final report

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Prepared by: Dr Bernard Doube
Dung Beetle Solutions International Pty Ltd

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Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

The release, and monitoring the establishment of, Onthophagus vacca and Bubas bubalus in high-care field rearing environment in southern Australia

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Abstract

B.ERM.0213 imported *Onthophagus vacca* and *Bubas bubalus* from Europe to fill a gap in dung beetle activity in early spring across southern Australia. B.ERM.0216 involved mass rearing and releasing those beetles (December 2016 to December 2017). Here B.ERM.1102 (September 2017 to February 2018) reports activities complementary to those carried out within B.ERM.0216.

The first large-scale field release of *O. vacca* in Australia (1,100 beetles, 19 September 2017) occurred at Manjimup, Western Australia. A release and monitoring protocol guided activities. The beetles bred and F1 beetles were recovered.

Adult *O. vacca* were provided to the Western Australia breeding hub on 22 September 2017 (n=119), 4 October (n=1,600) and 18 October (n=370). Adult *B. bubalus* were provided to the WA breeding hub on 18 October (n=185). Adult *O. vacca* (n=2,453) were released in field nurseries in Armidale, New South Wales, in early November, where breeding occurred.

The currently available data that could be used to define gaps in the distribution of introduced and native dung beetles in southern Australia were examined in principle and five complementary avenues for assessing the gaps in dung beetle activity were recognised. Each of these has been examined and recommendations for further action provided.

Three manuscripts reporting the biology and benefits of dung beetles were drafted.

Executive summary

Project B.ERM.0213 imported *Onthophagus vacca* and *Bubas bubalus* from Europe to fill a gap in dung beetle activity in early spring across southern Australia. The beetles were reared by CSIRO and in spring 2014 a small number of each species was released to high-care field nurseries in three locations in South Australia. This work was taken forward in Project B.ERM.0216 (1 December 2016 to 1 December 2017 inclusive), which covered the activities undertaken from February 2016 to November 2017, including a period of interim support from MLA for the mass rearing program. This document is the final report for Project B.ERM.1102, which covers the activities undertaken during the period 19 September 2017 to 15 February 2018.

Project B.ERM.1102 involves releasing the spring-active dung beetle *O. vacca* to one field location in Western Australia and to mass rearing facilities in New South Wales, and *O. vacca* and *B. bubalus* reared under Project B.ERM.0216 to breeding facilities in WA. The eight objectives detailed in the contract for the final report for B.ERM.1102 have been met and are reported here.

The rationale for initiating the project was, in brief, that multiple agricultural, environmental and social benefits are expected to arise from the widespread establishment across southern Australia of the two new spring-active species. These include biological control of spring breeding in the bush fly, improved agricultural production in pasture ecosystems, reduced fertiliser use, biological control of gut parasites of domestic livestock, and improved water quality.

At the beginning of the reporting period (19 September 2017), the imported dung beetles *O. vacca* and *B. bubalus* were emerging in the field nurseries at Strathalbyn (Project B.ERM.0216) and these needed to be distributed to the field and to mass rearing facilities in WA and NSW.

The first large-scale field release of *O. vacca* (1,100 adult F0 beetles) in Australia occurred at Manjimup, WA on 19 September 2017. A release and monitoring protocol for the two new spring-active species has been drafted and circulated for comment and is being used to guide the field releases and monitoring of *O. vacca* in WA. A small number of F1 adult *O. vacca* were recovered in late December 2017, suggesting that the beetle has bred successfully in the field in WA. Adult *O. vacca* were provided to the WA breeding hub on 22 September (n=119), 4 October (n=1,600) and 18 October (n=370). Adult *B. bubalus* were provided to the WA breeding hub on 18 October (n=185).

Three thousand *O. vacca* to be supplied to Armidale were harvested at Strathalbyn on 12 October 2017, ready for dispatch. Unfortunately, the nursery at Armidale was not complete at that time and so the beetles were stored and fed at Strathalbyn in tubs of soil with dung. A total of 2,700 beetles was recovered on 26 October and dispatched to Armidale, where on 27 October 2,453 live, healthy beetles were recovered following sieving of the carrier material. These were stored in tubs of soil with dung for 2 weeks and then placed in their field nursery in early November, where they resumed feeding and possibly breeding.

Two additional tasks were undertaken to summarise the currently available data that could be used to define gaps in the distribution of introduced and native dung beetles in southern Australia, AND prepare draft manuscripts reporting the biology and benefits of dung beetles. Five complementary avenues for assessing the gaps in dung beetle activity were recognised. Each of these has been briefly examined and recommendations for further action provided. The avenues are:

- anecdotal accounts from dung beetle workers with long experience in the field
- evaluating the published beetle trapping data

- evaluating the currently available unpublished beetle trapping data
- establishing a monitoring program at selected sites
- conducting local surveys at times of peak abundance to identify gaps due to the influence of local edaphic factors such as soil type, vegetation cover and water logging.

The second task was to prepare draft manuscripts reporting the biology and benefits of dung beetles. Three draft manuscripts are titled:

- The impact of dung burial by the dung beetle *Bubas bison* (L.) on phosphate levels in two contrasting subsoils on the Fleurieu Peninsula, South Australia
- Dispersal of the dung beetle *Bubas bison* (Linnaeus, 1767) (Coleoptera: Scarabaeidae) throughout the Adelaide–Mount Lofty region, South Australia, between 2002 and 2015
- Field evidence for the presence of a facultative adult reproductive diapause in the introduced European dung beetle *Bubas bison* in southern Australia.

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1 Background

Project B.ERM.0213 imported *Onthophagus vacca* and *Bubas bubalus* from Europe to fill a gap in dung beetle activity in early spring across southern Australia. The beetles were reared by CSIRO and in spring 2014 a small number of each species was released to high-care field nurseries in three locations in South Australia. This work was supported by Project B.ERM.0214, the final report of which was submitted on 22 March 2016. Interim support for the mass rearing program (February 2016 to January 2017) was provided by MLA. This work was taken forward in Project B.ERM.0216, the outcomes of which have been reported and cover the activities undertaken over the period February 2016 to November 2017 (revised final report submitted 15 January 2018).

