

**Cooperative Research Centre  
for National Plant Biosecurity**

# **Final Report**

**CRC50177**

**Australian Grain Insect Resistance Database –  
data mining**

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# 1. Executive Summary

The Australian phosphine resistance management strategy is underpinned by a national resistance monitoring program that provides continuous updates on the distribution and strength of resistance. This information is collected and stored in the Australian Grain Insect Resistance Database (AGIRD). This data warehouse now contains detailed information on the incidence of resistance in Australia for the past 20 years from thousands of sites monitored over this period.

The existence of AGIRD provides a unique opportunity for an analysis of the contribution of broader scale factors and practices to the occurrence of resistance that cannot be evaluated in local scale projects focussed on tactic development. However, the data in their current form are quite difficult to access and interrogate in a systematic way.

The aim of this pilot project was to develop a statistical methodology for analysing the database so that insights into resistance development might be obtained. We chose a limited set of the data from AGIRD with which to construct and test the methodology. The set chosen was detections of strong resistance to phosphine in all pest species over a 20 year period in Queensland. We analysed risk of resistance associated with factors such as storage type, site type, commodity, insect species, chemical treatment history and spatial-temporal elements. Our aim was to reveal trends in the development of strong resistance to phosphine over time and how these trends were affected by biological and environmental factors, biosecurity practices and agricultural context.

These factors (and/or constellations of factors) could affect strong resistance (SR) in two ways either SR has never occurred (absence) or SR occurred (SR present). After accounting for strata (of absence or potential presence of SR), we focused on trends in SR and how they evolved over the period in question. We used a hurdle model with Bayesian classification trees with a high penalty on false negatives to discover the hidden strata and underlying factors leading to pure absence or potential presence of SR (Strata). Generalized additive models with variable selector were used to reveal the relative importance of BEBA factors on incidence of SR within each BEBA stratum (Incidence). After adjusting for these strata and main effects within strata, we described the trends in development of SR (Trend).

The bioassay data held in AGIRD were recorded in a binary form (presence or absence of strong resistance to phosphine) along with several broader scale factors (covariates). Bioassays are usually analyzed using probit or logistic regression models. However, there are two features of these types of analyses for this dataset constraining the trend analysis: 1) prevalence of excess zeros (absence of strong resistance in most samples) and 2) the relationship between BEBA factors and strong resistance may not be linear. To address these limitations, we used a hurdle model with a classification tree to discover underlying stratification.

A hurdle model assumes there are two processes influencing the response: one generating the excess zeros (absences) and another generating the presences and absences in the remainder of the data. This translates to AGIRD in that some bioassay profiles (i.e. site types, storage types, geographic locations, etc., and combinations thereof) may not have been susceptible to strong resistance and the hurdle model allows these bioassays to be identified so our subsequent analysis can focus on the problematic profiles. While usually applied to count data, the 'two-step' nature of the hurdle model is suited to this dataset because excess zeros can dilute the analysis, lowering the explanatory power of the model and producing misleading trends.

We used Bayesian classification trees (BCART) for the first step of the hurdle model. These provide a decision tree where the sequences of decisions are defined by thresholds on factors. At the end of each decision path we arrive at a stratum (leaf node) where we model the chance of absence of strong resistance. We select trees with large numbers of observations in pure absence nodes so that the second step of the hurdle model can focus on those profiles leading to strong resistance.

For the second step in the model we used generalized additive models (GAMs) to determine if there were any trends in those strata identified in step one that contain some evidence of strong resistance. The use of GAMs overcomes the second problem listed above in that it allows for non-linear relationships between strong resistance and BEBA factors. As well as a nonparametric description of trends, we also used parametric covariates to adjust for varying incidence at different levels of factors. A variable selection model (spike and slab priors) was used to identify those factors having plausible influence on the probability/presence/risk of strong resistance. For binary response data such as ours we followed standard practice and related the log odds of probability of presence (logit) to the trends and adjusting factors.

The analysis revealed that patterns of resistance emergence and detection varied with species. Resistance was most often detected in *Cryptolestes ferrugineus* and *Rhyzopertha dominica* so that most implications from the data are associated with these species although some conclusions could be made about other species. Nevertheless, across all species, occurrence of strong resistance was associated with storages that had a history of phosphine use indicating that practices at particular storages are crucial.

Strong resistance in *C. ferrugineus* was associated with central storages and was very rarely detected on farm. Use of fenitrothion was very effective at preventing or eliminating phosphine resistance in this species and in *Tribolium castaneum*. However, use of pirimiphos-methyl was shown to be ineffective at controlling resistance in *R. dominica*. Detections of strong resistance in *C. ferrugineus* were also higher in unsealed storages while detections of *R. dominica* were significantly less frequent in aerated and bunker storages.

The analysis provided strong support for the implementation of resistance management tactics including use of alternative chemical and non-chemical treatments and methods.

## 2. Aims and objectives

Resistance to phosphine fumigant in insect pests is the most critical biosecurity threat to stored grain in Australia. This is because the Australian industry relies heavily on phosphine to ensure the insect free status of its product – an essential element to the maintenance of both domestic and overseas markets.

