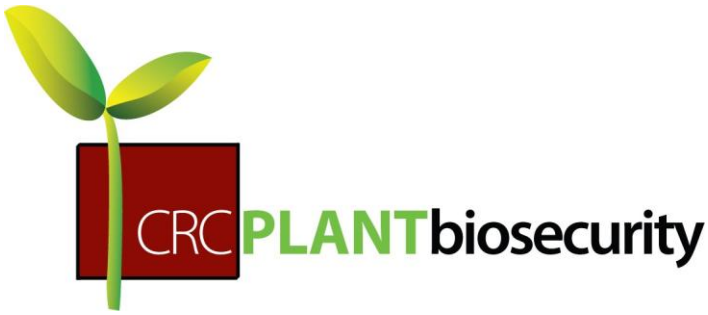

Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC50188

Dispersal and resource use by the lesser grain borer (*Rhyzopertha dominica*) in southern New South Wales

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1. Executive Summary

This project examined various aspects of the biology and ecology of the lesser grain borer (LGB), *Rhyzopertha dominica*, a key pest of stored grain that is rapidly developing resistance to the fumigant phosphine. The grains industry relies heavily on phosphine to ensure exports are free from insect contamination, and also free from detectable chemical residues. This project is a component of a concerted effort to develop a better understanding of how this pest survives and disperses in the field, and how ecologically-based recommendations on grain handling procedures can be used to reduce infestation risk in grain storages and thus slow the selection pressure for phosphine resistance. The project was funded as a 'turbo' project and ran for approximately 9½ months from August 2011 onwards. Experimental work conducted during the project fell into two categories – dispersal studies, and development studies on potential alternative host plants. Dispersal studies included experiments to validate fluorescent marking powders as a tool to discriminate between released and 'wild' beetles in trap captures, obtaining preliminary data on the residual efficacy of commercial LGB pheromone dispensers, development of techniques for quantitative LGB releases, and mark/release/recapture studies at distances of up to one kilometre.

Key findings are:

1. Fluorescent powders are effective markers, and allow the separation of released LGB from wild beetles even when trapping involves a liquid preservative (propylene glycol).

2. Storgard® LGB pheromone dispensers decline to 50% attraction capacity in around 4-5 days under summer/autumn conditions in southern NSW.

3. Quantitative releases of marked LGB showed that a large number of beetles (1.4%) can be recaptured at 1 km using a ring of eight traps fitted with pheromone dispensers, and that the maximum dispersal distance is likely to be substantially higher.

4. Alternating colours of fluorescent marking powders are required to discriminate between LGB from different release events conducted at weekly intervals, as marked beetles can persist in the release area for more than a week.

Studies on potential alternative host plants showed that;

1. LGB can develop from egg to adult on the fruit or seeds of several non-grain plant hosts, but adult production is much lower than on grains, development takes longer, and the adults may be smaller in size, which has implications for their dispersal and reproductive capacity. A faster, more labour-effective protocol for host plant screening has been developed that will facilitate future work.

2. Of the materials tested, native plant species either did not support LGB development, or were very poor hosts. The best non-grain hosts were introduced ornamental species, however further work is needed before broad generalisations can be made.

Field sampling of potential alternative host plants has been inconclusive. Only a small number of samples have been assessed so far, and no LGB have been recovered. One specimen of *Cryptolestes* sp., another serious stored grain pest, was recovered from fruit of the Canary Island date palm *Phoenix canariensis.*

The results of this project will primarily be of benefit to researchers developing more comprehensive studies on these aspects of LGB biology.

2. Aims and objectives

Grain and oilseed production form a major component of the Australian agricultural sector, with a gross value of production of 12.68 billion dollars in 2011/12. Wheat accounted for 7.6 billion dollars of this total, with wheat exports worth 6.1 billion dollars to the Australian economy (ABARES, 2012). Market access for wheat exported from Australia is dependent on freedom from live insects and freedom from chemical residues, and these criteria have been met through the use of grain fumigants, particularly methyl bromide and phosphine. With the withdrawal of methyl bromide due to its ozone-depleting effects, the grains industry has become increasingly reliant on phosphine, however strains of several key pests, particularly the lesser grain borer (LGB), *Rhyzopertha dominica*, and the flat grain beetle, *Cryptolestes* sp., have developed resistance to phosphine. This threatens market access, and research to overcome the problem is being conducted in several areas, including the development of new disinfestation techniques and new fumigants, reducing the rate of pest infestations through better grain hygiene, and improving grain handling guidelines based on a better understanding of pest ecology, particularly outside the storage environment.

Reducing the incidence of infestations will reduce the need to fumigate, effectively prolonging the life of phosphine while alternative disinfestation techniques are developed. Ensuring continuing market access for exports of wheat and other grains will provide direct economic benefits for farmers, grain handlers, and export authorities, and will provide flow-on economic benefits for all Australians.

Understanding the ecology of LGB in the field has the potential to lead to the development of pest management strategies that will help minimise the incidence of infestations in stored grain (Campbell *et al*., 2010; Mahroof *et al*., 2010), and two key aspects of LGB ecology that are receiving increasing attention are dispersal and potential alternative food sources. Studies in the USA are substantially more advanced than in Australia, and because of differences in our agricultural landscapes and native flora, conclusions drawn from overseas studies, particularly in regard to alternative food sources, cannot be directly transferred to our farming systems. For example, research in the USA has shown that acorns (*Quercus* spp.) support the development of LGB in the laboratory (Wright *et al*., 1990; Edde & Phillips, 2006; Jia *et al*., 2008) and LGB have been recovered from field collected acorns (Jia *et al*., 2008). This suggests that fallen acorns may act as reservoirs for LGB in American farming landscapes and this may to some extent account for higher captures of LGB in forested areas relative to open farmland. In Australia, trap captures of LGB have also been reported to be higher in areas of remnant native vegetation (Daglish, 2010), however *Quercus* spp. are only grown as ornamental trees in Australia, and if LGB are using non-grain hosts in Australian agricultural areas, then other host species must be involved.

This 9½ month project, supported by 'turbo' funding from the CRCNPB, was initiated in August 2011 and concluded in May 2012. The overarching aim of the project was to develop basic knowledge about how LGB persist and disperse outside the storage environment, with a view towards incorporating this ecological information into grain handling guidelines designed to reduce infestation risk. Specifically, the project aimed to:

- Quantify the dispersal capacity of LGB in SE Australian agricultural landscapes.
- Screen potential non-grain food sources for their capacity to support LGB development in the laboratory, including weeds and native plant seeds.
- Collect field samples of plant materials identified as supporting LGB development in the laboratory and determine whether they contain LGB.

3. Key findings

3.1 LGB dispersal studies

3.1.1 Marking techniques for LGB

Dispersal studies conducted as part of this project were based on standard mark/release/recapture techniques using commercial LGB aggregation pheromone dispensers. Two pilot studies were conducted to validate this approach; the first involved testing the techniques we proposed to use to mark laboratory-reared LGB prior to release, whilst the second looked at how long the pheromone dispensers retained their attractiveness to LGB under prevailing field conditions.