The rationale for initiating the beetle importation project has been fully described in the final reports for Project B.ERM.0213 and Project B.ERM.0214. In brief, multiple agricultural, environmental and social benefits are expected to arise from the widespread establishment across southern Australia of the two new spring-active species. These include biological control of spring breeding in the bush fly, improved agricultural production in pasture ecosystems, reduced fertiliser use, biological control of gut parasites of domestic livestock, and improved water quality.

At the beginning of the reporting period (19 September 2017), the imported dung beetles *O. vacca* and *B. bubalus* were emerging in the field nurseries at Strathalbyn (Project B.ERM.0216) and needed to be distributed to the field and to mass rearing facilities in WA and NSW.

The eight project objectives detailed in the contract have been met and are reported here.

2 Project objectives

By 15 January 2018:

- draft, and obtain and agreement with release partners, a release and monitoring protocol
- identify suitable co-operators for release of *O. vacca* in WA in spring 2017
- release additional *O. vacca* and *B. bubalus* beetles reared at Strathalbyn to Armidale and Western Australia
- inform and demonstrate monitoring actions and reporting requirements with co-operator - for a successful release
- package and release a known number of beetles to targeted sites
- undertake 1 monitoring action at the site
- identify available published and nonpublished monitoring data on species distribution, abundance and activity collected prior to 2018 and include in a report to be provided to MLA at the end of this project
- write in a form suitable for publication in a refereed journal research results obtained through previous activities quantifying the benefits of dung beetles to Australian agriculture and society.

3 Methodology

3.1 Maintenance and feeding

Project ERM.0216 involved rearing *O. vacca* and *B. bubalus* in large field nurseries over the period 1 December 2016 to 1 December 2017 and releasing the spring-emerged progeny to the field and to mass rearing facilities in NSW and WA. The final report for Project ERM.0216 has been submitted. Both projects relied on the same rearing facilities.

Newly emerged *O. vacca* adults were fed in the field nurseries from December 2016 to April 2017. At that time all of the beetles had ceased feeding and had entered a non-feeding, non-breeding diapause. It is likely that a proportion of these beetles bred for a time. Two new, large (3 m x 9 m) tunnel nurseries were constructed (one for each species) (Figure 1).

The dung collection, beetle feeding and activity monitoring have continued and follow the procedures detailed in Project ERM.0214 and Project ERM.0216.

Figure 1: MLA tunnel nurseries constructed for the two new species

All adult *B. bubalus* had died by February 2017 and so the *B. bubalus* nursery was not provided with dung during autumn or early winter.

Monitoring of the spring emergence began for both species in July 2017. Newly emerged beetles were counted and transferred to new nurseries. A detailed account of the breeding activities is provided in the final report for Project ERM.2016.

Creation Care (CC), Strathalbyn, were unable to continue maintaining and mass rearing *O. vacca* and *B. bubalus* beyond the contract for Project ERM.0216, so an arrangement was made to have the beetles vacate the nurseries in Strathalbyn by late September.

3.2 Field release and mass rearing program

A program for the release of the MLA *O. vacca* and *B. bubalus* to field situations in Western Australia, South Australia, Tasmania, Victoria and New South Wales was developed and approved but only one field release occurred. This was undertaken at the property at Manjimup, WA on 19 September 2017. Bernard Doube delivered the beetles in person and supervised the release. The release and monitoring protocol was discussed and agreed, including monitoring actions and reporting requirements.

The remaining beetles were released for mass rearing within the RR&DfP program at another site in WA (2,089 *O. vacca* and 185 *B. bubalus*) and Armidale (2,453 *O. vacca*). Delivery of beetles to WA and NSW was done in accordance with the quarantine regulations pertaining in each state. Beetles were dispatched in 5-litre plastic containers with small beetle-proof breather holes punched in the lid. The beetles were placed in the container with a supply of moist washed sand and the lid secured in place with tape.

Mass rearing of MLA *O. vacca* and *B. bubalus* will not continue at Strathalbyn beyond the term of Project B.ERM.0216. There were no remaining MLA *O. vacca* or *B. bubalus* at Strathalbyn at the time of writing this report.

4 Results

The objectives that are addressed in each section of the results are indicated in a box at the head of each section.

4.1 Release and monitoring protocols

Draft, and obtain and agreement with release partners, a release and monitoring protocol.

A release and monitoring protocol for the two new spring-active species has been drafted (Appendix 1). The protocol was used to guide the release and monitoring of the starter colony of MLA *O. vacca* in WA.

As indicated in the final report for ERM.0216, a program for the release of the MLA *O. vacca* and *B. bubalus* to field situations across southern Australia in September 2017 was developed and approved. However, only one field release occurred.

The MLA *O. vacca* at Strathalbyn had been destined for field releases but on Wednesday 27 September 2017, DBSI put on hold the field release program. A revised plan to mass rear beetles at Armidale, NSW and Mandurah, WA was quickly developed. New field nurseries were commissioned at Armidale and new nurseries were activated in WA to receive the spring-active beetles, which would be harvested and delivered from Strathalbyn.

4.2 Co-operators for release of *O. vacca* in WA in spring 2017

Identify suitable co-operators for release of *O. vacca* in WA in spring 2017.
Inform and demonstrate monitoring actions and reporting requirements with co-operator – for a successful release.
Undertake 1 monitoring action at the site.

The *O. vacca* for field release were sorted and counted at Strathalbyn on 18 September and taken by plane to WA by Bernard Doube: they were released to the field on 19 September. The beetles were healthy and active at the time of release (Figure 2). The release was organised through Warren Catchments Council.

Figure 2: O. vacca just prior to release

On 17 December 2017, it was reported that 1100 *Onthophagus vacca* beetles were released into 'south' paddock at junction of 'bull' and 'dam' paddocks on 19 September 2017.