As there is no practical, cost-effective replacement for phosphine, the CRCNPB has invested in the development and evaluation of tactics to support the effective management of resistance to phosphine. These efforts support the ongoing development of a national resistance management strategy (GTA website, [www.graintrade.org.au](http://www.graintrade.org.au)).

The Australian phosphine resistance management strategy is underpinned by a national resistance monitoring program that provides continuous updates on the distribution and strength of resistance. This information is collected and stored in the Australian Grain Insect Resistance Database (AGIRD) (AGIRD website, [www.agric.wa.gov.au/PC\\_92882.html](http://www.agric.wa.gov.au/PC_92882.html)). This data warehouse now contains detailed information on the incidence of resistance in Australia for the past 20 years from thousands of sites monitored over this period.

The existence of AGIRD provides a unique opportunity for an analysis of the contribution of broader scale biological and environmental factors, biosecurity practices, and agricultural context to the occurrence of resistance that cannot be evaluated in local scale projects focussed on tactic development. However, the data in AGIRD in their current form are quite difficult to access and interrogate in a systematic way.

The aim of this project was to develop and test a statistical methodology that could be used to interrogate the AGIRD database so that insights into resistance development might be obtained. We chose a limited set of the data from AGIRD with which to construct and test the methodology. The set chosen was detections of strong resistance to phosphine in all pest species over a 20 year period in Queensland. We analysed risk of resistance associated with factors such as storage type, site type, commodity, insect species, chemical treatment history and spatial-temporal elements. Our aim was to

reveal trends in the development of strong resistance to phosphine over time and how these trends were affected by biological and environmental factors, biosecurity practices and agricultural context.

### 3. Key findings

The Australian Grain Insect Resistance Database (AGIRD) is a data warehouse consisting of thousands of entries collected since 1992 of results of bioassay tests of grain insect pests for resistance to phosphine and other chemicals. AGIRD comprises three main tables (*Sites*, *Strains* and *Assays*) that link to each other and contain numerous fields. The fields of primary importance include information on insect species collected, date collected, bioassay results, geographic information, site type, storage type, commodity and treatments. The data vary considerably in quality and required a significant amount of preparation before it was suitable for statistical analysis. Because of the large amount of data and the need for extensive 'cleaning', it was decided that for this pilot project, the analysis would use information collected from Queensland sites in the Northern Region of the grain belt (northern NSW and Queensland) and focus on the development of strong resistance to phosphine. This phase also required detailed discussions with the contributors to the database, which was facilitated by using data from one laboratory. A national database analysis would also have posed significant challenges in data integration due to major differences between laboratories, particularly bioassay methodology and environmental conditions over the 20 year time period.

#### *Data preparation and ensuring consistency between segments of the data*

We found that many entries in AGIRD did not match other fields in tables or were not linked to logical diagnoses of resistance. This required identification of key assays used in diagnoses of resistance levels using a mixture of logical rules and historical contexts. In addition, individual collection sites required classification into sub-regions using GIS polygons that were constructed for this purpose (Fig. 1). Additionally, the treatment field in the database was arranged into multiple binary variables to allow analysis of all available combinations.