Our first experiment was to assess the effects of marking techniques on short-term survival of LGB. All LGB used were laboratory-reared in wheat at 30±1.5°C with a 15L:9D photoperiod and approximately 65% RH. Initial trials involved placing 20 LGB adults in tubes with a range of powdered microscopy dyes (brilliant blue G, methyl orange, Congo red, bromocresol green, bromocresol purple, etc, 0.15g of each) or marking individual beetles with 'glitter pens', white paint markers, or Liquid Paper® and placing them in kibbled wheat for 24 hours at 30±1.5°C. All of the microscopy dyes failed to adhere to the beetles, with none being detectable after 24 hours. Markings with 'glitter pens' also tended to fall off the beetles once they had dried, however both white paint markers and Liquid Paper® adhered well to the beetles. Studies with fluorescent marking powders (the type used for sterile fruit fly marking) were much more successful and in our detailed study two marking techniques were evaluated, dusting with fluorescent marking powder and (for field validation of fluorescent powder marking) painting a white dot on the pronotum of individual beetles using a white paint marker (Artline® 440XF, 1.2mm). Preliminary studies showed that 100% of beetles marked with either pink fluorescent marking powder or white paint pen retained their markings after four days in 100% propylene glycol under field conditions.

Four treatments were examined: an unmarked control, unmarked beetles subjected to simulated paint marking and associated handling but without any paint being applied, beetles marked on the pronotum with a white paint pen, and beetles dusted with pink fluorescent powder (Swada FEX Astral Pink, DIC International Australia Pty Ltd, 30-32 Kilkenny Court, Dandenong South, Victoria). There were four replicates of each treatment, each involving 25 LGB of between 14 and 18 days adult age. After treatment each group of 25 LGB were placed in a plastic cup with a piece of filter paper and kibbled wheat and maintained at 30±1.5°C with a 15L:9D photoperiod and approximately 65% RH for five days, when survival was assessed. Proportional mortality data was inverse-sine transformed prior to one-way ANOVA, with Tukey's HSD test used to separate means. Results are shown in Figure 1. Average mortality after five days varied from 2 to 11%, with the 'simulated' painting treatment showing significantly ($P<0.05$) lower mortality than all other treatments. Reasons for this treatment providing lower mortality than in the unhandled controls remain unclear, however there were no differences between the control and paint pen or pink powder treatments, indicating these approaches to LGB marking will have minimal impact on short-term beetle survival.

To test the durability of pink fluorescent powder marking under field conditions we set up four Lindgren funnel traps (four unit, Pherotech International, Delta, BC, Canada) at equidistant points on the perimeter of a 200 m diameter circle. Each trap was attached to a steel post hammered into the ground, with the top of each trap approximately 1.8m from ground level. LGB pheromone dispensers (Storgard® 3158, Tréce Incorporated, Salinas, CA, USA, Batch 55772299) were attached to each trap using metal clips and approximately 100mL of propylene glycol was placed in each collection container as a preservative/killing agent.

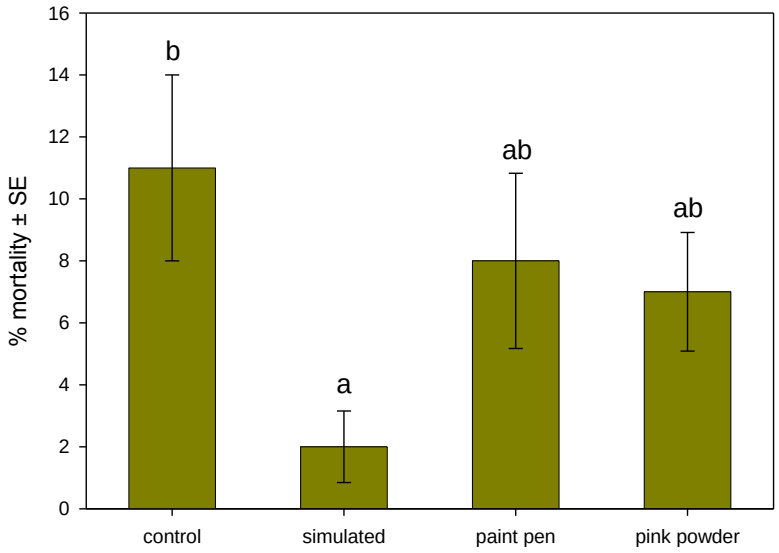

Figure 1. Survival of LGB adults five days after marking. Bars with different letters are significantly (*P*<0.05) different. ANOVA, inverse-sine transformed data, Tukey's HSD test.

Laboratory-reared LGB adults (*n*=390) of mixed ages were individually marked on the pronotum with a white paint pen and subsequently dusted with pink fluorescent marking powder. They were then released from the centre of the trap circle. Traps were checked at 24 and 48 hours and all captured beetles were returned to the laboratory for microscopic examination.

During the first 24 hours 125 LGB were captured, seven of which had paint markings. No further paint-marked beetles were captured at 48 hours. All captured LGB with paint markings also retained large quantities of the pink marking powder (figure 2). None of the LGB without paint markings (*i.e.*, 'wild' beetles) showed any evidence of the powder.

Figure 2. LGB adults recovered from Lindgren funnel traps 100m from their release point viewed under blue light, showing retained pink fluorescent powder particles as well as their white validation markings.

The results of these experiments demonstrate that:

- Marking LGB with pink fluorescent powder has no significant impact on short term survival.

- Pink fluorescent powder is retained on the bodies of LGB in good quantities during short range aerial dispersal.

- Pink fluorescent powder is retained on the bodies of LGB when they are captured into propylene glycol.

- Any powder particles lost in the propylene glycol do not adhere to 'wild' beetles and thus influence the accuracy of recovery estimates.

Marking with fluorescent powders is, as a consequence, considered as an appropriate approach to use in LGB mark/release/recapture studies, although the behavioural impact of the dye powders does warrant more detailed investigation.

3.1.2 Residual efficacy of Storgard® LGB pheromone dispensers in the field

The effective field life of pheromone dispensers used in ecological studies is a critical factor in experimental design and can be particularly important in studies where efforts are being made to correlate insect activity with factors such as temperature and rainfall. This is because pheromone volatilisation from dispensers (particularly impregnated rubber septa) declines over time, so in a weekly sampling period without dispenser renewal the differential level of attraction over time can lead to difficulties in result interpretation. For example, it will prove difficult to correlate weekly trap captures with weekly weather conditions if the pheromone dispenser has effectively 'run dry' well before the end of the sampling interval. Despite this fact, little work has been done to evaluate how the efficacy of commercially available pheromone dispensers declines over time, or to develop an understanding of how this needs to be taken into account in experimental work.