All beetles released were very active and disappeared into the dung pats they were released onto quickly. There were no dead beetles remaining on top of dung pats. The cattle on the farm have had no veterinary chemicals applied in their lives. The dung is free of any chemical residue. The release paddock and the two adjoining paddocks have a heavy vertisol loam soil with a grass / legume pasture which is legume dominant. Today, 17 December 2017, the pasture in these three paddocks is still lush and green providing very high quality dung. The cattle herd has rotated around these three paddocks since the release date, keeping fresh dung available to the beetles continuously.

The F0 beetles have been sought in pads surrounding the release site. They have spread from the central release point and have been observed in adjacent paddocks. They appeared to be breeding soon after release. Monitoring has been conducted and beetles recovered in the traps. On 18 December 2017, it was reported that beetles in the weeks post release could easily be found in dung

in all three paddocks up to a radius of approximately 100 meters from the site of original release. When found the beetles were always frenetically burrowing, pushing up soil.

Monitoring for the emergence of the next generation has commenced. The beginning of this emergence was noted and the presence of newly emerged beetles (no tibial wear) was observed in the field in late December 2017. This suggests that a successful first generation in the field has been achieved in WA.

4.3 Release of *O. vacca* and *B. bubalus* for mass rearing in NSW and WA

Release additional *O. vacca* and *B. bubalus* beetles reared at Creation Care to Armidale, NSW and to Western Australia.

Package and release a known number of beetles to targeted sites.

4.3.1 O. vacca

Beetles were provided to the WA breeding hub on 22 September, 4 October and 18 October. An account of the supply of dung beetles to the Western Australia breeding hub is provided in Table 1.

Table 1: The supply of dung beetles to the Western Australia breeding hub in spring 2017

Consignment #/ To	Notes 1	Species	Number received	Date received	Notes 2
1: B. Doube	Spring Cohort 3 dead in transit	*O. vacca*	119	20 September 2017	In washed river sand B. Doube delivered. Held overnight for collection .
2: Creation Care	Spring Cohort 12 dead in transit	*O. vacca*	1600	4 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth.
3: Creation Care	Autumn Cohort 8 dead in transit	*O. vacca*	370	18 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth.
3 Creation Care consignment	Aug. 2017 eclosion 5 dead in transit	*B. bubalus*	185	18 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth.

Three thousand *O. vacca* to be supplied to Armidale were harvested at Strathalbyn on 12 October 2017, ready for dispatch. Unfortunately, the nursery at Armidale was not complete at that time and so the beetles were stored for a time at Strathalbyn in tubs of soil with dung. The beetles were sieved from their holding tubs on 26 October (2,700 recovered) and dispatched to Armidale, where on 27 October 2,453 live, healthy beetles were recovered following sieving of the carrier material.

They were maintained in plastic tubs for two weeks at Armidale until the construction of the nursery was completed (Figure 4), whereupon they were introduced to the nursery. At Armidale, the beetles were fed with fresh spring dung which was frozen (to kill contaminating organisms) and then thawed and fed to the beetles. The beetles were introduced to the Armidale nursery in early November and began actively burying dung: they were presumed to be breeding.

Figure 4: The nursery at Armidale

The mass rearing of *O. vacca* at Armidale was transferred to Charles Sturt University. The transfer of beetles occurred by firstly trapping the next generation of newly emerged adult beetles as they arrived at the soil surface in the field nursery. Since egg to adult takes about 6–8 weeks in warm weather, it seemed reasonable to expect that the new generation of *O. vacca* might begin emerging in January–February 2018 following a November breeding event. We recommended that beetles be either trapped at that time and transferred to nurseries at CSU or maintained at Armidale until spring 2018.

The spring 2017 emergence of *B. bubalus* began in August 2017 and continued for about 6 weeks. Most newly emerged beetles were trapped, counted and transferred to a new nursery, where they were provided with a regular supply of fresh dung.

The overall pattern of emergence in 2017 was considerably extended, compared with that observed in 2016 (Figures 5 and 6). The numbers of beetles that emerged were also considerably lower than expected. All the trappable *B. bubalus* (N=185, Table 1) were transferred to WA on 18 October 2017. *B. bubalus* began burying dung soon after arrival and is considered to be breeding in the WA field nursery.

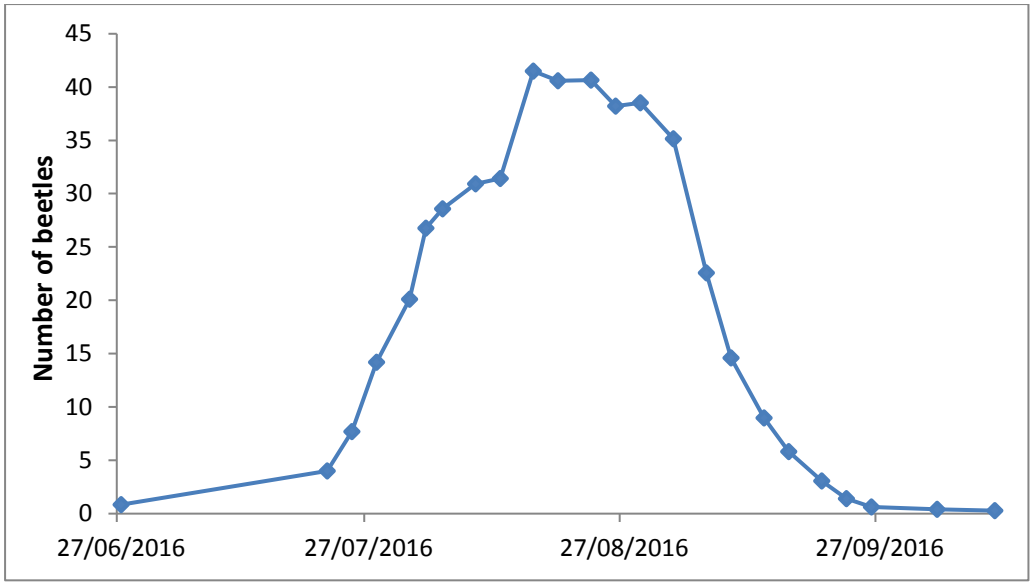

Figure 5: ***B. bubalus*** **emergence patterns in late winter – spring 2016. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 3 to 5 days.**