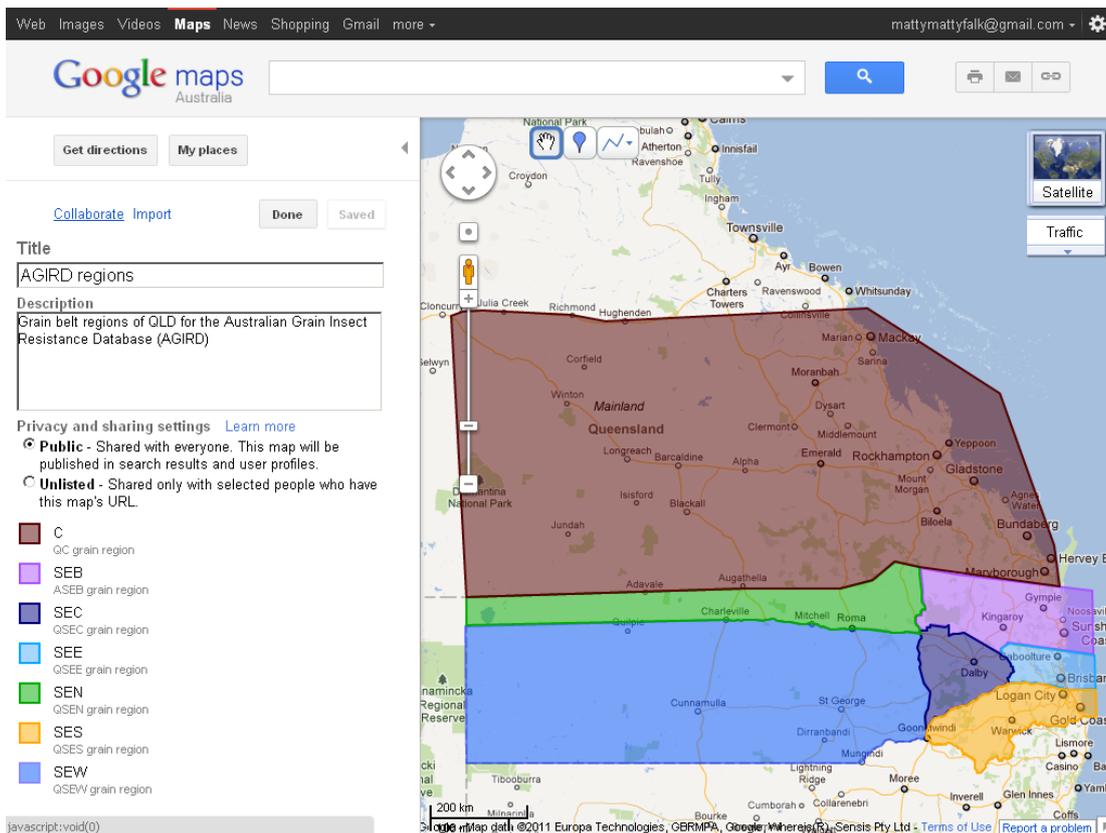


Fig 1: Map of sub-regions for Queensland sites in the Northern grain growing region.

### Analysis

The aim of the analysis was to reveal trends in the development of strong resistance to phosphine over the two decades covered by AGIRD. This was evidenced by insect species surviving a high discriminating dose of phosphine in laboratory bioassays. Of interest is how these trends are affected by biological and environmental factors, biosecurity practices and agricultural context (BEBA). The key factors considered were:

Biological Factors	Environmental Factors	Biosecurity Practices	Agricultural Context
Insect species Host Type (commodity)	Sub-region	Storage type Use of chemical treatments	Site type

These factors (and/or constellations of factors) could affect strong resistance (SR) in two ways:

1. Defining BEBA strata where either:
  - a. SR has never occurred (records of pure absence of SR in the same BEBA stratum), or;
  - b. SR may have occurred (records of both absence and presence that share the same BEBA factors).
2. Within a stratum, particular values of a BEBA factor will have varying influence on the incidence of SR.

Finally, after accounting for strata (of absence or potential presence of SR), we can focus on trends in SR and how they have evolved over the period in question. So, we used a hurdle model with:

1. Bayesian classification trees with a high penalty on false negatives to discover the hidden strata and underlying BEBA factors leading to pure absence or potential presence of SR (Strata).
2. Generalized additive models with variable selector were used to reveal the relative importance of BEBA factors on incidence of SR within each BEBA stratum (Incidence).
3. After adjusting for these strata and main effects within strata, we describe the trend in development of SR (Trend).

The bioassay data held in AGIRD were recorded in a binary form (presence or absence of strong resistance to phosphine) along with several broader scale BEBA factors (covariates). Bioassays are usually analyzed using probit or logistic regression models. However, there are two features of these types of analyses for this dataset constraining the trend analysis: 1) prevalence of excess zeros (absence of strong resistance in most samples) and 2) the relationship between BEBA factors and strong resistance may not be linear. To address these limitations, we used a hurdle model with a classification tree to discover underlying stratification.

A hurdle model assumes there are two processes influencing the response: one generating the excess zeros (absences) and another generating the presences and absences in the remainder of the data. This translates to AGIRD in that some bioassay profiles (i.e. site types, storage types, geographic locations, etc., and combinations thereof) may not have been susceptible to strong resistance and the hurdle model allows these bioassays to be identified so our subsequent analysis can focus on the problematic profiles. While usually applied to count data, the 'two-step' nature of the hurdle model is suited to this dataset because excess zeros can dilute the analysis, lowering the explanatory power of the model and producing misleading trends.

We used Bayesian classification trees (BCART) for the first step of the hurdle model. These provide a decision tree where the sequences of decisions are defined by thresholds on BEBA factors. At the end of each decision path we arrive at a stratum (leaf node) where we model the chance of absence of strong resistance. We select trees with large numbers of observations in pure absence nodes so that the second step of the hurdle model can focus on those profiles leading to strong resistance.

For the second step in the model we used generalized additive models (GAMs) to determine if there were any trends in those strata identified in step one that contain some evidence of strong resistance. The use of GAMs overcomes the second problem listed above in that it allows for non-linear relationships between strong resistance and BEBA factors. As well as a nonparametric description of trends, we also used parametric covariates to adjust for varying incidence at different levels of BEBA factors. A variable selection model (spike and slab priors) was used to identify those factors having plausible influence on the probability/presence/risk of strong resistance. For binary response data such as ours we followed standard practice and related the log odds of probability of presence (logit) to the trends and adjusting BEBA factors.

## Results

Statistical modelling was repeated at three different scales.

Regional: All species were considered together. Of interest were the BEBA factors determining general strata and adjustments ignoring biological specifics of each pest species.

	Absent	Present
All Species	3854	217

Grain Specific: We separately considered primary feeders that can compromise the integrity of whole grains, as opposed to secondary feeders that can attack fragmented grain.

	Absent	Present
Primary Feeders	2034	55
Secondary Feeders	1820	162

Pest Specific: We consider each pest separately.

	Species	Absent	Present
Primary Feeders	<i>Rhyzopertha dominica</i>	1177	46
	<i>Sitophilus oryzae</i>	857	9
Secondary Feeders	<i>Cryptolestes ferrugineus</i>	580	138
	<i>Oryzaephilus surinamensis</i>	274	10
	<i>Tribolium castaneum</i>	966	14

These three investigations focussed on increasing biological resolution (from region to grain impacts to pest) but at the expense of evidence (from 217 down to nine bioassays indicating presence of strong resistance) and therefore power to detect trends when present. We present analyses of the data using species grouped as either primary or secondary pests as this scale represents a suitable trade off between biological resolution and trend detection.

