamensis).

Key activities to achieve the aims of the proposed project are as follows:

1. Strategic monitoring – insect samples were systematically collected from key points throughout the supply chain in Australia and North America and diagnosed for strong resistance to phosphine. A statistically robust and nationally agreed resistance monitoring protocol was followed. Sample collection was based on a standardised survey to provide data on overall trends in frequency, distribution and strength of resistance, and was important in the identification of new resistances. As data were analysed, areas of high resistance intensity were further examined to understand the development of resistance and to link it to biological and operational factors.

2. Tactical monitoring - insect samples were received from members (mostly bulk handlers) of the supply chain seeking an immediate diagnosis to inform timely decisions on treatment options. Recently developed 'quick tests' were used to provide industry same day (within 6 hours of receival of the sample) advice on the resistance status of the pest population.

3. Data management - All testing results were entered into the AGIRD database. AGIRD was further modified to improve data entry and retrieval, as well as supplying basic statistical analysis to monitor trends and patterns of key interest. It was imperative that the data custodians and researchers from state agencies (QDAF, DAFWA and NSWDPI) were regularly consulted for this research component. This ensured adequate understanding of existing data, guided its analysis, and also provided the basis for recommending improvements to sampling protocols, database proformas and sampling strategies.

4. Establish a cost-effective, accurate, responsive and self-sustainable resistance monitoring program – Based on the first 12 months activities, an analysis was undertaken to provide industry with a cost-effective, accurate, self-sustainable resistance monitoring program model. We aim to implement this model in the second phase of the project that will continue until the end of the current PBCRC investment.

Data generated across all components will be subjected to modern statistical analysis ensuring the publication of research findings in peer-reviewed research journals.

4. Aims

Key elements of the transition process are envisaged as:

1. Detection of high levels of resistance to phosphine across the grain value chain in Australia, evaluation and optimisation of rapid bioassay methods to detect phosphine resistance in key pest species and timely advice to industry for appropriate intervention/eradication strategies.

2. Partnership activity with industry to transition the activity to a sustainable base, including an assessment of introducing a fee-for-service component for companies including those outside the PBCRC structure.

3. A final season of field collection and resistance monitoring across Australia while exploring with industry bodies such as GRDC how to move this element of the program to a more sustainable model.

5. Results

Strong resistance trend in the northern region

Over the last 12 months, in the northern region, a total of 106 farms were visited randomly, from which, a total of 185 samples were collected and processed in the Brisbane laboratory for resistance testing. In addition, 47 samples collected through survey of 27 central storages were also subjected to resistance diagnosis (Table 1).

The 185 farm samples were sorted to the following key pest species, colonies of which were established for resistance testing: 97 strains of lesser grain borer (LGB) (*Rhyzopertha dominica*), 95 strains of red flour beetle (RFB) (*Tribolium castaneum*), 40 strains of rice weevil (RW) (*Sitophilus oryzae*), 54 strains of rusty grain beetle (RGB) (*Cryptolestes ferrugineus*) and 34 strains of sawtoothed beetle (SGB) (*Oryzaephilus surinamensis*) (Table 2).

Strong resistance was detected in 16 of the 97 strains of LGB (17%), 7 of the 95 strains of RFB (7%), 5 of the 54 strains of RGB (9%) and 5 of the 34 strains of SGB (15%); whereas all 40 strains of rice weevils were tested negative for strong resistance to phosphine (Table 2). When added together the frequency of strong resistance remained around 10% in the farm sector (Table 2).

The 47 samples collected from central storages were sorted into 15 strains of LGB, 19 strains of RFB, 16 strains of RW and 45 strains of RGB, which were tested for strong resistance test. No populations of SGB were encountered in the samples from the central storages surveyed in this region. The strong resistance was detected only in 6 of the 15 strains of LGB (40%) and 14 of the 45 strains of RGB (31%) (Table 2). The RFB and RW populations collected from these facilities returned with negative results for the strong resistance test.

As part of the tactical monitoring, we received 33 samples directly from GrainCorp, of which, 24 RGB populations were subjected to quick test. Of these, 10 populations (30%) were diagnosed as strongly resistant to phosphine. The results were communicated to GrainCorp managers, the same day of receipt of these samples for implementation of eradication strategies.

When added together, the bulk handling sector experienced a strong resistance frequency of 21%.

When strong resistance detections in both farm and central storages added together, the northern region returns with a frequency of 13%.