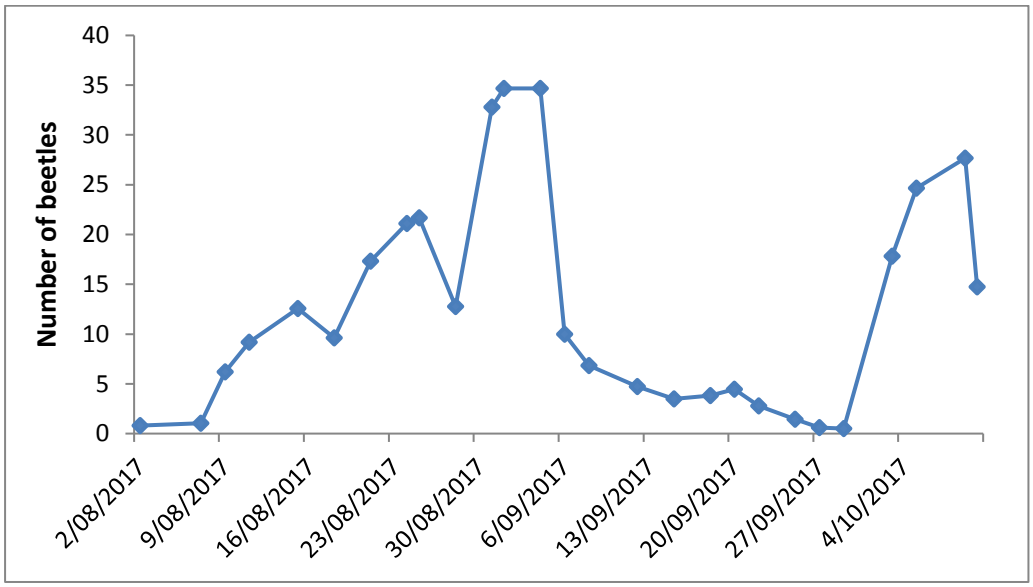

Figure 6: ***B. bubalus*** **emergence in spring 2017. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 1 to 5 days.**

4.4 Published and unpublished dung beetle distributions and activity data

Identify available published and nonpublished monitoring data on species distribution, abundance and activity collected prior to 2018.

Collecting and collating available pre-2018 monitoring data on species distribution, abundance and activity of introduced dung beetles in southern Australia will enable the identification of gaps in distribution and activity, information that can be used to inform release locations for newly imported dung beetle species or species currently available but which have not reached the natural limits of their distribution.

There are five avenues for assessing the gaps in dung beetle activity:

- anecdotal accounts from dung beetle workers with long experience in the field
- evaluating the published beetle trapping data
- evaluating the currently available unpublished beetle trapping data
- establishing a monitoring program at selected sites
- conducting local surveys at times of peak abundance to identify gaps due to the influence of local edaphic factors such as soil type, vegetation cover and water logging.

I strongly recommend that a working party be established to collate and analyse these data, and then to report on the revealed gaps in dung beetle species distribution.

4.4.1 Anecdotal accounts of dung beetle workers with long experience

A number of dung beetle workers in many regions of Australia have had long experience in the field and have gathered important anecdotal evidence about the distribution, abundance and activity of their local dung beetle fauna. These individuals have been approached and asked to provide an account of where they see the major gaps in the current dung beetle fauna.

4.4.2 Evaluating the published data

There is a substantial literature on the activity and impact of dung beetles in Australia, primarily published through CSIRO. In this literature there are numerous references to beetle abundance and where they occurred. Much of this information is not known to the current suite of dung beetle workers, and needs to be carefully reviewed and summarised.

4.4.3 Evaluating the currently unpublished beetle trap data

Over the past decade or so, Landcare and other organisations, as well as private landholders, have initiated and carried out dung beetle trapping programs aimed at identifying the gaps in dung beetle distribution and activity in their region. In addition, there have been numbers of government-agency dung beetle surveys aimed at identifying those same gaps. The Lucyvale Better Beef Group Dung Beetle Resource Kit[1] was produced in 2008 was a substantial step forward in assembling some of these data. The data from the Resource Kit surveys have been provided to and incorporated in the Atlas of Living Australia, but occurrences are represented only by presence or absence. None of these data have been collated and analysed to identify gaps in the Australia dung beetle fauna: they need proper scrutiny.

This subproject should focus on the winter- and even-rainfall regions. The work of Dr Penny Edwards provides a very useful, but perhaps dated, view of the dung beetle situation in northern Australia, with particular reference to Queensland.

4.4.4 Establishing a monitoring program at selected sites

Once the review of the currently available data is complete, and the limitations of those data are recognised and documented, a targeted monitoring program could be established to rectify the

[1] Dung Beetle Resource Kit, https://www.landcarevic.org.au/groups/northeast/dungbeetle/dung-beetle-resource-kit, viewed 15 January 2018.

regional deficiencies in data on beetle abundance and activity. I recommend that three, not two, traps be set at each monitoring location.

I do not recommend repeating the trapping at locations that have already been assessed for dung beetle activity in recent times, but locations sampled a decade or more ago could well deserve sampling in order to assess whether the populations had stabilised and to monitor new arrivals.

4.4.5 Local surveys at times of peak abundance

Local opportunistic surveys at times of peak dung beetle abundance could identify gaps due to the influence of local edaphic factors such as soil type, vegetation cover and water logging.