### *Analysis by species grouped as primary or secondary pests*

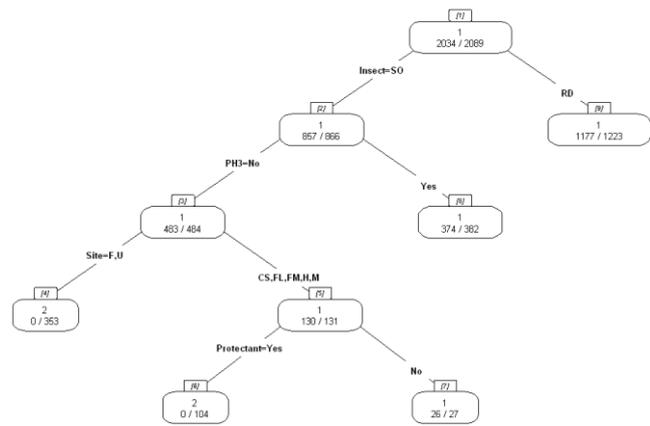
The major insect pests of grain can be divided into either Primary or Secondary pests based on their life history. Primary pests feed on whole seeds, completing most of their immature development inside the grain. These species include *R. dominica* and *S. oryzae*. Secondary pests spend their entire life history on the outside of the seed, feeding mostly on broken grain particles, mould spores and hyphae, and the germ of the seed. Secondary pests include *C. ferrugineus*, *O. surinamensis* and *T. castaneum*.

The results of our analyses are summarised in the following tables:

Primary Pests

Strata – overall the key broad-scale factors affecting resistance in primary pests were related to:

- (1) Insect – whether the RD (*R. dominica*) species;
- (2) Biosecurity practice - whether PH3 or another chemical treatment was known to have been applied; and
- (3) Site types – CS (central storage), FL (feed lot), FM (flour mill), H (household) and M (merchant)



Incidence – the majority of Strong Resistance was found in the stratum classified by the RD (*R. dominica*) species. Models on other stratum with incidence of SR failed due to insufficient data. The following BEBA factors were important to the incidence of SR:

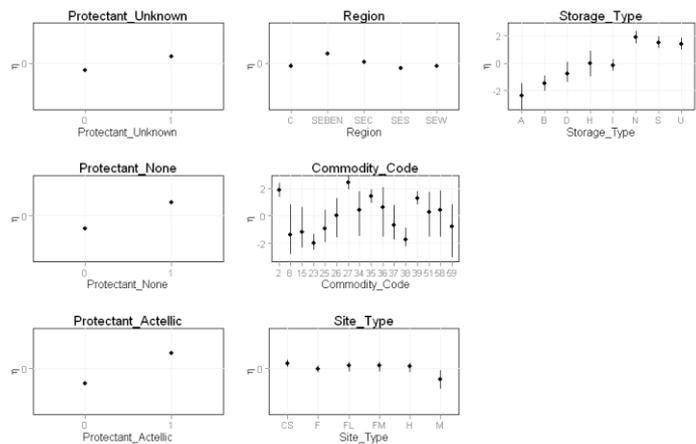
Treatments – unknown, none or actellic were associated with high SR

Region – slightly higher incidence of SR for SEB (compared with C, SEC, SES, SEW)

Commodity – higher incidence with 2 (barley), 27 (oats), 35 (sorghum) and 39 (wheat); lower incidence with 23 (maize) and 38 (unknown)

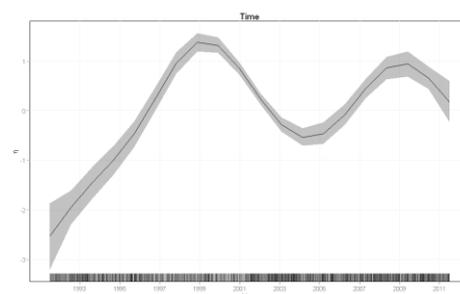
Site Type – CS (central storages) have slightly higher incidence of SR; M (merchants) slightly lower

Storage Type – U (unsealed), S (sealed) and N (unknown) have higher incidence, whereas A (aerated) and B (bunker) have lower incidence



Trend

Possible cyclical trend in SR – steady increase to peaking in 1999, trough around 2004, peak around 2010 and a recent decline



Secondary Pests (1)