To investigate the residual life of LGB pheromone dispensers, we set up two Lindgren funnel traps on steel posts (as for the previous experiment) approximately 25m apart in a paddock outside Leeton, NSW. Propylene glycol was placed in the collection containers and LGB pheromone dispensers (Storgard® 3158, Tréce Incorporated, Salinas, CA, USA, Batch 59112231) were fitted to each trap. One trap was designated as 'fixed' and the initial pheromone dispenser was left in place for 10 days; the other as 'renewed' and had the pheromone dispenser replaced daily. LGB were removed from each trap daily and counted. After 10 days both pheromone dispensers were removed and several days were allowed to pass before the next replicate was established, with the trap designations reversed. Efficacy of the pheromone dispenser on the 'fixed' trap was calculated on a percentage basis relative to captures in the 'renewed' trap on each sampling day. Due to high overall daily variation in captures it was necessary to censor the resulting data, discarding data points where <10 LGB were captured in the 'renew' trap on any individual day. Preliminary results are summarised in Figure 3.

A total of 1,628 LGB were trapped during the study. Maximum daily air temperatures during the first five days of each trapping cycle averaged 28.6^{o}C (range 20.5 – 35.3^{o}C). An analysis of the data currently on hand indicates that the capacity of Storgard® LGB pheromone dispensers to attract beetles declines over time and that after approximately four to five days potency has declined to around 50%, relative to a new dispenser. There are, however, limitations with our data set as it now stands, most notably at one day after establishment. At this point, the paired dispensers were of the same age and theoretically the three points in Figure 3 should all be around 100%, however only one is. Had all three points been at approximately this level the pattern of declining catches over time would have more closely followed an exponential decay curve, rather than a straight line. Fitting a non-linear regression to the data as it now stands provides little improvement over the linear regression shown in Figure 3 in regard to goodness-of-fit.

There are two possible explanations for why the points one day after initiation of each replicate show so much variation. It is possible that there are fine spatial differences in LGB dispersal

patterns, however this is considered unlikely because the replicates that returned 101% and 40% were both conducted using the same trap allocations to the two treatments, and also because the traps were in such close proximity to each other. A more disturbing possibility is that there may be considerable variation in the pheromone load on individual dispensers and this could have significant impacts on ecological studies in which these dispensers are used. Further replication of the experiment is necessary and this will determine whether there is a need to conduct chemical analyses of the variation in pheromone content between individual dispensers.

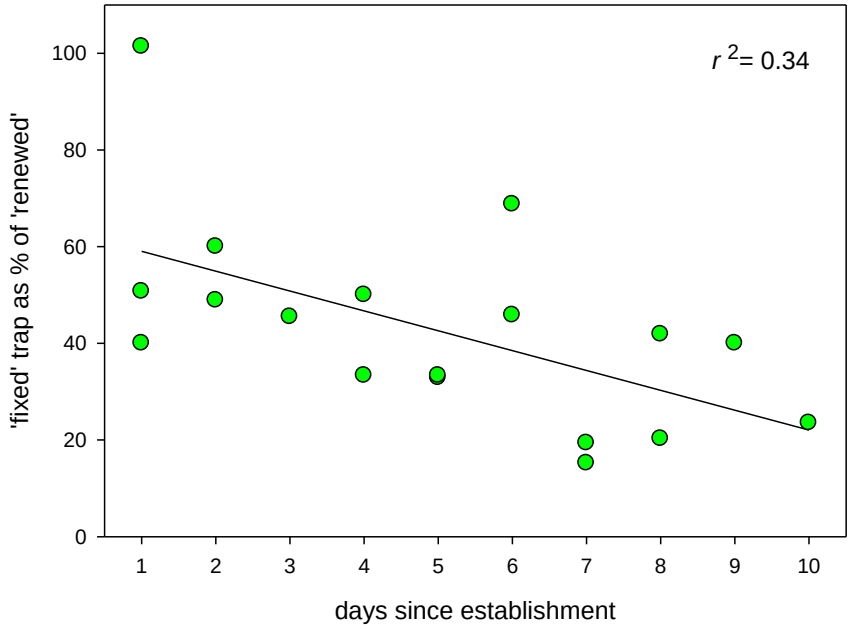

Figure 3. LGB adults recovered from a Lindgren funnel trap where the pheromone dispensers were allowed to age for 10 days, relative to an adjacent trap where the dispensers were renewed daily.

The weather conditions that occurred during this experiment could be considered to be reasonably representative of those expected to occur during summer/autumn in southern Australia (the three trapping cycles were run between February and April 2012). Our results have several important implications for research work using LGB pheromone dispensers:

- Under representative field conditions in southern Australia the attraction of LGB to Storgard® pheromone dispensers declines to around 50% after 4-5 days. Experimental work designed to correlate weather to dispersal activity will therefore be compromised unless the dispensers are replaced more frequently (every 1-2 days). A one week trapping period without dispenser renewal will result in catch data strongly biased towards the conditions prevailing during the initial part of the sampling period when the pheromone release rate is presumably highest.

- Although further work is needed, it would be unwise to assume that the pheromone load of individual dispensers is consistent until this has been proven through chemical analysis.

3.1.3 Dispersal of marked LGB adults over open farmland

An initial study was conducted at Yanco Agricultural Institute (34°37'S, 146°26'E) in southern NSW to evaluate the short-range dispersal of LGB at different distances. To do this, we prepared a

release/recapture site in sparsely wooded mixed farmland with a view to creating concentric circles of 4-unit Lindgren funnel traps set on steel posts at 100, 200, 300, 400 and 500m from a common central release point. The traps were placed equidistantly along the perimeter of each circle, so there would be four traps at 100m, eight traps at 200m, 12 traps at 300m and so on, but with only one circle of traps being operated at each release event. Due to the time constraints of the project, however, only circles up to 300m radius were established (by GPS), and flooding in early 2012 meant that only 100 and 200m radius trap circles could be used in this project. The objective was to collect sufficient data to model dispersal rates across different distances and use this model to predict longer range dispersal.

Because we wished to determine the dispersal of LGB quantitatively, we designed a 'release station' to allow LGB to initiate flight by climbing vertically to the top of small posts. Initial experiments showed that some materials were unsuitable for use in release stations, for example Styrofoam® coffee cups could not be used because significant numbers of LGB would actually burrow into the Styrofoam® rather than disperse. It was also important to avoid designs which had lots of cracks or crevices, because the beetles would enter the cracks and stay there, rather than initiate flight. The final design of the release station is shown in Figure 4.

An inverted plastic jar was inserted through a hole cut in the underside of a standard PostPak® mailing box and all edges on the box were sealed with packing tape. Four waxed paper cups were then glued to the top of the box and post assemblies were prepared using ice cream sticks obtained from a craft shop. The post assemblies were then glued to the inside base of each cup, and when the glue had dried, gap-filler was carefully smoothed around the base of the post and along the inner and outer bottom edges of each cap to remove any crevices. A raised edge was also constructed around the upper box margins to discourage any beetles climbing out of the cups from falling to the ground. The completed release station could be mounted onto a fence post or wooden stake by inserting the top of the post into the jar opening on the underside.