Quick and efficient survey methods are available (see the report 'The distribution and abundance of summer-active dung beetles in the Tarago Water Catchment', funded by Melbourne Water, Doube 2004). A substantial region can be evaluated over a 12-hour trapping period, with additional time required for beetle counting, data analysis and reporting.

This type of evaluation of regional gaps in the distribution of local species can readily identify gaps in the distribution of established species and is illustrated by two examples:

- Soil type preferences: The winter-active dung beetle *Bubas bison* prospers in loam and clay but cannot breed successfully in deep sand, although it can breed in regions of shallow sand over clay (refer pp. 103–104 and Figure 10.1 in *Dung down under*[2]). As a consequence *B. bison* is absent from the extensive sandy soil regions of southern Australia.
- Creation Care and DBSI introduced numbers of starter colonies of *B. bison* to the Fleurieu Peninsula, SA, in 2002–2003 (NRM funded). A detailed survey of the distribution and abundance of *B. bison* in this region was undertaken at the time of peak abundance in 2015 (June) (NRM funded). This survey revealed that the species was widespread but there were two major regional gaps in distribution. Establishment will be monitored over 2+ years. These beetle releases are expected to see the Fleurieu Peninsula thoroughly populated by *B. bison*.

5 Discussion

All eight project objectives have been met and the manner in which this has occurred is largely detailed in the results, where the project objectives are presented in boxes and the manner in which each has been met is detailed thereafter.

5.1 Inferences and insights from the data relative to previous research

This project and the allied and complementary ERM.0216 have taken forward our understanding of the mass rearing and biology of both *O. vacca* and *B. bubalus*, with the result that over the course of the two projects the numbers of *O. vacca* in the Strathalbyn nurseries increased from 76 F0 beetles to 16,795 F3 and F4 beetles and the number of *B. bubalus* has increased from about 600 F0 beetles to 2,250 F2 adult beetles plus an unknown number of F2 and F3 larvae underground.

[2] Doube, BM and Marshall, T 2014, *Dung down under: Dung beetles for Australia*, Dung Beetle Solutions Australia, Adelaide.

While this is commendable, we point out that if the generation-to-generation increase had been 20-fold each year, the total emergence in spring 2017 would have been in the order of 570,000 O*. vacca* and 200,000 *B. bubalus*.

With appropriate understanding and control of the factors limiting reproductive performance and larval and adult survival a generation-to-generation increase in the order of 20-fold is considered feasible. For example, for *O. vacca* a 12.6-fold increase from F1 to F2 was achieved and field observations on *Bubas bison* indicate a 16-fold annual increase in the field in the Bool Lagoon populations during the early establishment phase.

The marked contrast between what was achieved and what could have been achieved illustrates the priority need to understand and resolve the constraints on beetle breeding in the nursery environment. We consider the primary factors constraining reproductive performance in the field nurseries to be overcrowding (adult and larval density) and soil moisture/soil type interactions.

The reproductive constrains discussed above apply not only to *O. vacca* and *B. bubalus*, but also to all new species that are to be brought to Australia. If the RR&DfP program is to meet its stated objectives, serious attention must be given to the constraints limiting reproductive success of dung beetles in the laboratory and in field nurseries.

The detailed monitoring conducted at Strathalbyn has identified the possibility of *O. vacca*'s achieving two generations per year. Such a biennial breeding pattern could double the rate of increase of the captive population, to great effect when attempting to increase nursery numbers quickly.

The documentation of the 1-, 2- and 3-year cycles in *B. bubalus* is also important. Understanding of the factors inducing diapause may allow us to avoid the expression of diapause and so cause all *B. bubalus* to adhere to a 1-year cycle, thereby greatly increasing the rate of increase of populations in the nurseries.

5.2 Practical implications for industry

The successful rearing of both species in field nurseries brings closer the field release and widespread establishment of these two spring-active species. Again we emphasise that resolving the mass rearing constraints will allow the benefits to be realised much earlier than currently anticipated. Increasing the generation-to-generation rate of increase from the currently relatively low level (3-fold to 12-fold for *O. vacca* and 1.4-fold to 2.8-fold for *B. bubalus*) to about 20-fold could reduce by a decade the time taken to get these two species established across southern Australia.

Widespread establishment of these two species across southern Australia will bring multiple agricultural, environmental and social benefits. These include biological control of spring breeding in the bush fly, improved agricultural production in pasture ecosystems, reduced fertiliser use, biological control of gut parasites of domestic livestock, and improved water quality. Achieving these benefits a decade earlier will bring a considerable financial bonus to the Australian livestock industry, and the farming and urban communities that are currently troubled by spring-active bush fly populations.

6 Conclusions/recommendations

- Mass adoption of new dung beetles on-farm can be met only if beetles are mass reared for release in controlled environments and not in the field. The field release program was terminated and 2,000-2,500 *O. vacca* were dispatched to mass rearing facilities in WA and NSW. In addition 185 *B. bubalus* were dispatched to WA. None of either species were retained in Strathalbyn.
- Successful mass rearing of both species in field nurseries can generate huge numbers of beetles in a few years with 2,000 beetles becoming 16 million in three years with a 20-fold increase per generation.
- The goal of a 20-fold increase per generation is considered feasible in ideal conditions. But we also conclude that the current breeding technology in the laboratory and field nurseries falls far short of that goal. There are a range of candidate factors that appear to restrain the generation-to-generation increase in numbers.
- Defining ideal conditions for brood production and larval and adult survival will yield major benefits for all new dung beetle species being introduced to Australia.
- We strongly recommend financial support for experimental analysis of mass rearing conditions designed to develop mass rearing protocols that can consistently produce a 20-fold generation-to-generation increase in numbers in field nurseries. The impact of larval and adult crowding and the interactions between soil moisture and soil type on the reproductive performance and survival of both spring-active species of dung beetles deserve special attention.
- We recommend that the biennial reproductive pattern in *O. vacca* be experimentally manipulated with a view to achieving two generations per year.
- We recommend that diapause in the larval *B. bubalus* be examined experimentally with a view to eliminating the expression of diapause in the mass rearing program.
- We conclude that *O. vacca* released in the field under the guidance of the high-care release and monitoring protocol is likely to have established in WA from an inoculum of 1,100 beetles. This bodes well for further releases of *O. vacca* into suitable locations with suitable co-operators.
- We conclude that there is a substantial body of information concerning the current distribution and abundance of introduced dung beetles in Australia that needs to be collated, summarised and synthesised.
- We strongly recommend that a working party be established to collate and analyse these data, and then to report on the revealed gaps in dung beetle species distribution.
- We recommend that *O. vacca* and *B. bubalus* be reared in large numbers and released to field locations across southern Australia, and their field performance monitored.