<p>Strata – overall the key broad-scale factors affecting resistance in secondary pests were related to:</p> <p>(1) Region – whether in the south east quadrants NESW (SEE, SEN, SES, SEW);</p> <p>(2) Biosecurity practice - whether fenitrothion was known to have been applied; and</p> <p>(3) TC (<i>T. castaneum</i>) is a special case in certain sub-regions</p>	<pre> graph TD     Root["1 1820 / 1982 Region=C,SEB,SEC,XXXXX"]     Root --&gt; L["1 939 / 1040 FEN=Yes"]     Root --&gt; R["1 881 / 942 SEE,SEN,SES,SEW"]     L --&gt; L1["2 0 / 28"]     L --&gt; L2["1 911 / 1012 No"]     R --&gt; R1["2 0 / 460 Insect=TC"]     R --&gt; R2["1 421 / 482 CRY,OS"]     </pre>
<p>Incidence – SR was found in two strata. Firstly we consider the stratum classified by Regions C, SEB, SEC and unmapped locations and no application of fenitrothion. The following BEBA factors were important to the incidence of SR:</p> <p>Treatment with phosphine (PH3) presents higher incidence of SR</p> <p>Insect – highest incidence for CRY (<i>C. ferrugineus</i>), lowest for OS (<i>O. surinamensis</i>)</p> <p>Region – higher SR in SEC compared to C</p> <p>Commodity – higher incidence for 2 (barley), 15 (feed), 25 (mixed grain), 35 (sorghum), 36 (sunflower) and 39 (wheat); 59 (corn) much lower incidence</p> <p>Site Type – high SR for FM (flour mills) and M (merchants); low for (F) farmers</p> <p>Storage Type – higher SR for S (sealed); lower SR for U (unsealed)</p>	<p>The figure consists of six dot plots arranged in a 3x2 grid, each showing the relationship between a specific BEBA factor and SR incidence. The y-axis for all plots is SR, ranging from 0 to 1.0. The x-axis for each plot represents the factor being analyzed.</p> <ul style="list-style-type: none"> <li><b>Protectant_PH3:</b> Shows SR incidence for 0 (no phosphine) and 1 (phosphine applied).</li> <li><b>Commodity_Code:</b> Shows SR incidence for various commodity codes (2, 8, 10, 15, 23, 25, 26, 27, 35, 36, 37, 38, 39, 51, 58, 59).</li> <li><b>Insect_Code:</b> Shows SR incidence for CRY, OS, and TC.</li> <li><b>Site_Type:</b> Shows SR incidence for CS, F, FM, and M.</li> <li><b>Region:</b> Shows SR incidence for C and SEC.</li> <li><b>Storage_Type:</b> Shows SR incidence for A, B, D, H, I, N, S, and U.</li> </ul>
<p>Trend</p> <p>Increasing steadily until around 2003 with the increase slowing recently (and potentially asymptotic)</p>	<p>The graph shows the trend of SR incidence over time from 1990 to 2011. The x-axis is labeled 'Year' and ranges from 1990 to 2011. The y-axis is labeled 'SR' and ranges from 0 to 1.0. The data points show a steady increase in SR incidence from approximately 0.4 in 1990 to about 0.8 in 2003, after which the increase slows down and the incidence appears to be approaching an asymptote around 0.9.</p>

## Secondary Pests (2)

<p>Strata – overall the key broad-scale factors affecting resistance in secondary pests are related to:</p> <p>(1) Region - whether in the south east quadrants NESW (SEE, SEN, SES, SEW);</p> <p>(2) Biosecurity practice - whether FEN (fenitrothion) was known to have been applied; and</p> <p>(3) TC (<i>T. castaneum</i>) is a special case in certain sub-regions</p>	<pre> graph TD     Root["#1 1 1820 / 1982"] -- "Region=C,SEB,SEC,XXXXX" --&gt; Node1["#1 1 939 / 1040"]     Root -- "SEE,SEN,SES,SEW" --&gt; Node2["#1 1 881 / 942"]     Node1 -- "FEN=Yes" --&gt; Node1_1["#1 2 0 / 28"]     Node1 -- "No" --&gt; Node1_2["#1 1 911 / 1012"]     Node2 -- "Insect=TC" --&gt; Node2_1["#1 2 0 / 460"]     Node2 -- "CRY,OS" --&gt; Node2_2["#1 1 421 / 482"]     </pre>
<p>Incidence – the SR is found in two strata. Here we consider the stratum classified by Regions SEE, SEN, SES and SEW for insects CRY (<i>C. ferrugineus</i>) and OS (<i>O. surinamensis</i>). The following BEBA factors were important to the incidence of SR:</p> <p>Treatment with an unknown protectant or phosphine (PH3)</p> <p>Insect – higher incidence for CRY (<i>C. ferrugineus</i>)</p> <p>Commodity – higher incidence for 2 (barley), 25 (mixed grain), 35 (sorghum), 36 (sunflower) and 39 (wheat); 59 (corn) much lower incidence</p> <p>Site Type – high for CS (central storages) and M (merchants); low for F (farms)</p> <p>Storage Type – higher incidence for I (silos) and U (unsealed) but lower for A (aerated), D (shed), N (unknown) and S (sealed)</p>	<p>The figure consists of six dot plots arranged in a 3x2 grid, each showing the incidence of SR (Y-axis, ranging from 0 to 3) across different levels of a BEBA factor (X-axis). The plots are:</p> <ul style="list-style-type: none"> <li><b>Protectant_Unknown:</b> Shows incidence for 0 and 1.</li> <li><b>Commodity_Code:</b> Shows incidence for codes 2, 8, 10, 15, 23, 25, 26, 27, 34, 35, 36, 37, 38, 39, 59.</li> <li><b>Protectant_PH3:</b> Shows incidence for 0 and 1.</li> <li><b>Site_Type:</b> Shows incidence for CS, F, FL, H, M.</li> <li><b>Insect_Code:</b> Shows incidence for CRY and OS.</li> <li><b>Storage_Type:</b> Shows incidence for A, B, D, I, N, S, U.</li> </ul>
<p>Trend</p> <p>Fairly steady increase over time, with a slowing increase from 1997 to 1999, before a strong increase until 2005. After 2005 the trend appears to plateau and possibly decrease in 2011 (subject to wide credible intervals)</p>	<p>The graph shows the trend of SR incidence over time from 1993 to 2011. The Y-axis represents incidence (ranging from 0 to 2), and the X-axis represents the year. A solid line shows the trend, which increases steadily from 1993 to 2005, then plateaus and shows a slight decrease in 2011. A shaded area around the line represents the credible interval, which is wider in the early years and narrows towards the end of the period.</p>