Strong resistance trend in the southern region

In this region, a total of 182 farms were visited and samples sorted to the following populations of major stored grain pest species: 179 strains of LGB, 165 strains of RFB, 98 strains of RW, 68 strains of RGB and 100 strains of SGB. Of these strains, the following were diagnosed with strong resistance: 24 of the 179 strains of LGB (13%), 27 of the 165 strains of RFB (16%), 13 of the 98 strains of RW (13%), 5 of the 68 strains of RGB (7%) and 4 of the 100 strains of SGB (4%) (Table 2).

When added together, the overall frequency of strong resistance in farm sector in the southern region comes around 12% for the last 12 months.

Samples collected from 56 central storages on this region were sorted to the following strains of major pest species: 21 strains of LGB, 28 strains of RFB, 13 strains of RW, 92 strains of RGB and 6 strains of SGB. The strong resistance diagnosis returned negative results for the RFB and SGB strains, whereas strong resistance was confirmed in 6 strains of the 21 strains of LGB (29%), 2 strains of the 13 strains of RW (15%) and 80 of the 92 strains of RGB (87%) (Table 2).

When added together, the frequency of strong resistance in the bulk handling sector (central storages) in this region over the last 12 months determined to be at a staggering 55%.

When results from both sectors were added, the overall frequency of strong resistance translates to 21% for the southern region.

Strong resistance trend in the western region

Samples collected from the 164 farms surveyed over the last 12 months in this region were sorted to the following pest populations: 118 strains of LGB, 248 strains of RFB, 35 strains of RW, 26 strains of RGB and 112 strains of SGB. Resistance tests revealed strong resistance in only 5 of the 248 strains of RFB (2%) and in one of the 35 strains of RW (3%), with no strong resistance detections in populations of LGB, RGB and SGB (Table 2).
When added together, the frequency of strong resistance on farm in the western regions calculated at a very low level of 1% only.

Samples collected from the 19 CBH storages surveyed, returned with 5 strains of LGB, 20 strains of RFB, 59 strains of RW and only one strain of RGB; whereas no population of SGB was found in this sector over this period. Of these strains, only one strain of RFB (5%) was returned positive with strong resistance diagnosis (Table 2).

The frequency of strong resistance in this bulk handling sector (central storages) remains incredibly low at the 1% level for the last 12 months.

Unsurprisingly, when resistance detections from both the farm and bulk handling sector (central storages) added together, the frequency of strong resistance in the western region comes to a very low level of 1% only.

Highlights at the national level

When resistance detections are added up for all three regions for establishing a frequency at the national level, for the farm sector, it was determined at the 8% level (112 detections from the 1469 strains tested). There was an increase of at least 3% in frequency of strong resistance in the farm sectors during the 2015-16 season compared to that recorded for the 2014-15 cycle. This year we experienced a very high level of 32% frequency (109 detections from the 340 strains tested) in the bulk handling system (central storages) compared with the very low 6% recorded for the 2014-15 period. When both sectors combined together, the frequency of strong resistance for the last 12 months comes to approximately 12%, this is nearly double the frequency recorded for 2014-15. A major contributory factor to this dramatic increase in the frequency can be attributed to the very high incursion of strong resistance in the southern region that upset the national statistics. It is important to note that resistance monitoring was not undertaken for 2 consecutive seasons (2012-14) in South Australia and Victoria that may have masked several strong resistance incidences. Without early detection followed by implementation of an eradication strategy, these populations would have spread in the region. This has happened particularly in the bulk handling sector (central storages), for example, in South Australia, 24 of the 25 samples of RGB were detected with strong resistance that itself accounts to 96%.

The above result is self-explanatory in that, for the first time, the strong resistance frequency for fumigant phosphine has gone past the 10% level in Australia. This should serve as a serious warning to the industry in that though strong resistance to phosphine has been a serious industry issue over the last two decades, Australia has an enviable record in managing them in a proactive way through early detection of strong resistant populations and their timely eradication. We have been highly successful in maintaining the strong resistance frequency at the 7-8% level till this year. This is in comparison to very high frequencies of above 90% in several overseas countries including India, Turkey and Brazil.