7 Key messages

- High levels of generation-to-generation increase in beetle numbers in field nurseries is essential if mass adoption of new dung beetles on-farm is to be achieved.
- Resolving the factors that constrain brood production and larval and adult survival will yield major benefits for the success of the current spring-active species and for all dung beetle species being introduced to Australia.
- Widespread establishment of these two species across southern Australia will bring multiple agricultural, environmental and social benefits. These include biological control of spring breeding in the bush fly, improved agricultural production in pasture ecosystems, reduced fertiliser use, biological control of gut parasites of domestic livestock, and improved water quality.
- Defining the gaps in the current distribution and abundance of dung beetles in Australia is an essential pre-requisite for choosing the location of the proposed test sites.

8 Appendix 1: Protocols for release, and monitoring the establishment of, *Onthophagus vacca* and *Bubas bubalus* in high-care field rearing environment in southern Australia

Bernard Doube, DBSI, Adelaide, 9 September 2017

1. Summary

- The 2017 release locations for the two new spring-active dung beetles are detailed.
- Releases will occur in all southern states.
- *Onthophagus vacca* will be released at all 6 sites in September.
- *Bubas bubalus* is expected to be released at only some of the 6 selected sites.
- *B. bubalus* will be released in September–October.
- Release strategies for both species are detailed.
- Monitoring procedures for both species are detailed.
- Monitoring of beetle abundance for both species will occur.
- Monitoring of larval diapause in *B. bubalus* will occur.
- Model data sheets are provided (Appendix 1).
- The field biology of both species is summarised (Appendix 2).

Two new European spring-active dung beetles, *Onthophagus vacca* and *Bubas bubalus*, have recently been introduced to Australia. After being reared in the CSIRO quarantine laboratories some were transitioned to field nurseries at Strathalbyn, South Australia, where they have multiplied to the extent that this spring we will be conducting field releases of *O. vacca* and expect to be conducting field releases of *B. bubalus*. Fewer sites will receive *B. bubalus* and they will be released at some of the same locations as *O. vacca*; that is, both species will be released at some locations.

Fewer than expected *O. vacca* and *B. bubalus* had emerged by early September 2017. Spring 2017 has been cooler than was spring 2016. The emergence of *B. bubalus* commenced about 4 weeks later than in 2016, while that of *O. vacca* occurred at the same time of year as in 2016. The numbers that emerge over the next few weeks will determine the numbers to be released at each site. *O. vacca* are currently being accumulated in small nurseries prior to release. They have begun to breed.

We anticipate releasing *O. vacca* at six locations (Table 1) in September 2017. *B. bubalus* will be released at fewer locations, if at all. *B. bubalus* numbers are not yet determined for spring 2017. *B. bubalus* releases are likely in late September or early October.

Table 1: Release locations for MLA *O. vacca* in spring 2017

State	Region
Western Australia	Manjimup
South Australia	Kangaroo Island
South Australia	Adelaide Hills
Victoria	Mitta Mitta
New South Wales	Armidale
Tasmania	Launceston

This document presents a protocol for release and managing the beetles in high-care field environments and monitoring their increase in abundance over the first three years following release to the field.

The biology of the two species in Europe and Australia is reviewed in Appendix 2, along with my view of the anticipated life cycles of *Onthophagus vacca* and *Bubas bubalus* in the field in southern Australia, and a list of the 'danger points' in their life cycles.

2. Protocol for release, management and monitoring of *O.* vacca

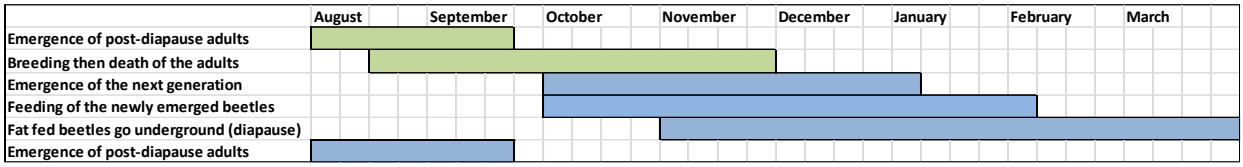

Figure 1: Life cycle of *O. vacca* in the field

Choosing release and monitoring sites

- Choose a release paddock, preferably friable loam or sandy loam.
- Avoid waterlogged soils.
- Cattle must be present in the paddock, or nearby, from the time of release until February the following year in order to provide a continuous source of dung for the beetles. The following situations are appropriate:
    - a relatively small paddock with permeant stocking Aug–Feb, e.g. a bull paddock
    - at the junction of several paddocks that will be occupied Aug–Feb
    - near a watering point for a series of paddocks, e.g. in the middle of a cell-grazing wagon wheel
- Cattle must not be treated with beetle-toxic chemicals (for parasite control) for 6 weeks before the release of beetles and during their subsequent activity period.
- Depending upon rainfall, intermittent irrigation of the paddock in late spring would be useful to maintain dung quality (green grass) and to help maintain soft soil texture.
- Place a few bales of hay at the release site a few days before the dung beetle release date in order to provide a substantial supply of dung for the beetles.
- Spring irrigation may assist by providing high-quality dung and softening the soil.
- Construct a beetle monitoring site near the proposed beetle release site in an area that can be kept free of cattle, e.g. a small fenced area, to contain the dung beetle traps.
- Maintain the grass in the monitoring site at a few cm high or less. Grass can interfere with beetle flight activity. Cutting the grass may be required; poisoning with glyphosate would be acceptable.