We can summarize the appearance of each biological and environmental factor, biosecurity practice, and agricultural context in terms of whether they define pockets (strata) where strong resistance is completely absent, or whether they are associated with higher or lower levels of strong resistance (impact). This is assessed for primary and secondary pests.

#### STORAGE TYPE

Scale	Impact of BEBA factor on incidence of strong resistance	Stratum
Primary pests	No apparent effect on absence of SR	All
Primary pests	Higher SR for U (unsealed), S (sealed) and N (unknown); Lower SR frequency for A (aerated) and B (bunker)	<i>R. dominica</i>
Secondary pests	Higher for U (unsealed)	<i>C. ferrugineus</i> and <i>O. surinamensis</i>  Regions SEE, SEN, SES and SEW
Secondary pests	Higher for I (silos) and U (unsealed) but lower for (S) sealed, D (shed), N (unknown), S (sealed) and A (aerated)	<i>T. castaneum</i>

#### TREATMENTS

Scale	Impact of BEBA factor on incidence of strong resistance	Stratum
Primary pests	Pirimiphos-methyl and unknown treatments are ineffective, as is no treatment	<i>R. dominica</i>
Secondary pests	No strong resistance when fenitrothion used	Regions C, SEB, SEC and unmapped locations
Secondary pests	No strong resistance when phosphine used	<i>S. oryzae</i>
Secondary pests	Increased strong resistance when phosphine used	Regions C, SEB, SEC and unmapped locations No fenitrothion used
Secondary pests	Increased strong resistance when phosphine or unknown (likely some chemical) treatment used	<i>C. ferrugineus</i> and <i>O. surinamensis</i> in Regions SEE, SEN, SES and SEW

#### SITE TYPE

Scale	Impact of BEBA factor on incidence of strong resistance	Stratum
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Primary pests	No strong resistance for F (farmers) and U (unknown) but some strong resistance for CS (central storage), FL (feed lot), FM (flour mill), H (households) and M (merchants)	<i>S. oryzae</i> and phosphine not used
Primary pests	Low for M (merchants)	<i>R. dominica</i>
Secondary pests	High for FM (flour mills) but low for F (farmers)	Regions C, SEB, SEC and unmapped locations using fenitrothion
Secondary pests	High for CS (central storage) but low for F (farmers) and M (merchants)	Regions SEE, SEN, SES and SEW for <i>C. ferrugineus</i> and <i>O. surinamensis</i>

#### REGIONS

Scale	Impact of region on incidence of strong resistance	Stratum
Primary pests	No apparent effect on absence of strong resistance	All
Primary pests	Slightly higher for SEBEN (compared to C, SEC, SES, SEW)	<i>R. dominica</i>
Secondary pests	No strong resistance	Regions C, SEB, SEC and unmapped locations when fenitrothion used
Secondary pests	Slightly higher incidence of SR in SEC compared to C	Regions C, SEB, SEC and unmapped locations when fenitrothion not used
Secondary pests	No strong resistance	Regions SEE, SEN, SES and SEW for <i>T. castaneum</i>

#### COMMODITY

Scale	Impact of region on incidence of strong resistance	Stratum
Primary/Secondary pests	No apparent impact on absence of strong resistance	All
Primary pests	Lower incidence in 20 (maize) and 38 (unknown) but higher incidence in 2 (barley), 27 (oats), 35 (sorghum) and 39 (wheat)	<i>R. dominica</i>
Secondary pests	Higher incidence for 2 (barley), 15	Regions C, SEB, SEC and XXXXX

	(feed), 25 (mixed grain), 35 (sorghum), 36 (sunflower) and 39 (wheat). 59 (corn) has a much lower incidence of SR	using fenitrothion
Secondary pests	Higher incidence for 2 (barley), 25 (mixed grain), 35 (sorghum), 36 Sunflower, and 39 (wheat) with 59 (corn) much lower incidence of SR	Regions SEE, SEN, SES and SEW for <i>C. ferrugineus</i> and <i>O. surinamensis</i>

## INSECTS

Scale	Impact of region on incidence of strong resistance	Stratum
Primary pests	Highest incidence for <i>R. dominica</i>	Own stratum
Secondary pests	Highest incidence for <i>C. ferrugineus</i>	All