The following are the highlights from the findings from our last one year (2015-16) of resistance survey:

- There was a significant increase in frequency of strong resistance in LGB, in central storages of both in the northern and southern regions it has gone up to nearly 4-times of that recorded in the previous season (Figure 1).
- For RFB, there was a 3-fold increase in frequency of on farm detection of strong resistance in the southern region compared to previous year, but the frequency was lowered this year for the northern and western region (Figure 2).
- While no strong resistance in RFB was reported from central storages in the southern and northern regions, there was a 5% frequency recorded for the western region (Figure 2).
- Although no strong resistance was recorded in RW in the northern region over the 2015-16 season, there was 4-fold increase in frequency of strong resistance in this pest on farm in the southern region and 3% frequency on farm in the western region (Figure 3).
- The strong resistance frequency in RW in central storages in the southern region was higher than the previous season (Figure 3).
- Frequency of strong resistance in RGB, specifically in central storages in the southern region have gone past 87% level over the last season compared to a meagre 5% recorded in the previous year (Figure 4).
- Although several strongly resistant populations of RGB have either been eradicated completely or being managed by using sulfuryl fluoride as an alternative to phosphine in central storages, we are experiencing a major problem in this sector through new strong resistant populations being detected in several new sites.

- While we have just seen the early incidences of strong resistance in RFB and SW (remained at 1% level only) in Western Australia, the state with the enviable of record of not harbouring strong resistance for several decades should be more vigilant now on.
- Industry across Australia also experiencing the emergence of SGB as a new pest problem, particularly, on farm in the southern and western region, although there are only few cases of strong resistance (Figure 5).

Table 1. Total number of storages surveyed during 2015-16 across the three grain growing regions in Australia.

Northern region		**Southern region**		**Western region**	
Farms	Central storages	Farms	Central storages	Farms	Central storages
106	27	182	56	164	19

Table 2. Total number of population samples of five major stored grain pests collected from two major storage types and the numbers detected with strong resistance to phosphine (in brackets) over 2015-16 across the three grain growing regions across Australia .

Pest species	Northern region		Southern region		Western region	
	Farm	CS*	Farm	CS	Farm	CS
Lesser grain borer (*R. dominica*)	97 (16)	15 (6)	179 (24)	21 (6)	118 (0)	5 (0)
Red flour beetle (*T. castaneum*)	95 (7)	19 (0)	165 (27)	28 (0)	248 (5)	20 (1)
Rice weevil (*S. oryzae*)	40 (0)	16 (0)	98 (13)	13 (2)	35 (1)	59 (0)
Rusty grain beetle (*C. ferrugineus*)	54 (5)	45 (14)	68 (5)	92 (80)	26 (0)	1 (0)
Sawtoothed beetle (*O. surinamensis*)	34 (5)	0 (0)	100 (4)	6 (0)	112 (0)	0 (0)
Total	320 (33)	95 (20)	610 (73)	160 (88)	539 (6)	85 (1)

*Central storages

Figure 1. A two year comparative percentage frequency of strong resistance to phosphine in lesser grain borer *R. dominica* across the three grain growing regions of Australia (CS: Central storages).

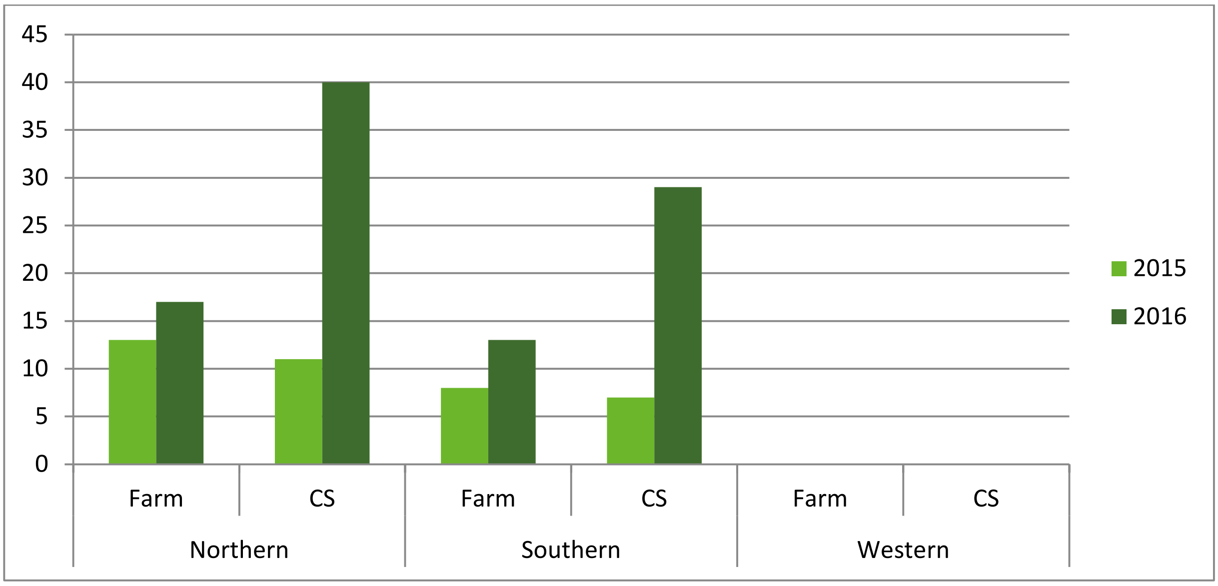


Figure 2. A two year comparative percentage frequency of strong resistance to phosphine in red flour beetle *T. castaneum* across the three grain growing regions of Australia (CS: Central storages).