Figure 4. LGB 'release station' developed for quantitative studies on LGB dispersal. LGB adults marked with gold fluorescent powder can be seen climbing the wooden posts in the centre of each cup.

LGB used in this study were laboratory-reared in wheat at 30±1.5^{o}C with a 15L:9D photoperiod at approximately 65% RH. The standard protocol involved placing single Storgard® LGB pheromone dispensers (Batch 59112231) on each trap in the circle and adding 100mL of propylene glycol to the

capture container. Laboratory reared LGB (*n*=2,500) of mixed age and sex were then dusted with pink fluorescent powder (Swada FEX Astral Pink) and placed in the release station. The release station was recovered on day 3 and the remaining LGB were counted; the traps were emptied and the pheromone dispensers were removed on day 5, and the following replicate was initiated two days later. Recoveries were calculated as percentages of the number of beetles that had actively dispersed from the release point. This program was initiated on 11 November 2011.

After three replicates at 100m with gradually increasing recovery percentages, we became suspicious that there may have been 'carry-over' of LGB from one release event still being captured in subsequent replications, so we suspended further releases, but continued the trapping cycle for another three weeks. Results of this investigation are shown in Figure 5.

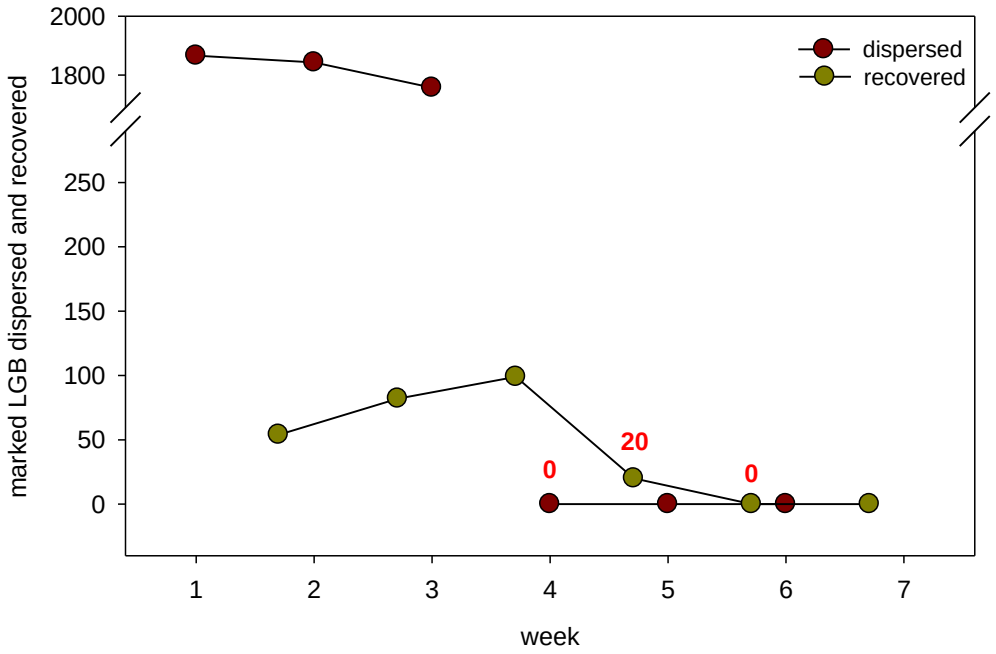

Figure 5. LGB dispersal and recovery during initial six weeks of mark/release/recapture trials with traps at 100 metres radius. Despite no releases in week 4, marked LGB were still recovered up to five days later. Red numbers are absolute values for those data points.

When releases were suspended after week 3, but traps established in week 4 as normal, a further 20 marked LGB were captured, however no further marked beetles were captured in weeks 5 and 6. This demonstrates that carryover does occur from consecutive releases made at weekly intervals, and this carryover could have serious impacts on the interpretation of trap catches in quantitative studies.

To overcome this issue we constructed additional release stations for use with different coloured fluorescent dye powders and alternated dye colours (Swada FEX Astral Pink, Arc Chrome and Stellar Green, DIC International Australia) on a three week cycle for the rest of the study. Due to time constraints in this study it was only feasible to conduct a further six replications, three at 100m and three at 200m. Recovery data is summarised in Figure 6.

Average recovery rates dropped only slightly between 100 and 200m, from 1.62 to 1.50%. Although the data is at present extremely limited, we investigated factors that could account for data variability, but found no correlation between % recovery of released beetles and catch of wild

beetles, % dispersal from the release station, maximum air temperature in the first three days after release, or maximum wind speed during that period.

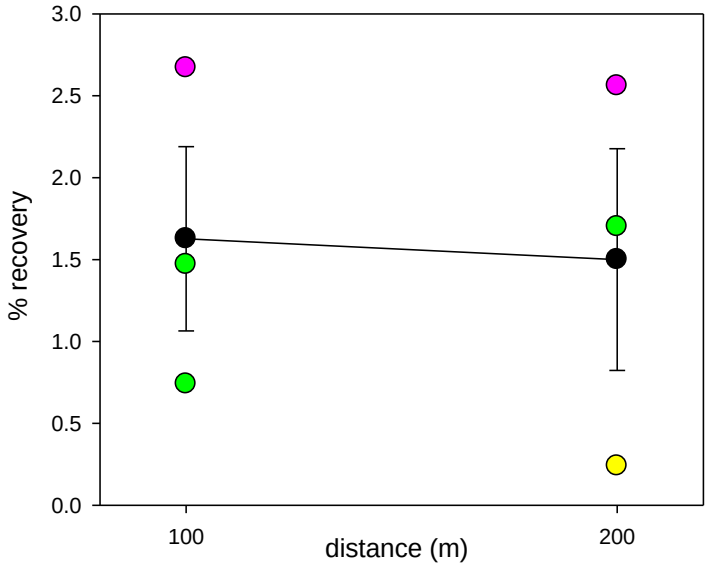

Figure 6. Marked LGB dispersal and recovery at 100m (four traps) and 200m (eight traps) from a central release point in open farm land. Symbol colour indicates colour of fluorescent marking powder used in that replicate. Black points are means ± standard error.

There was also no correlation between % dispersal and catch of wild beetles. More data is needed, particularly at greater trap distances, to facilitate a more meaningful analysis. The apparent differences in % recovery associated with the use of different marking powder colours is particularly interesting, however more data is needed before conclusions can be reached about the possible effects of different coloured marking powders on LGB behaviour. During this short-range dispersal study 22,500 marked beetles were placed in the release stations; of these 18,236 (81%) dispersed and of these 458 (2.5%) were recovered. A further 5,163 wild (unmarked) LGB were captured.

