final report

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Rearing Onthophagus vacca and Bubas bubalus for release in southern Australia

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Abstract

Project B.ERM.0213 imported the spring-active dung beetles *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. Small numbers were released to high-care field nurseries in South Australia in spring 2014 (Project B.ERM.0214). Projects B.ERM.0216 and B.ERM.1102 deal with the transfer of these species to the field and to mass rearing facilities in WA and NSW.

Newly emerged *O. vacca* adults were fed in the field nurseries from December 2016 to April 2017, when they stopped feeding and entered diapause. A small number bred in late summer, producing an autumn emergence, the second generation in a year. All adult *B. bubalus* had died by February 2017, leaving larvae underground. Monitoring of the spring emergence began in July 2017. Newly emerged beetles were transferred to new nurseries.

The planned field release of the beetles was cancelled in favour of breeding beetles. One field release of 1,100 adult *O. vacca* occurred in Manjimup, Western Australia. The remaining beetles were released for mass rearing in WA (2,089 *O. vacca*; 185 *B. bubalus*) and at Armidale, New South Wales (2,453 *O. vacca*).

Mass rearing of *O. vacca* and *B. bubalus* will not continue at Strathalbyn beyond the term of Project B.ERM.0216.

Executive summary

Rationale and background

Project B.ERM.0213 imported the spring-active dung beetles *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. The outcomes of the current project (B.ERM.0216, 1 December 2016 to 1 December 2017 inclusive) are reported here.

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 of the two new spring-active species across southern Australia. 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.

Procedures

The current project (B.ERM.0216) involves 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 New South Wales and Western Australia.

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 stopped feeding and had entered a non-feeding, non-breeding diapause. However, it is likely that a proportion of these beetles bred for a time in February.

All adult *B. bubalus* were at the end of their life cycle and 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.

Outcomes

Onthophagus vacca

The adult *O. vacca* present in summer 2016–2017 were F3 beetles, the progeny of F2 beetles that emerged and bred in spring 2016. The presence of F2 and F3 adults in the nurseries overlapped in late spring 2016 and early summer 2017. Because of this it was not possible to assess the number of F3 beetles that were present in summer 2016–2017, but they were numerous.

All F2 adults had died by mid-summer.

Of the spring-emerged 2016 *O. vacca*, a total of 1,100 was released in the field in WA. The remaining beetles were held and fed in a large nursery from emergence until they were trapped for delivery to NSW and WA for mass rearing in field nurseries. These beetles were dispatched via airport-to-airport delivery to Armidale (n=2,453) and WA (n=2,089). Beetles were healthy on arrival at both locations.

A small amount of summer breeding in F3 *O. vacca* gave rise to a second 2016–2017 emergence (F4 beetles) in May 2017 (1,706 adults, of which 98% were collected in the weeks from 22 May to 19 June 2017). They were maintained in a separate nursery. The newly emerged F4 adults fed in May–

June but did not breed. After some time they stopped feeding and entered the soil, where they waited until spring 2017 to emerge again. Their temporal pattern of emergence was largely parallel to that of the F3 beetles in a separate nursery. Upon emergence the F4 beetles fed for some weeks, where after breeding commenced. These beetles were trapped in October 2017 and sent to the Western Australian mass rearing facility. Only 377 of the original 1,706 F4 beetles were recovered. The reason for the substantial mortality as adults in the period May to August is not known.

The F4 beetles represent a second generation of *O. vacca* within the 12-month period covered by B.ERM.0216.

The timing of the spring emergence of the F3 *O. vacca* in 2017 parallels that of the F2 beetles in spring 2016. Upon emergence, they were placed in separate nurseries, where they began breeding soon after arrival.

The generation-to-generation increase in numerical abundance in the Strathalbyn nurseries was 6.8-fold for F0 to F1, 12.6-fold for F1 to F2 and 3.0-fold for F2 to F3. In addition, there was a summer breeding event in the F3 beetles that produced an autumn-emerged cohort of F4 beetles. Over that 3-year period the total number of *O. vacca* at Strathalbyn has increased from 76 adults to 16,795.