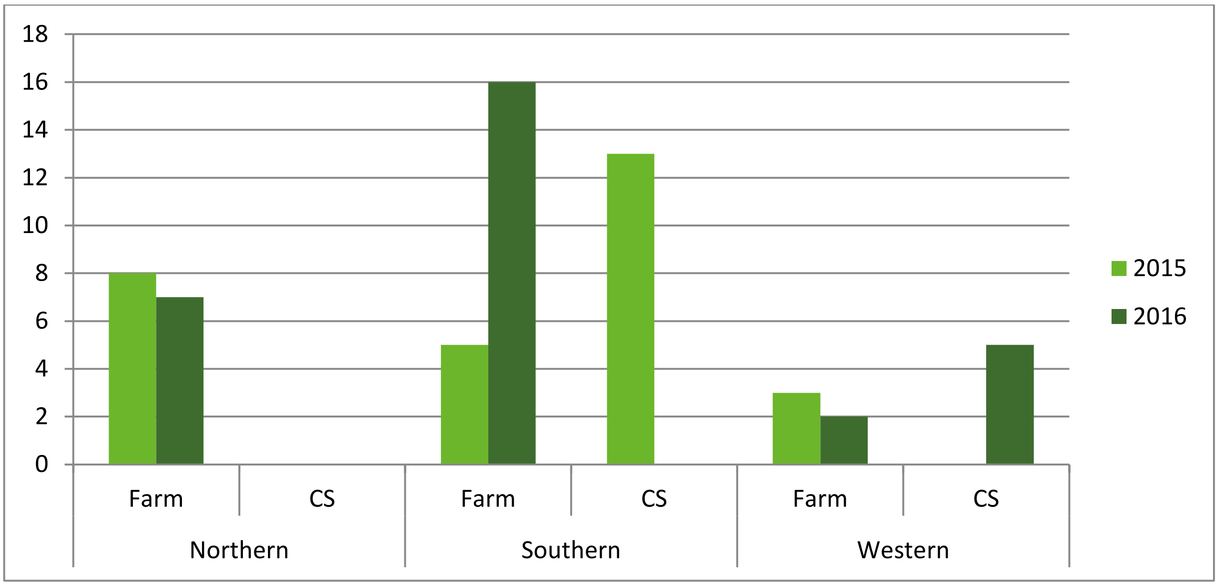


Figure 3. A two year comparative percentage frequency of strong resistance to phosphine in rice weevil *S. oryzae* across the three grain growing regions of Australia (CS: Central storages).