To evaluate the capacity of LGB adults for medium-range dispersal, we established a ring of eight Lindgren funnel traps (4-unit on steel posts, as described previously in section 3.1.1) equidistantly along the perimeter of a 1 kilometre radius circle at Yanco Agricultural Institute. The site was a mixture of flat and gently undulating hill country with mixed farming activities and sparse irregular remnant trees, predominantly eucalypts. The trap positions were initially plotted using Google Earth® and trap positions were identified on-ground using a handheld GPS system. Trap distances from the central release point were confirmed using measurement algorithms provided by Geoscience Australia (2011). Trap positions and the location of the central release point are shown in Figure 7.

LGB used in this mark/release/recapture study were laboratory-reared in wheat at 30±1.5^{o}C with a 15L:9D photoperiod and approximately 65% RH. Four quantitative releases were made at the central release point between 13 and 21 February 2012 involving a total of 10,000 LGB adults of mixed age and sex, all dusted with pink fluorescent marking powder. Two days after each release of 2,000 to 3,000 beetles the release station was recovered and the remaining beetles counted – of the 10,000 beetles placed in the release station 5,690 actively dispersed (56.9%). Dispersal rates from the four releases ranged from 9.4 to 84.5%. Single Storgard® LGB pheromone dispensers

(Batch 59112231) were placed on each trap immediately prior to the first release and propylene glycol was added to the collection containers as a preservative. Traps were emptied and dispensers replaced at least every four days during the trial and the traps were operated for a further 14 days after the final release event.

Figure 7. Trap arrangement for medium-range dispersal tests of LGB adults at Yanco Agricultural Institute.

A total of 3,855 beetles were recovered from the traps, of which 3,775 were wild beetles and 80 were marked. This represents a recovery rate of 1.41%, which is higher than we initially anticipated and indicates that LGB have the capacity to move long distances over Australian farming landscapes. Reasons for the high variability in departure rates from the release station remain unclear, however there was a weak but statistically non-significant ($P>0.05$) positive correlation between the departure percentage of marked LGB from the release station and the captures of wild (unmarked) LGB in the traps when they were next emptied. This suggests that the factors which stimulate the dispersal of wild LGB in the field may be exerting a similar influence on the laboratory-reared LGB placed in the release station, provided pheromone sources are not in close proximity to the release point as they were in the short-range dispersal study.

The recovery data we obtained in this medium-range dispersal study shows close parallels with studies conducted in the USA. Ching'oma (2006) used a circular grid of 46 Lindgren funnel traps at distances of 50 to 1,000m from a central release point, and also recovered 1.4% of released beetles, however the differing geometry of his trapping grid and the fact that all trap radii were operated simultaneously indicates the similarity in our recovery percentages is largely coincidental, and if anything our data suggests higher dispersal capabilities because we had no operational traps at distances below 1,000m to contribute to the total recovery percentage. Of 62,400 marked LGB released by Ching'oma (2006), three were recovered from traps at 3,600m from the release site, indicating that when populations are high enough a very small number of beetles are capable of dispersing much further than 1,000m. Mahroof *et al*. (2010) recovered LGB at 1,600m from a central release point, however releases made in her study were not quantitative (marking powder was mixed with infested grain placed at the release point), so the number of beetles dispersing from the release point is unknown and recovery percentages cannot be determined, other than relative to the different trap distances.

The results of our dispersal studies show that:

- In quantitative experiments where it is necessary to discriminate between LGB captured from separate release events (e.g. our short-range dispersal study) and releases are made at short time intervals, alternating colours of fluorescent marking powder must be used to identify beetles from individual releases because of the persistence of marked beetles in the environment.

- Significant numbers of LGB can disperse at least 1km across Australian agricultural landscapes. We recovered 1.4% of released LGB in our medium-range study and this is likely to be an underestimate of the proportion actually dispersing over this distance because we can assume not all LGB dispersing this far were intercepted in the traps. Based on our high recovery percentage and overseas data, a proportion of released beetles would be likely to have dispersed considerably further, especially if not intercepted by the 1km trap grid.

3.2 LGB host plant studies

3.2.1 Development of LGB on grains and potential alternate host plants

Trapping studies conducted as part of Project CRC50089 (Daglish, 2010) showed that LGB adults were more commonly trapped in remnant native vegetation than in cereal fields or adjacent to on-farm storages. Similar observations have been made in the USA (Campbell *et al*., 2010), and whilst LGB have significant dispersal capacity by flight (see previous section), these observations indicate that LGB may be surviving, and possibly reproducing, on non-grain hosts in the field (Jia *et al*., 2008). This may have a significant impact on how LGB disperse and how to best manage phosphine resistance. Field populations on non-grain hosts may act as reservoirs for susceptible or resistant LGB and may act as a source of new infestations in storages – new infestations may not be arising directly from LGB emigrating from infested grain bulks. This hypothesis is supported by studies in the USA which show that deciduous woodlands, rather than grain storages, have a dominant role in driving LGB activity (Campbell *et al*., 2010). If LGB are using alternate food sources in the field, removing these food sources from around storages may be an effective way to reduce infestation risk.

In the first part of this study, we looked in detail at the development of LGB on potential alternative food sources and compared adult production and adult size (as a measure of fitness) to LGB reared on wheat, maize and sorghum.

LGB were subcultured from wheat into jars containing rolled oats supplemented with 5% wholemeal wheat flour, as this medium was found to facilitate the easy removal of adult beetles, allowing adults of known age range to be removed at subsequent intervals. The cultures were maintained at 30±1.5^{o}C and approximately 65% RH with a 15L:9D photoperiod. LGB adults 0 to 4 days old were removed from the jars and sexed using the technique described by Crombie (1941). This is considered the only reliable method of sexing adult LGB, but it can have adverse effects on the beetles (Sinclair 1981), so we maintained the sexed beetles overnight with a small quantity of coarsely ground wheat, and any beetles that showed reduced levels of activity were discarded.

Six plant materials were compared in each of the three experiments, three standards (wheat, sorghum, and maize) common to all experiments, and nine test materials, three of which were used in each experiment. Bread wheat (*Triticum aestivum*) and maize (*Zea mays*) were obtained from commercial farms in the Yanco area of southern New South Wales, whilst grain sorghum (*Sorghum bicolor*) was obtained from a commercial farm south-west of Dalby, Queensland. The test materials were seeds of Cootamundra wattle (*Acacia baileyana*), seeds of knifeleaf wattle (*Acacia cultriformis*)

and seeds of kurrajong (*Brachychiton populneus*) in Experiment 1, Canary Island date palm fruit (*Phoenix canariensis*), dried orange peel (*Citrus sinensis*), and dried olive fruit (*Olea europaea*) in Experiment 2, and English oak acorns (*Quercus robur*), dried flowers and seeds of capeweed (*Arctotheca calendula*) and seeds of Flinders Range wattle (*Acacia iteaphylla*) in Experiment 3. All of these plants are common in the mixed farming area around Leeton, NSW, with the exception of *Q.robur* which was included in the study to determine whether our results are consistent with studies conducted in the USA. *Quercus* sp. acorns are known to be viable hosts for LGB in American farming landscapes (Jia *et al*., 2008).