Release of beetles

Adult beetles which are ready to breed will be released to the field in August–September.

- Beetles will be delivered by DBSI staff or couriered to the release location.
- Beetles will be delivered in a 5-litre plastic box with damp potting mix or vermiculite.
- Release the beetles into fresh cattle dung in the release paddock and close to the monitoring site.
- On the day of beetle release add 20+ beetles to each dung pad.
- Release all of the beetles in one area close to the monitoring site.

Monitoring abundance

In the long term (3 years) dung beetle trapping will be conducted using pitfall traps.

In the short term (while traps are being built and delivered) an alternative trap will be used.

- **The trap**: At the monitoring site dig a small depression in the soil about 30 cm square by 10 cm deep. Place a piece of weed mat in the depression large enough to cover it, overlapping the depression by a few cm on each side. Fill the depression with sieved sand. Construct three traps in this manner.
- Once the pitfall traps are installed, both types of trap will be set on four occasions to standardise one against the other.
- At weekly (or fortnightly) intervals (I recommend weekly), bait three traps by placing about 1 litre of fresh cattle dung onto the sand in the morning (before 9 am).
- Empty the trap before dusk the same day. (The beetles fly during the day and come to fresh dung.)
- **Emptying the traps**: Before dusk on the day the traps were baited, lift each pad off the sandy surface. Collect, count and record any beetles on the underside of the pad. Sieve the sand and collect, count and record the number of beetles recovered. Check each beetle to ensure that it is *Onthophagus vacca* (black belly, mottled cream wing covers – quite distinct).
- If you are uncertain about the identity of trapped beetles, email a photograph of trapped beetles to Bernard for confirmation of their identity.
- Release the live beetles onto nearby dung pads.
- Data sheets will be provided along with contact details in case of queries.
- After each trapping occasion, email the data sheet to Bernard Doube, who will enter the data in a register. A summary of the progress of sampling across southern Australia will be made available to all participants as the season progresses.
- Continue weekly trapping from August to February.

We anticipate low numbers in September to December, but larger numbers (the next generation) from November to January–February.

Please do not be disheartened by low numbers in the first year. A herd of 50 cattle will produce about 6000 pads over 10 days and so it is not surprising that only a few of those pads will contain

beetles derived from a release of say 1000 beetles in their paddock. Beetle trap data from the year of release is important because it provides a base-line from which we can estimate the populations in subsequent years.

If there is no mortality of the adult beetles, a similar number is expected to be trapped in spring 2018 as were trapped in late spring–summer 2017.

The monitoring must be continued between August and February for three years following the release of the beetles.

3. Protocol for release, management and monitoring of *B. bubalus*

Adult beetles which are ready to breed will be released to the field in August–October.

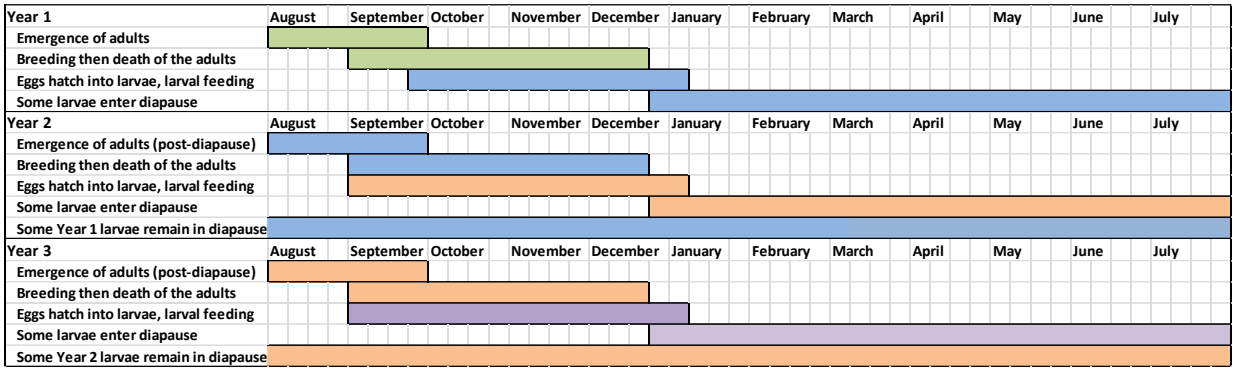

Figure 2: Life cycle of *B. bubalus* in the field

Adult beetles will feed for a few weeks then begin breeding. Eggs will be produced from September until the adult beetles die (November–December in the same year). Eggs hatch into larvae which feed on the buried dung, develop over spring and summer and turn into mature third instar larvae. At this stage they have consumed most of the buried dung (called the brood material) and they then partially empty their guts (intestines) and in so doing produce a capsule (made from gut material) in which they live until they emerge as adults. This is called a faecal shell. Some larvae turn into adults in that first winter and are ready to emerge in spring – a one year cycle. Others remain underground as third instar larvae for a further 12 months – a two year life cycle. Yet others stay underground as third instar larvae for a further 12 months – a three year life cycle. This is termed a larval diapause and can delay emergence for up to 2 years.

The proportion of the population in a 1- or 2- or 3-year life cycle is likely to vary with temperature, with cold conditions inducing and increasing the incidence of diapause. The incidence of diapause in *B. bubalus* will be monitored at each release site (protocol below).