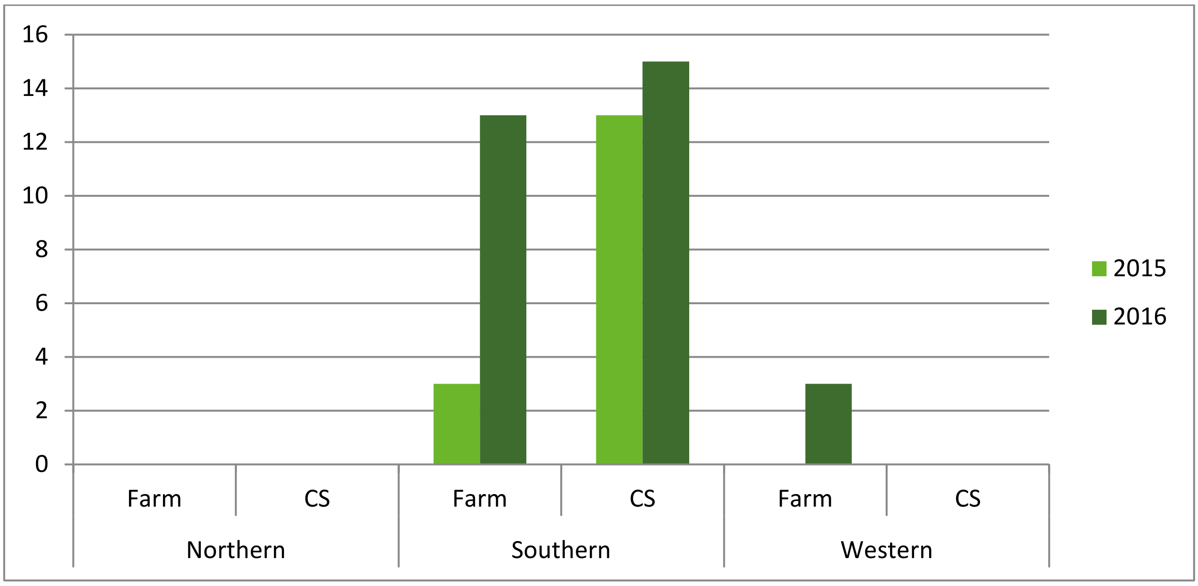


Figure 4. A two year comparative percentage frequency of strong resistance to phosphine in rusty grain beetle *C. ferrugineus* across the three grain growing regions of Australia (CS: Central storages).

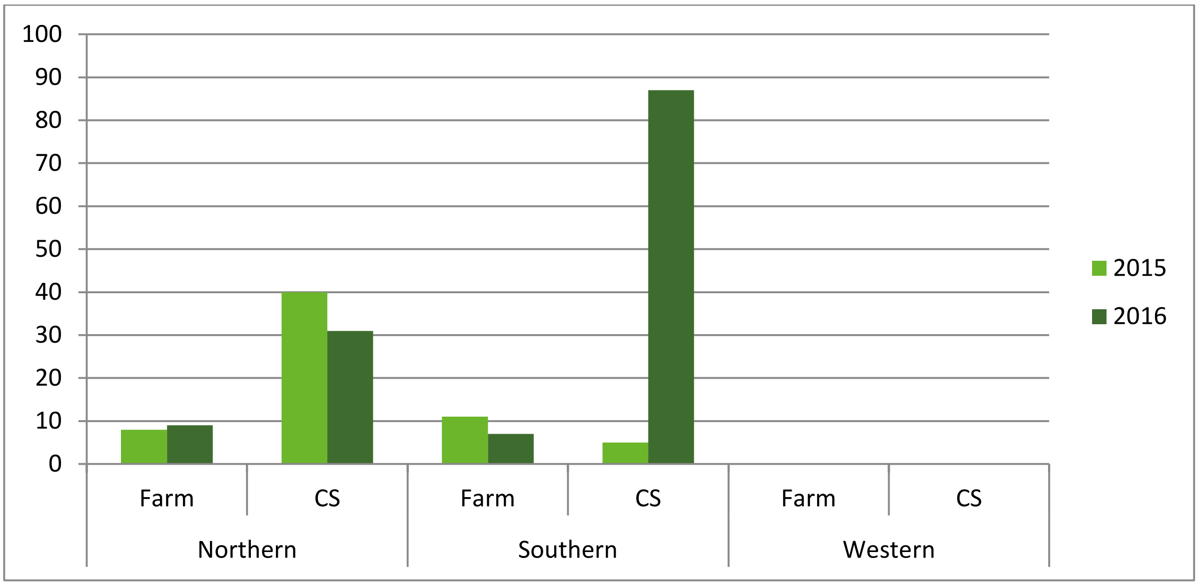
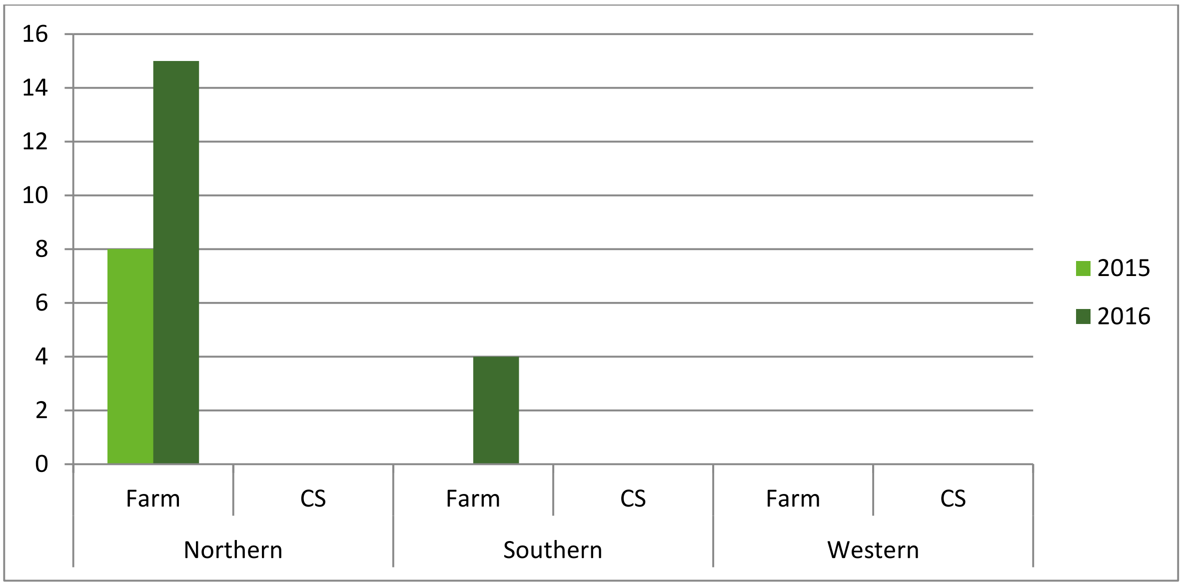