A common protocol was used for the preparation and exposure of plant materials to LGB, although in some instances this had to be varied to accommodate particular characteristics of some samples. Plant samples were exposed to LGB in 60mL capacity screw-top glass jars of approximately 37mm internal diameter. The lids were drilled with a 26mm diameter hole and filter papers were cut to fit inside the lids to allow air exchange. For wheat, sorghum and the three *Acacia* species, 15g of whole seeds were placed in test jars and then a further 7g of the same material was added after being finely ground using a Wiley Mill (Arthur H. Thomas Co. Scientific Apparatus, Philadelphia) fitted with a size 20 screen (20 holes per inch). With maize and kurrajong the protocol was essentially the same, however the 15g of kernels were split manually prior to addition of the corresponding ground material. Acorns, olives, citrus peel and date fruit were cut into small sections and air dried on stainless steel trays, before addition (15g) to the jars along with 7g of finely ground material. The high oil content of the olive fruit meant that it had to be manually cut into fine pieces, rather than ground. Capeweed flowers and seeds were not ground, but were coarsely crushed by hand.

Once prepared, the unlidded containers containing plant materials were placed in a desiccator above saturated NaCl at 30±1.5°C to allow moisture equilibration overnight at approximately 75% RH (Winston and Bates, 1960). Fifteen adult LGB (10 ♀, 5 ♂) no more than four days old were then added to each jar. The lids were placed on the jars and each replicate (six jars in total) was placed on a stainless steel tray and maintained at 30±1.5°C and approximately 65% RH with a 15L:9D photoperiod. Parent beetles were removed by hand three weeks later and survival rates were recorded. Routine weekly monitoring for new adult emergence commenced immediately, however no F1 adults were recovered until 2-3 weeks after the parent beetles were removed. Once emergence commenced, monitoring and removal of adult beetles from the samples continued at weekly intervals for a further five weeks, followed by a final removal of adults at nine weeks after the first appearance of adults. LGB removed during this final period were sexed and adult size was assessed by taking a digital photograph at fixed magnification of each individual in dorsal view using a Canon Powershot® S45 camera mounted on a Wild M7S stereomicroscope. Calibration images were also taken and ImageTool 3.0 software (UTHSCSA, 2002) was then used to measure maximum pronotal width in pixels prior to conversion to millimetres using the calibration images. The final sampling period was chosen for the comparison of adult sizes because the majority of LGB arising from the non-grain test materials emerged during this period.

This protocol does not reflect the overall maximum production that may arise from a given food source, since the ongoing removal of LGB during the course of the experiment reduces reproductive capacity in the system, assuming food volumes are not limiting. We chose to use this approach because in addition to obtaining a relative measure of the capacity of the food materials to support LGB development, we also wished to determine the time taken for the first adults to emerge from different food sources to facilitate the design of faster and more efficient protocols for host plant testing and also segregate groups of LGB at a fixed period after emergence began for size measurement. Because of potential handling effects and the risk of damaging LGB whilst searching through the plant material, we chose not to return the recovered adults to the jars because to then portray the final numbers of LGB recorded as being representative of an undisturbed system would have been potentially misleading.

Preliminary data analyses were conducted on total cumulative LGB recoveries nine weeks after emergence began, and on the size of adults recovered during the final sampling interval. Where necessary data were square-root transformed prior to ANOVA to conform to the assumption of variance homogeneity; where this transformation failed, ANOVA results were considered to be significant at $P<0.01$ rather than at the standard level of $P<0.05$. For adult size, females and males were analysed separately. No size analyses were conducted for Experiment 1 because only two LGB were recovered from a non-grain host during the final sampling interval. Analyses in Experiments 2 and three were restricted to comparing the grain hosts and *P.canariensis* and the grain hosts and *Q.robur* respectively because too few LGB were recovered from other non-grain hosts to allow valid comparisons. In some instances LGB were only recovered from two or three of the four experimental replicates. Results of the study are summarised in Table 1. A total of 8,457 LGB were reared through to adult emergence on the various host materials.

No LGB emergence was recorded from *Acacia cultriformis* seeds, *Brachychiton populneus* seeds (Experiment 1), *Citrus sinensis* rind or *Olea europaea* fruit (Experiment 2). All other non-grain hosts yielded at least some LGB, and could be grouped into two categories – moderate host capacity (*P.canariensis* fruit, *Q.robur* acorns) and marginal host capacity (*Acacia baileyana* and *A.iteaphylla* seeds and *Actotheca calendula* seeds/flowers). In terms of LGB production from grains, the results of the three experiments were extremely consistent, with wheat and sorghum producing the most adults and maize always producing significantly ($P<0.05$) less, although the times to first adult detection were largely consistent across all grain types. In Experiment 2 there was a close relationship between adult recoveries and adult size, with adults from maize being significantly smaller than those from wheat and sorghum, and adults from *P.canariensis* being significantly smaller than adults from any of the grains. In Experiment 3 the results were less clear-cut, with females from maize and *Q.robur* being no different in size to those from either wheat or sorghum. In Experiment 3 differences in male adult size were not statistically significant. Materials with moderate host capacity (*P.canariensis*, *Q.robur*) yielded less than half the adults of maize, and it took longer for the first adults to emerge. Marginal hosts yielded <10 LGB on average per replicate, and not all replicates produced adult beetles.

Table 1. LGB recoveries from different grain and non-grain food sources previously exposed to LGB adults under laboratory conditions.

Host	Material*	Total LGB recovered $\bar{x}$ (SE)	# of replicates producing LGB	Days to first emergence detection** $\bar{x}$ (range)	♀ pronotal width $\bar{x}$ (SE), mm	♂ pronotal width $\bar{x}$ (SE), mm
			Experiment 1			
Wheat	S	237 (19.9) [a]	4	42	NM	NM
Sorghum	S	247 (7.4) [a]	4	42	NM	NM
Maize	S	158 (10.6) [b]	4	42	NM	NM
A.baileyana	S	4 (1.3) [c]	3	60 (48 – 77)	NM	NM
A.cultriformis	S	0	0	-	-	-
B.populneus	S	0	0	-	-	-
			Experiment 2			
Wheat	S	211 (19.4) [a]	4	44 (42 – 49)	0.792 (0.004) [a]	0.797 (0.004) [a]
Sorghum	S	234 (13.1) [a]	4	47 (42 – 49)	0.793 (0.004) [a]	0.799 (0.005) [a]
Maize	S	124 (3.2) [b]	4	47 (42 – 49)	0.768 (0.006) [b]	0.767 (0.007) [b]
P.canariensis	F	55 (7.2) [c]	4	66 (63 – 70)	0.741 (0.004) [c]	0.739 (0.005) [c]
C.sinensis	F (rind)	0	0	-	-	-
O.europaea	F	0	0	-	-	-
			Experiment 3			
Wheat	S	301 (38.1) [a]	4	45 (42 – 49)	0.806 (0.003) [a]	0.809 (0.003)[NS]
Sorghum	S	365 (25.0) [a]	4	44 (35 - 49)	0.790 (0.003) [b]	0.805 (0.003)[NS]
Maize	S	122 (3.8) [b]	4	44 (35 - 49)	0.792 (0.006) [ab]	0.795 (0.003)[NS]
Q.robur	Acorns	47 (2.0) [c]	4	53 (42 – 63)	0.810 (0.010) [ab]	0.840 (0.016)[NS]
A.iteaphylla	S	5 (2.8) [d]	3	61 (49 – 70)	NM	NM
Ar.calendula	S/FL	7 (3.9) [d]	2	49 (42 – 56)	NM	NM