While this is commendable, we point out strongly that if the generation-to-generation increase had been 15-fold each year, the total emergence in spring 2017 would have been in the order of 240,000 beetles (or 570,000 beetles with a 20-fold increase), rather than the achieved 16,795 beetles.

Bubas bubalus

Adult beetles emerged in spring 2015. These bred for about 3 months, producing immatures that stayed underground for about 6–8 months and eventually gave rise to the F2 adults that emerged and bred in spring 2016, and a second cohort of F2 beetles that emerged and bred in spring 2017.

The 2016 adult F2 *B. bubalus* finished breeding in December 2016 and then died. The progeny of that spring-breeding F2 cohort were present as larvae underground at the beginning of Project B.ERM.0216 (1 December 2016). The number of these larvae was not known.

By January 2017 the stocks of *B. bubalus* comprised exclusively immature beetles underground in broods. These were the progeny of the F2 spring-breeding of *B. bubalus* in 2016 (a 1-year life cycle) and F1 breeding in 2015 (a 2-year life cycle). There was no emergence of adult *B. bubalus* in the period January to August 2017, after which adult emergence commenced. The 2-year life-cycle beetles were pooled with the 1-year spring emergence (from the 2016 spring breeding).

The spring 2017, F3 emergence of *B. bubalu*s was not parallel with the temporal pattern observed in the 2016 F2 beetles, in that the emergence appeared to have two peaks (late August, early October).

In October 2017 the nursery contained only diapausing larvae (the progeny of the 2016 breeding, due to emerge in spring 2018). The developmental stage of some larvae suggested that they might be on the verge of becoming pupae and, as such, might provide an unexpected emergence of new adults in November. This did not occur. Additional sampling in November indicated that all larvae present were in diapause.

The generation-to-generation increase in numerical abundance in the Strathalbyn nurseries was 2.8-fold for F0 to F1 and 1.4-fold for F1 to F2. A 20-fold increase per generation would have yielded over 200,000 F2 beetles in spring 2017 with one generation per year (no diapause) instead of the current crop of 2,250 adults. Achieving a high reproductive output must be a high research priority.

The issues raised in the above discussion apply not only to *O. vacca* and *B. bubalus*, but also to all new species that are to be brought to Australia.

Releases from Strathalbyn to other locations

A program for the release of the *O. vacca* and *B. bubalus* to field situations in Western Australia, South Australia, Tasmania, Victoria and New South Wales was developed and approved, but a subsequent decision to hold the breeding stock for mass rearing altered these plans and only one field release occurred. One starter colony of 1,100 adult *O. vacca* occurred in Manjimup WA (19 September 2017). The beetles have spread from the central release point and have been observed in adjacent paddocks. The F0 beetles appear to be breeding and some F1 beetles have been recovered.

The remaining beetles were released for mass rearing in WA (2,089 *O. vacca*; 185 *B. bubalus*) and at Armidale (2,453 *O. vacca*). Delivery of beetles to WA and NSW was done in accord 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 *O. vacca* and *B. bubalus* will not continue at Strathalbyn beyond the term of this project (B.ERM.0216). There were no remaining *O. vacca* or *B. bubalus* at Strathalbyn at the time of writing this report.

Insurance nurseries

Small numbers of *O. vacca* and *B .bubalus* have been reared at the Port Elliot site and these are forming the basis of a field release at the southern tip of the Fleurieu Peninsula in South Australia.

Small numbers of *B .bubalus* have been reared at the Bool Lagoon site and these are forming the basis of a field release in the south-east of South Australia. *O. vacca* failed to breed successfully in two successive years in the heavy clay soil of the Bool Lagoon test location.