Figure 5. A two year comparative percentage frequency of strong resistance to phosphine in sawtoothed grain beetle *O. surinamensis* across the three grain growing regions of Australia (CS: Central storages).



6. Discussion & Conclusion

While the project successfully achieved all its Milestones, following are the highlights from the end users' perspective:

- The project has been proven to be a highly successful program as the outputs from this form the basis for development and deployment of major pest and resistance management strategies. It is clearly evidenced by the fact that the frequency of strong resistance to phosphine in key pests have been managed at a much lower level in Australia (approximately 7-8%) till the 2015-16 period than that recorded in overseas countries such as Brazil, India and China (approximately more than 90%).
- For the first time, the frequency of strong resistance has gone past the 10% level, currently sitting at 12% at the national level, a major factor that may have contributed towards this increase in frequency was the lack of resistance monitoring in the states of Victoria and South Australia in the southern region for two consecutive years (2012-14). Without monitoring, the early detection of strong resistant populations and their timely eradication would have not been possible.
- A dramatic increase in frequency of strong resistance in multiple species in the southern region over the last 12 months has altered the national statistics.
- While we have just seen the early incidences of strong resistance in RFB and SW (remained at 1% level only) in Western Australia, the state with the enviable of record of not harbouring strong resistance for several decades should be more vigilant now on
- Industry across Australia also experiencing the emergence of SGB as a new pest problem, particularly, on farm in the southern and western region, although there are only few cases of strong resistance.
- Transmission of critical information on resistance trends and frequencies and available alternative strategies to biosecurity officers and extension networks to get the message across to all stake holders along the grain value chain
- The project helped in providing strategic direction on performance of resistance management strategy
- Information from the project was used for decision making on a range of issues related to treatments and storage
- The project enhanced our knowledge on incidences and selection for resistance
- The project delivered a number of technical improvements in recent years including development of a world-first molecular assay and a re-structuring of the survey based on diagnostic and biometric support. This work has placed Australia as world leaders in understanding the genetic basis of resistance.
- The current program developed and deployed a 'quick tests' that provide 'same day advice' to industry for decision making. Industry has acted on this knowledge, in a number of instances pro-actively eradicating populations of storage pests with high levels of resistance. Industry strongly supports the service and their advice is that it is a key component of decision making at grain aggregation sites.
- Findings from the resistance monitoring program have driven demand for other PBCRC investments such as the recent deployment of new phosphine fumigation protocols and alternatives to phosphine such as nitrogen technology and sulfuryl fluoride.
- It is timely that findings from this study be used to modify the 'National Resistance Management Strategy' that has been last modified in 2009.

International recognition of the research team:

Over the tenure of the project the team had several international recognitions by peers:

- Manoj Nayak received invitation by the Scientific Committee of the International Controlled Atmosphere and Fumigation in Stored Products Conference (CAF) (held during 7-11 November 2016 in New Delhi, India):
    - delivered oral presentation titled 'Recent advances in phosphine resistance detection methods'
    - co-chaired (with Prof. Tom Phillips from KSU) Scientific Session 7 titled 'Resistance to fumigants and controlled atmosphere treatments'

- Manoj Nayak delivered two invited keynotes at the 12th Fumigants and Pheromone Conference, held in Adelaide, 7-9 March 2016
    - Phosphine Resistance in Grain Insects and their Management
    - Psocids: Why are they difficult to control?