* S = seeds, F = fruit, FL = flowers. ** after initial addition of parent LGB. NM, not measured due to inadequate sample sizes. Within each experiment numbers in the same column with different letters are significantly different ($P<0.05$, ANOVA, Tukey's HSD test, or $P<0.01$ where transformation failed to correct variance heterogeneity).

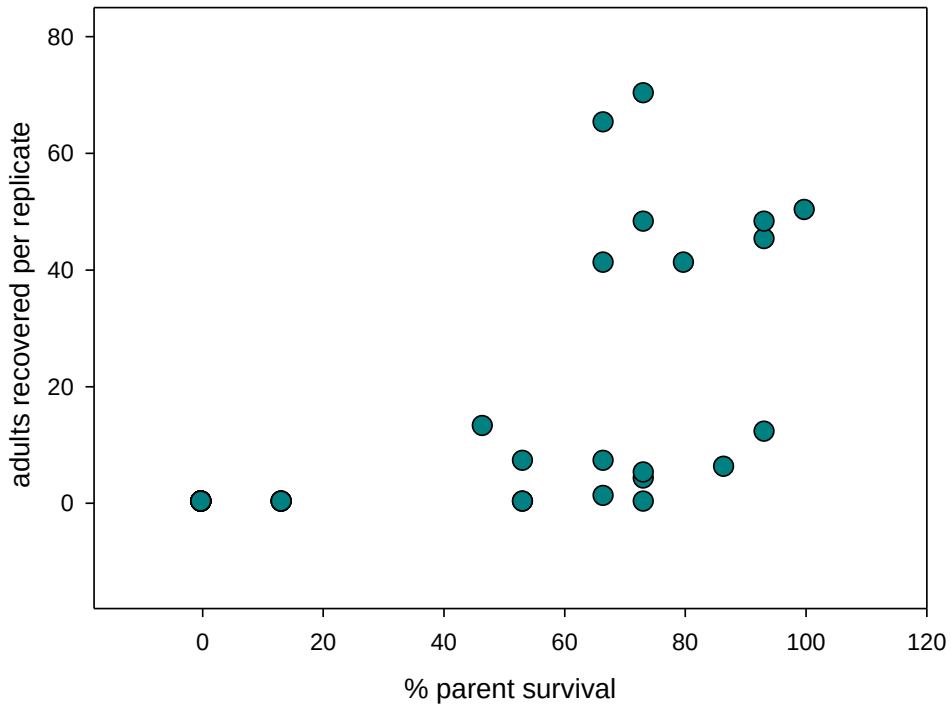

Figure 8. Scatter plot showing the relationship between the survival of parent LGB during the oviposition period and subsequent adult recoveries from non-grain test materials (data from individual replicates).

The relationship between LGB recoveries and the survival of parent beetles during the oviposition period on non-grain test materials is shown in Figure 8. Although parental survival below 20% resulted in no adults being produced, at higher rates of survival the relationship is not well defined; a test replicate with parental survival of 47% led to F1 adults, however other replicates with parental survival up to 73% did not produce adults at all.

This study, although preliminary, has yielded some important information relevant to the design of a faster-throughput protocol that is currently being developed and tested:

- A single grain standard (either wheat or sorghum because of their greater productivity relative to maize) should be sufficient in routine LGB host plant screening.
- Our exposure period to adults of known age, sex and number is appropriate to provide good levels of initial oviposition.
- A total exposure period of 15 weeks under our conditions and without routine removal of adults will provide an effective test of host development potential, since LGB reared on even the most marginal hosts emerged within 11 weeks.
- Although the simplified protocol will speed sample throughput, replication is still necessary to identify materials with marginal host potential, as not all replicates will necessarily produce adult LGB.
- Although there is a relationship between the survival of parent beetles and subsequent development success, that relationship is too weak to allow parent beetle survival to be used alone as an indicator of host potential.

In one instance a non-grain host (*P.canariensis*) with moderate host potential produced adults with significantly reduced adult size relative to those that developed on grain hosts. Since adult size can correlate to fitness, this may reflect reduced dispersal and reproductive capacity.

A further significant outcome from this study is that the Australian native plants tested either did not support LGB development, or had very poor host potential. Substantial further screening is needed before any generalisations can be made about the relative host potential of native and introduced plant species.

3.2.2 Use of non-grain host plants by LGB in the field

Laboratory testing of potential host plants can identify whether plant materials can support development of LGB through its complete life cycle and therefore whether these plants have the potential to be acting as reservoirs for LGB in the field, possibly facilitating dispersal between grain storages. This testing does not, however, demonstrate that plant materials identified as having host potential are actually being used as hosts in the field.

In an effort to identify alternate host plant utilisation by LGB in the field, we collected plant samples from *Acacia baileyana* (litter, seed pods) and *Phoenix canariensis* (fallen damaged fruit) from the Yanco area, southern NSW. The samples were initially sieved to collect any active adult insects, and then placed in glass jars with filter paper lid inserts in a controlled temperature room set at 25 ± 1^{o}C and maintained at approximately 65% RH. After eight weeks the samples were sieved again and then transferred to Tullgren funnels (Figure 9) for 48 hours under 100 watt bulbs for further insect extraction. Plant samples were then weighed to determine dry weights and the recovered insects were counted and identified.

Figure 9. Tullgren funnels used for extracting insects from plant samples. Tests with wheat spiked with LGB adults demonstrated high levels of recovery over 48 hours.

To date 440g dry weigh of *A.baileyana* litter and pods have been processed and have returned 195 insects and other invertebrates (excluding psocids), none of which have been recognised stored product pests. Of 243g dry weight of *P.canariensis* fruit processed so far, over 800 insects have been recovered, almost all of which have been adults and larvae of the palm seed borer, *Coccotrypes carpophagus* (Hornung) (Coleoptera: Curculionidae: Scolytinae). One specimen of *Cryptolestes* sp. has also been recovered from *P.canariensis*. Extraction and identification work is continuing.