## TRENDS

Scale	Impact of region on incidence of strong resistance	Stratum
Primary pests	Possible cyclical trend in SR – steady increase to peaking in 1999, trough around 2004, peak around 2010 and a recent decline	<i>R. dominica</i>
Secondary pests	Increasing steadily until around 2003 with the increase slowing recently (and potentially asymptotic)	Regions C, SEB, SEC and unmapped locations using FEN
Secondary pests	Fairly steady increase over time, with a slowing increase from 1997 to 1999, before a strong increase until 2005. After 2005 the trend appears to plateau and possibly decrease in 2011 (subject to wide credible intervals)	Regions SEE, SEN, SES and SEW for <i>C. ferrugineus</i> and <i>O. surinamensis</i>

## Conclusions

Despite covering only a limited amount of data held in the AGIRD database, we are able to make a number of conclusions. Over the 20-year period, Strong Resistance (SR) was detected in most frequently in the rusty grain beetle, *Cryptolestes ferrugineus*, (138 samples) followed by the lesser grain borer, *Rhyzopertha dominica* (46 samples), and much less frequently in the rice weevil, *Sitophilus oryzae* (9), red flour beetle, *Tribolium castaneum* (14) and sawtoothed grain beetle, *Oryzaephilus surinamensis* (10). SR occurred in storages with a history of phosphine use indicating that practices at particular storages are selecting for resistance rather than resistant insects invading from other locations.

Strong resistance in *C. ferrugineus* occurred most frequently in Central Queensland including the Emerald and Biloela districts (C), Kingaroy/South Burnett (SEB) and the region around Dalby (SEC) in storages not using fenitrothion. SR was not detected in storages using fenitrothion. Frequency of SR in this species was high in central storages and low on farm and at merchant premises and higher in unsealed storages. As expected SR occurred in insects infesting commodities associated with the central handling

system. This analysis revealed that incipient resistance was first detected in *C. ferrugineus* in 1992 and 1993 from single observations. There were then no detections of fully expressed strong resistance until 2007 and since that time detection rate has plateaued.

SR in *R. dominica* occurred in most regions but was a little more frequent in Burnett region. Detections were significantly less frequent in aerated and bunker storages. Use of pirimiphos-methyl was shown to be ineffective at controlling resistance in this species. Resistance was most frequent in central storages and on farm with relatively few detections from merchant premises. SR was first detected in this species in 1997 and there was a rapid increase in frequency to 1999 which was followed by a decline to 2004, another peak around 2010 and a recent decline.

Some conclusions could also be made about species with lower frequencies of SR. Strong resistance in *O. surinamensis* was highly associated with grain merchant premises and unsealed storages. Resistance in *S. oryzae* occurred in all sectors of the grain industry but was not detected on farm in Queensland. Use of fenitrothion appears to have prevented development of SR in *T. castaneum* as this resistance occurred only in storages where fenitrothion was not used and never where it was used.

### 3. Implications for stakeholders

The Australian phosphine resistance management strategy is underpinned by a national resistance monitoring program that provides continuous updates on the distribution and strength of resistance. This information is collected and stored in the Australian Grain Insect Resistance Database (AGIRD). This data warehouse now contains detailed information on the incidence of resistance in Australia for the past 20 years from thousands of sites monitored over this period.

The existence of AGIRD provides a unique opportunity for an analysis of the contribution of broader scale factors and practices to the occurrence of resistance that cannot be evaluated in local scale projects focussed on tactic development. However, the data in AGIRD in their current form are quite difficult to access and interrogate in a systematic way.

The aim of this pilot project was to develop and test a statistical methodology that could be used to interrogate the AGIRD database so that insights into resistance development might be obtained. We chose a limited set of the data from AGIRD with which to construct and test the methodology. The set chosen was detections of strong resistance to phosphine in all pest species over a 20 year period in Queensland. We analysed risk of resistance associated with factors such as storage type, site type, commodity, insect species, chemical treatment history and spatial-temporal elements.

The analysis revealed that patterns of resistance emergence and detection varied with species. Resistance was most often detected in *Cryptolestes ferrugineus* and *Rhyzopertha dominica* so that most implications from the data are associated with these species although some conclusions could be made about other species. Nevertheless, across all species, occurrence of was associated with storages that had a history of phosphine use indicating that practices at particular storages are crucial in limiting resistance development.

Strong resistance in *C. ferrugineus* was associated with central storages and was very rarely detected on farm. Use of fenitrothion was very effective at preventing or eliminating phosphine resistance in this species and in another external feeder, *Tribolium castaneum*. However, use of pirimiphos-methyl was shown to be ineffective at controlling resistance in *R. dominica*. Detections of SR in *C. ferrugineus* were also higher in unsealed storages while detections of *R. dominica* were significantly less frequent in aerated and bunker storages.

## 4. Recommendations

Despite the limited geographical extent of the data analysis, the project demonstrated that very useful information relevant to improving resistance management strategies can be mined from the AGIRD database. The statistical methodology developed was able to analyse the data in a systematic way to obtain significant trends.

Even though this was essentially a demonstration analysis, the analysis provided quite practical outputs. The results demonstrated that the integrated use of alternative chemical treatments and physical methods such as grain cooling will reduce the incidence of resistance. The results also revealed that each pest species must be considered when developing resistance management tactics as each has different behaviours and can react in different ways to various treatments.