- Manoj Nayak and Greg Daglish published a paper titled 'Base-line susceptibility of field populations of *Rhyzopertha dominica* (F.) to spinosad in Australia', that established a discriminatory dose for future detection of resistance to spinosad in this pest as they arise in the field populations.

- For the first time we have published two research papers on the analysis of trends and frequencies of phosphine resistance developed in two major pest species (lesser grain borer and rice weevil) across a vast geographic spread of Australia over a period of 20 years (three manuscripts are under review by co-authors and will soon be submitted)

Challenges and issues arising from this research

- Risks on the horizon
    - Phosphine resistance
        - *rice weevil and rusty grain beetle - on farm*
        - *Rusty grain beetle – central storages*

    - Sulfuryl fluoride
        - *all species – bulk and farm sectors*

    - Spinosad, deltamethrin
        - *all species – bulk and farm sectors*

- Evaluate role and function of molecular resistance diagnostics

- A self-sustaining/cost effective resistance monitoring program
    - Industry (bulk handling sector)
    - GRDC (farm sector)

Peer reviewed publications

1. Nayak, M.K. and Daglish G.J. (2017). Base-line susceptibility of field populations of *Rhyzopertha dominica* (F.) to spinosad in Australia. Journal of Stored Products Research 70: 1-6.
2. Collins, P.J.; Falk, M.G.; Nayak, M.K.; Emery, R.N. and Holloway, J.C. (2017): Monitoring resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica* in Australia: a national analysis of trends, storage types and geography in relation to resistance detection. Journal of Stored Products Research 70: 25-36.
3. Holloway, J.C.; Falk, M.G.; Emery, R.N.; Collins, P.J. and Nayak, M.K. (2016): Resistance to phosphine in *Sitophilus oryzae* in Australia: A national analysis of trends and frequencies over time and geographical spread. Journal of Stored Products Research 69: 129-137.
4. Schlipalius, D. I., Tuck, A.G., Jagadeesan, R., Nguyen, T., Kaur, R., Subramanian, S., Barrero, R., Nayak, M.K. and Ebert, P.R. (submitted to Genetics). The rph1 phosphine resistant locus in insects is a cytochrome-b5 related fatty acid desaturase.

Impact Delivery Plan

- National Resistance Management Strategy
    - network of pest and resistance managers, researchers
- GRDC National Extension Network
- National Working Party on Grain Protection
- Industry Workshops/Field Days
- Scientific and extension publications
    - Peer-reviewed journal papers
    - GRDC Publications (e.g. Growers Update, Ground Cover)

Conclusion:

This project has clearly demonstrated the value and impact of a national resistance monitoring program on the grain industry. The project outputs contributed significantly to resistance management by providing industry with both strategic and tactical information on the frequency, distribution and strength of resistance in key pest species.

Furthermore, the information provided by this project emphasises the critical need for industry to adopt the nationally agreed 'Phosphine Resistance Management Strategy'. Results from this round of monitoring clearly demonstrates that the selection pressure on phosphine is continuing and there have been instances of strong resistance in saw toothed grain beetles, which is a new resistance for the industry. It is imperative that judicial use of alternative strategies including the use of sulfuryl fluoride be adopted by industry to reduce this ongoing pressure on phosphine.

The ongoing detections of strong resistances in the rice weevil and rust red flour beetle requires that industry should confirm and ensure their control through the currently developed new fumigation protocols targeted for strongly resistant rusty grain beetles. If needed further adjustments in these high dosages would be required.

As highlighted by the end user advocate (see below), the future resistance monitoring program should include the monitoring of resistance to spinosad and sulfuryl fluoride, discriminating dosages for both of which were established through this project.

Looking at the future, the research team is in consultation with the industry (bulk handlers and growers) and funding bodies to develop and implement a transition to a cost-effective, responsive and self-sustainable resistance monitoring program.

7. End User Impact Assessment

The following impact assessment was undertaken by representative key end-user Mr Robin Reid from GrainCorp Australia Pty Ltd.

- *What does your sector hope to/what has your sector gained from this project?*

This project is critical to understand the level of resistance in all the grain insect species. The 'Quick tests' allow the correct decisions to be made as to what treatment is required for the next pest control treatment to control all insects. The project has highlighted detection of resistance in all parts of the supply chain and the emergence of strong resistance in rice weevils. The information from this data allows the industry to prioritise research directions.