4. Implications for stakeholders

Due to the short duration of this project, the principal benefits from it will be derived by researchers developing more in-depth studies on dispersal and resource use by LGB and other stored grain pests. Many of the findings reported here are too preliminary in nature to result in recommendations to industry on immediate practice change. However, if further data supports the theory that native plants are unsuitable or very poor hosts for LGB and that introduced plant species are being used as hosts by LGB in the field, then there may be a real opportunity to reduce infestation risk in commercial storages through selective management of introduced plant species in the surrounding areas.

The results of this project demonstrate that LGB have strong dispersal capacity across Australian agricultural landscapes which parallels that found in overseas studies, with recovery of 1.4% of released beetles in a ring of eight pheromone traps 1km from the release point. The maximum dispersal distance remains unknown and there would be considerable merit in repeating the work with a 2km radius trap circle and also evaluating dispersal potential across more densely vegetated landscapes. Overseas studies suggest that recoveries at 2km would still occur, although at reduced rates, and that the maximum dispersal distance may be between 3 and 4 kilometres. If this holds true, then ensuring that major grain storages have no smaller on-farm storages within 4km would help to mitigate infestation risk, provided alternative food sources in the field are not acting as reservoirs for LGB.

This study has shown that some native and introduced plant species found in Australian agricultural landscapes have the potential to support LGB development in the field. The best potential hosts identified so far have been introduced ornamental species, with native plant materials either being inadequate or marginal in host potential. The development of an effective, less labour-intensive testing method for LGB host suitability is one of the major outcomes of this project. Application of this approach (using a single grain standard and end-point assessment only at 15 weeks) will enable greater sample throughput in future research projects, and by extending this work to other stored grain pests using a 'reference set' of potential host materials, we can also get a better idea of the *relative* breadth of potential host suitability across different pest species. This may be a particularly valuable approach, given that it is not feasible to assess all (or even most) potential host materials across the different grain producing regions.

Work on field samples is yet to demonstrate that food materials found suitable for LGB in the laboratory are actually being used by LGB in the field, with no LGB and only one specimen of *Cryptolestes* recovered from field samples to date. This section of the investigation needs to be continued before conclusions can be made about what is occurring in the field.

The validation of fluorescent powder marking conducted in this study demonstrates the utility of this technique for LGB dispersal studies. The recognition that LGB released during mark/release/recapture studies can survive in the release area long enough to 'carry-over' to subsequent replicates has important implications for future work, with rotational use of different coloured marking powders being necessary to discriminate between beetles from different release events when replication is being attempted at short intervals. Although further work on the residual efficacy of commercial pheromone lures is needed, the preliminary finding that attraction declines to around 50% in 4-5 days under summer/autumn conditions has particular importance for future studies to be conducted under the auspices of the Plant Biosecurity CRC. Trapping studies designed to correlate LGB flight activity with weather conditions will need to have pheromone dispensers renewed at much shorter intervals than previously thought to prevent LGB captures being strongly skewed towards the initial part of each sampling period.

5. Recommendations

This research has led to development of some preliminary insights into dispersal and potential alternative hosts of LGB in Australian agricultural systems, but more importantly it has led to the development of techniques for use in subsequent studies. Marking techniques have been validated, quantitative methods of LGB release have been devised and tested, a less labour-intensive protocol for host plant testing has been developed and preliminary data on the residual life of LGB pheromone dispensers has been generated. The short time frame of this project (9½ months), coupled with the fact that LGB mark/release/recapture studies in southern NSW are only viable from November through to March, has meant that many of these techniques have only been applied to understanding the biology of LGB in the field in a relatively preliminary way. To gain the maximum benefits from this project the techniques developed here need to be used in future research projects, with a focus on the following areas:

- Quantitative mark/release/recapture studies at increased distances (2, 3 and possibly 4km from the release point), and parallel studies in more heavily wooded landscapes.
- Use of the modified host plant testing protocol across a wide range of potential food sources.
- Ongoing efforts to isolate LGB and other stored grain insects from field-collected plant material known to be capable of supporting LGB development.

6. Acknowledgments

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7. Publications arising

Stevens, M. and Warren, N. 2012. The secret lives of grain beetles. IREC Farmers' Newsletter (Large Area) 186, 22-25.

8. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
CSIRO	Commonwealth Scientific and Industrial Research Organisation
LGB	Lesser grain borer
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
RH	Relative humidity
SE	Standard error

^{o}C	Degrees Celsius
$\bar{x}$	Mean (average)

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10. Plain English website summary

CRC project no:	CRC50188
Project title:	Dispersal and resource use by the lesser grain borer (*Rhyzopertha dominica*) in southern New South Wales
Project leader:	Dr Mark M Stevens
Project team:	Dr Mark M Stevens, Mr Glen N Warren
Research outcomes:	This project looked at two aspects of the biology of lesser grain borer, dispersal ability and potential use of food sources other than stored grains. We showed that fluorescent powders are effective for marking beetles used in field studies and that alternating colours are necessary to discriminate between beetles released at weekly intervals, as individuals from previous releases can persist in the local environment for 7-14 days. Recovery of marked beetles from pheromone traps 1 km from the release point was 1.4%, showing this pest has strong dispersal ability over open agricultural landscapes. Host plant studies showed that lesser grain borers can develop on a range of other plants, however most native plants tested were either unsuitable or very poor hosts. The best alternative hosts were introduced ornamental species. Development of beetles on non-grain hosts takes longer and the adult beetles may also be smaller. So far no lesser grain borers have been recovered from field samples of plants known to support development of this pest in the laboratory.
Research implications:	If further data supports the theory that native plants are unsuitable or very poor hosts for lesser grain borers, and that introduced plant species are being used by lesser grain borers in the field, then there may be a real opportunity to reduce infestation risk in commercial storages through selective management of introduced plant species in the surrounding areas. The results of this project demonstrate that lesser grain borers have strong dispersal capacity in Australian agricultural landscapes, with close parallels to the situation in the USA. The maximum dispersal distance remains unknown and there would be considerable merit in repeating the work over greater distances and also evaluating dispersal potential across more densely vegetated landscapes. Overseas studies suggest that the maximum dispersal distance for lesser grain borers may be 2-4km. If this holds true, then ensuring that major grain storages have no smaller on-farm storages within 4km would help to mitigate infestation risk, provided alternative food sources in the field are not acting as reservoirs for the beetles.
Research publications:	Stevens, M. and Warren, N. 2012. The secret lives of grain beetles. *IREC Farmers' Newsletter (Large Area)* 186, 22-25.

Acknowledgements:	The authors thank Tom Weir and Rolf Oberpreiler, Australian National Insect Collection, CSIRO, Canberra, for the identification of *Coccotrypes carpophagus.* Joe Valenzisi, Farm Manager, Yanco Agricultural Institute, and his staff are thanked for working around the large number of insect traps that have been set up all over the Institute. Bernie Dominiak and Laura Jiang, NSW DPI, provided the fluorescent marking powders used in the dispersal studies.