Choosing release and monitoring sites

- Choose a release paddock of friable loam or loam over clay.
- Avoid deep sand.
- Avoid waterlogged soils.
- Avoid irrigation of the paddock in summer.
- Cattle must be present in the paddock, or nearby, from the time of release until December in order to provide a continuous source of dung for the beetles. The following situations are appropriate:

  - a relatively small paddock with permeant stocking Aug–Feb, e.g. a bull paddock
  - at the junction of several paddocks that will be occupied Aug–Feb
  - near a watering point for a series of paddocks, e.g. in the middle of a cell-grazing wagon wheel
- Cattle must not be treated with beetle-toxic chemicals (for parasite control) for 6 weeks before the release of beetles and during their subsequent activity period.
- Place a few bales of hay near the trap site a few days before the dung beetle release date in order to provide a substantial supply of dung for the beetles.
- Construct a beetle monitoring site near the proposed beetle release site in an area that can be kept free of cattle, e.g. a small fenced area, to contain the dung beetle traps.
- Maintain the grass in the monitoring site at a few cm high or less. Grass can interfere with beetle flight activity. Cutting the grass may be required; poisoning with glyphosate would be acceptable.

Release of beetles

- Beetles will be delivered by DBSI staff or couriered to the release location.
- Beetles will be delivered in a 5-litre plastic box with damp potting mix or vermiculite.
- Release the beetles into fresh cattle dung in the release paddock and close to the monitoring site
- On the day of beetle release add 20+ beetles to each dung pad.
- Release all of the beetles in one area close to the monitoring site

Monitoring abundance

In the long term (3 years) dung beetle trapping will be conducted using pit-fall traps.

In the short term (while traps are being built and delivered) an alternative trap will be used.

- **The trap**: At the monitoring site dig a small depression in the soil about 30 cm square by 10 cm deep. Place a piece of weed mat in the depression large enough to cover it, overlapping the depression by a few cm on each side. Fill the depression with sieved sand. Construct three traps in this manner.
- Once the pitfall traps are installed, both types of trap will be set on four occasions to standardise one against the other.
- At weekly (or fortnightly) intervals (I recommend weekly), bait three traps by placing about 1 litre of fresh cattle dung onto the sand in the evening (before dusk)
- Empty the trap following morning. (The beetles fly at dusk and dawn and come to fresh dung.)
- **Emptying the trap**: In the morning of the day after the traps were baited, lift each pad off the sandy surface. Collect, count and record any beetles on the underside of the pad. Sieve the sand and collect, count and record the number of beetles recovered. Check each beetle to ensure that it is *Bubas bubalus* (very similar to *Bubas bison*. Check against reference specimens until you are sure of separating *B. bubalus* from *B. bison*).

- If you are uncertain about the identity of trapped beetles, email a photograph of trapped beetles to Bernard for confirmation of your identity.
- Release the live beetles onto nearby dung pads.
- Data sheets will be provided along with contact details in case of queries.
- After each trapping occasion, email the data sheet to Bernard Doube, who will enter the data in a register. A summary of the progress of sampling across southern Australia will be made available to all participants as the season progresses.

We anticipate low numbers in September to December, but larger numbers (the next generation) in spring the following year.

Please do not be disheartened by low numbers in the first year. A herd of 50 cattle will produce about 6000 pads over 10 days and so it is not surprising that only a few of those pads will contain beetles derived from a release of say 800 beetles in their paddock. Beetle trap data from the year of release is important because it provides a base-line from which we can estimate the populations in subsequent years.

Monitoring larval diapause

The incidence of diapause in *B. bubalus* will be monitored at each release site.

In winter each year the population of *B. bubalus* in the soil will comprise adult beetles (waiting to emerge in spring) and third instar larvae (in diapause): no pre-pupae or pre-adults are expected to be present at that time of year. Each larva or adult will be found enclosed within a faecal shell, most of which will be found 20–40 cm below the soil surface. In order to assess the incidence of diapause, all recovered faecal shells need to be broken open to assess whether larvae or adults are present. The adults can be released but the larvae will die. The proportion the population that remains in the larval stage (inside a faecal shell) is the proportion of the population that has entered diapause.

- At the time of beetle release, set up six soil cores about 0.5 m apart in the beetle monitoring site. Establish the soil cores in the following way:
    - Dig a hole in the ground 50 cm deep by about 25 cm in diameter.
    - Place a double mesh bag (supplied) in the hole and back-fill the bag with the excavated soil. Compact the soil on a number of occasions while filling the bag.
- Add five pairs of *B. bubalus* to the surface of each soil core.
- Add 5 litres of fresh dung on top of the beetles in each core.
- Tie off the mesh bag securely.
- Assess the incidence of diapause as follows:
    - About 10 months after setting up the cores (and after one year and 10 months) (i.e. in June–July), remove three soil cores from the soil.
    - Cut open the mesh bags enclosing the soil.
    - Break open the soil core and recover and count and record the faecal shells (each containing an adult or a larva) in each core. Most will be located at the base of the core.

- Break open each faecal shell and count and record the number of adult beetles and third instar larvae: no pre-pupae or pre-adults are expected to be present.
- In the second year repeat the above process.
- On the second occasion there will again be faecal shells (each containing an adult or a larva) in each core. but there will also be a number of empty faecal shells (vacated by adults during the previous year). These may be somewhat decomposed but, if possible, count and record the number of empty faecal shells.

The monitoring will continue for three years following the release of the beetles.

BM Doube Cave Ave, Bridgewater SA 5155, 9 September 2017

Appendix 1: Model data sheets

Data recording sheet for monitoring diapause of *B. bubalus*

Set-up date			
Date of first excavation (~ 10 m after set-up)			
	No. faecal shells	**No. larvae**	**No. adults**
Core 1			
Core 2			
Core 3			
Date of second excavation (~ 1 year and 10 m after set-up)			
	No. faecal shells	**No. larvae**	**No. adults**
Core 4			
Core 5			
Core 6			

Data recording sheet for monitoring abundance in the field

Date	***Onthophagus vacca***		***Bubas bubalus***	
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				
Date				
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				
Date				
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				
Date				
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				
Date				
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				
Date				
	No. under pad	**No. in sand**	**No. under pad**	**No. in sand**
Trap 1				
Trap 2				
Trap 3				