Finally, this pilot project has demonstrated the potential benefits of expanding this research to include the analysis and modelling of all data in AGIRD. This would allow for similarities and differences to be robustly identified for different regions of Australia and to determine whether the same biological and environmental factors, biosecurity practices, and agricultural context are important. Also the modelling could be expanded to assess whether geographical movement of insects contributes to strong resistance.

## 5. Abbreviations/glossary

<b>ABBREVIATION</b>	<b>FULL TITLE</b>
AGIRD	Australian Grain Insect Resistance Database
BEBA	Biological and environmental factors, Biosecurity practices, and agricultural context
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
EPP	Emergency plant pest
SR	Strong resistance

## 6. Plain English website summary

CRC project no:	CRC50177
Project title:	<b>Australian Grain Insect Resistance Database data mining</b>
Project leader:	Patrick J. Collins
Project team:	Samantha Low-Choy, Matthew Falk
Research outcomes:	<p>The Australian phosphine resistance management strategy is underpinned by a national resistance monitoring program that provides continuous updates on the distribution and strength of resistance. This information is collected and stored in the Australian Grain Insect Resistance Database (AGIRD). This data warehouse now contains detailed information on the incidence of resistance in Australia for the past 20 years from thousands of sites monitored over this period.</p> <p>The existence of AGIRD provides a unique opportunity for an</p>

	<p>analysis of the contribution of broader scale factors and practices to the occurrence of resistance that cannot be evaluated in local scale projects focussed on tactic development. However, the data in their current form are quite difficult to access and interrogate in a systematic way.</p> <p>The aim of this pilot project was to develop a statistical methodology for analysing the database so that insights into resistance development might be obtained. We chose a limited set of the data from AGIRD with which to construct and test the methodology. The set chosen was detections of strong resistance to phosphine in all pest species over a 20 year period in Queensland. We analysed risk of resistance associated with factors such as storage type, site type, commodity, insect species, chemical treatment history and spatial-temporal elements.</p> <p>Despite the limited geographical extent of the data analysis, the project demonstrated that very useful information relevant to improving resistance management strategies can be mined from AGIRD. For example, our analysis provided strong support for the implementation of resistance management tactics. The results demonstrate that the integrated use of alternative chemical treatments and physical methods such as grain cooling will significantly reduce the incidence of resistance.</p> <p>Finally, this pilot project has demonstrated that the statistical methods developed could be used successfully to interrogate AGIRD. A whole database analysis is now feasible and this would allow much stronger trends and conclusions to be made. It would also allow for similarities and differences to be robustly identified for different regions of Australia and to determine whether the same biological and environmental factors, biosecurity practices and agricultural context are important. Also the modelling could be expanded to assess whether geographical movement of insects contributes to strong resistance.</p>
<p>Research implications:</p>	<p>Despite covering only a limited amount of data held in the AGIRD database, we are able to make a number of conclusions. Over the 20-year period, Strong Resistance (SR) was detected most frequently in the rusty grain beetle, <i>Cryptolestes ferrugineus</i>, (138 samples) followed by the lesser grain borer, <i>Rhyzopertha dominica</i> (46 samples), and much less frequently in the rice weevil, <i>Sitophilus oryzae</i> (9), red flour beetle, <i>Tribolium castaneum</i> (14) and sawtoothed grain beetle, <i>Oryzaephilus surinamensis</i> (10). SR occurred in storages with a history of phosphine use indicating that practices at particular storages are selecting for resistance rather than resistant insects invading from other locations.</p> <p>Strong resistance in <i>C. ferrugineus</i> occurred most frequently</p>

	<p>in Central Queensland including the Emerald and Biloela districts (C), Kingaroy/South Burnett (SEB) and the region around Dalby (SEC) in storages not using fenitrothion. SR was not detected in storages using fenitrothion. Frequency of SR in this species was high in central storages and low on farm and at merchant premises, and higher in unsealed storages. As expected SR occurred in insects infesting commodities associated with the central handling system. This analysis revealed that incipient resistance was first detected in <i>C. ferrugineus</i> in 1992 and 1993 from single observations. There were then no detections of fully expressed strong resistance until 2007 and since that time detection rate has plateaued.</p> <p>SR in <i>R. dominica</i> occurred in most regions but was a little more frequent in Burnett region. Detections were significantly less frequent in aerated and bunker storages. Use of pirimiphos-methyl was shown to be ineffective at controlling resistance in this species. Resistance was most frequent in central storages and on farm with relatively few detections from merchant premises. SR was first detected in this species in 1997 and there was a rapid increase in frequency to 1999 which was followed by a decline to 2004, another peak around 2010 and a recent decline.</p> <p>Some conclusions could also be made about species with lower frequencies of SR. Strong resistance in <i>O. surinamensis</i> was highly associated with grain merchant premises and unsealed storages. Resistance in <i>S. oryzae</i> occurred in all sectors of the grain industry but was not detected on farm in Queensland. Use of fenitrothion appears to have prevented development of SR in <i>T. castaneum</i> as this resistance occurred only in storages where fenitrothion was not used and never where it was used.</p> <p>Overall, it was clear from the results that each pest species must be considered individually when developing resistance management tactics as each can react in different ways to various treatments. As we improve our understanding of the biology and ecology of these insects we will be better able to target more effective management strategies.</p>
Research publications:	
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