- *How should the project be altered to maximise impact?*

As there is a need for large numbers if insects to be collected for the current testing methods, work needs to be done on the use of DNA testing to allow individual insects to be tested. This along with cost effective collection methods need to be developed to ensure that resistance data along the supply chain continues to be collected.

- *Should the project be extended to include further delivery activities?*

The project is currently not monitoring grain protectants; we have a new grain protectant (spinosad) and a new fumigant sulfuryl fluoride (SF) that needs to be monitored for level of resistance along with exiting products. It is noted that base-line response of key pest species to spinosad (*R. dominica*) and sulfuryl fluoride (all species) have now been established at the QDAF laboratory for this purpose. Monitoring resistance to SF is critical, specifically for the fact that the current label use of SF will not lead to a satisfactory break fumigant outcome. Resistance status of psocids and saw-toothed grain beetle to phosphine need to be investigated. There is a gap in knowledge of resistance of key beetle pests to deltamethrin, which also need to be addressed.

- *Who are key end-users that need to be engaged during delivery?*

Bulk handlers, grain farmers, grain processers (millers, animal feed producers), storers and exporters.

- *What other delivery pathways and mechanisms do we need to consider?*

Press releases and contact by extension staff are important mechanisms for this project may need to look at ways we can better communicate with farm advisors, suppliers and other key decision influencers

8. Project Leader Response to End User Impact Assessment

It is heartening to see very positive feedback on our research from the end-user advocate Mr Robin Reid. The points raised in his assessment will be incorporated in the future monitoring program.

On behalf of the Team, the Project Leader acknowledges and appreciates this. This also highlights the importance of the national resistance monitoring program that helps industry in early detections of existing as well as new resistances so that timely remedial actions are taken to meet the market access for Australia grain.

The Project Leader fully agrees on the observation by the end-user advocate in that the rice weevils are emerging as a new problem along the grain value chain. Moreover, the saw toothed grain beetles are also been detected on some farms over the last couple of years. These are being highlighted in the 'Challenges and issues arising from this research' section above. If industry and funding bodies such as PBCRC and GRDC support this research into the foreseeable future, the research team is in a good position to characterise these new strong resistances and develop appropriate eradication strategies.

Currently, with the uncertainty in funding for resistance monitoring, we are unable to address industry's priority to test individual insects in a sample using DNA markers for accurate determination of strong resistant alleles. However, if industry supports and funds such research in the future, the research team is technologically well-equipped to address this.

We fully agree with the suggestion of end user advocate in that the future resistance monitoring program should include the monitoring of resistance to spinosad and sulfuryl fluoride, discriminating dosages to detect resistance for both of these were established through the current project. It is important to note here that both these treatments are being used by both growers and bulk handlers across the eastern grain regions since last two years and we should keep them under our watch list for early detection of resistance.

9. Recommendations

The research and development information generated by this project emphasises the critical need for industry to adopt the nationally agreed 'Phosphine resistance management strategy'. Results from this round of monitoring clearly demonstrates that the selection pressure on phosphine is continuing and there have been instances of strong resistance in saw toothed grain beetles, which is a new resistance for the industry. It is imperative that judicial use of alternative strategies including the use of sulfuryl fluoride be adopted by industry to reduce this ongoing pressure on phosphine.

The ongoing detections of strong resistances in the rice weevil and rust red flour beetle requires that industry should confirm and ensure their control through the currently developed new fumigation protocols targeted for strongly resistant rusty grain beetles. If needed further adjustments in these high dosages would be required.

As highlighted by the end user advocate (see above), the future resistance monitoring program should include the monitoring of resistance to spinosad and sulfuryl fluoride, discriminating dosages for both of which, were established through this project. It is also important to note that while we utilise the 'quick tests' to provide industry same day advice on resistance status of a pest population; with the development of the molecular diagnostics, industry is keen on molecular screening of individual insects to get a much comprehensive picture of the frequency of resistance alleles in a pest population.

It is timely that findings from this study be used to modify the 'National Resistance Management Strategy' that has been last modified in 2009.

Looking at the future, the research team is currently in negotiation with key end users (GRDC, Bulk grain handling authorities) for funding for the purpose of transitioning the resistance monitoring program to a long term, self-sustainable model and establishing an enduring legacy.

10. References

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Plant Biosecurity
Cooperative Research Centre

Level 2, Building 22, Innovation Centre
University Drive, University of Canberra
Bruce ACT 2617

LPO Box 5012
Bruce ACT 2617

P: +61 2 6201 2882
F: +61 2 6201 5067
E: info@pbcrc.com.au
www.pbcrc.com.au