Future field releases of *O. vacca* and *B. bubalus*

The mass rearing of large numbers of *O. vacca* and *B. bubalus* is now exclusively reliant upon the breeding success in WA and at Armidale and Charles Sturt University (CSU), NSW. A release and monitoring protocol has been developed. Attention must be given to the constraints limiting reproductive success of dung beetles in the laboratory and in field nurseries.

Table of contents

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. The outcomes from the current project (B.ERM.0216) are reported here and cover the activities undertaken over the period1 December 2016 to 1 December 2017.

The rationale for initiating the 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 current project (B.ERM.0216) (that is, December 2016) the numbers of F2 *O. vacca* at Strathalbyn were estimated at several thousand adults: small numbers were also present at Port Elliot but none were present at Bool Lagoon, where breeding in spring 2014 had failed (Project B.ERM.0214 final report).

At the beginning of the reporting period (that is, in December 2016), the numbers of F1 *B. bubalus* from cohort 1 were minimal and the numbers derived from cohort 2 at Strathalbyn (brought from Canberra in early spring 2015) were estimated at several thousand larvae in one large nursery. Small numbers were present at Port Elliot and Bool Lagoon.

1.1 Activities undertaken between completion of Project B.ERM.0214 (31 March 2016) and initiation of Project B.ERM.0216 (1 December 2016)

1.1.1 Activity of *O. vacca* (April to December 2016)

The beetles that gave rise to the estimate of 2,000+ *O. vacca* in April 2016 (Project B.ERM.0214 final report) were the progeny of the beetles that bred in spring 2015 in two small nurseries. The nursery arrangements and breeding performance of *O. vacca* for the period April to December 2016 are listed here and discussed in the discussion. The key points are:

- Before this period:
  - The F0 to F1 (2014–2015) generation change (65 to 443 beetles) comprised a 6.8-fold increase in numbers.
  - The F1 adults fed over the summer of 2014–2015 and then entered diapause and went underground, where they waited until spring 2015 before emerging.
  - The F1 adults emerged again in spring 2015 and bred, producing the F2 generation that emerged in late spring–early summer 2015. The F1 and F2 generations overlapped and were not counted at that time.
  - The F2 adults fed over the summer of 2015–2016 and then entered diapause and went underground, where they waited until spring 2016 before emerging.

- We estimate that at the beginning of the period under review (April to December 2016) there were several thousand adult *O. vacca* underground and in diapause.
- One new large *O. vacca* tunnel nursery was constructed during this period (Figure 1).
- In spring 2016 F2 adults (n=5,421) that emerged from a range of nurseries were placed in the two new tunnel nurseries (n=2,857 and n=2,216) and into two small nurseries (n=95 and n=253).In addition, 160 beetles were allocated to the 'insurance' locations..
- The F2 adults emerged in spring 2016 and bred, producing the F3 generation that emerged in late spring–early summer 2016. The F2 and F3 generations overlapped and were not counted at that time.
- The F1 to F2 (2015–2016) generation change (443 to 5,581 beetles) comprised a 12.6-fold increase in numbers.
- The F3 adults fed over the summer of 2015–2016 then entered diapause and went underground where they waited until spring 2017 before emerging.
- Project B.ERM.0216, reported in this final report, began on 1 December 2016. At that time the nurseries contained breeding F2 adults (that emerged in spring 2016 and bred, producing the F3 generation) and the initial emergence of the F3 generation. The F2 and F3 generations overlapped and were not counted at that time. Many thousand F3 beetles were considered to be present.
- Some F2 beetles were transferred in spring 2016 to small field nurseries with either sand or local soil at each of the two minor test (insurance) sites in South Australia. Some breeding occurred at both sites.
- A third 'insurance' test site was established with F2 adults at Wilsons Inlet in Western Australia with one loam and one sandy soil nursery. Beetles appeared to breed in both soil types. Cattle destroyed the cages after some months and the trial was abandoned.

1.1.2 Activity of *B. bubalus* (April to December 2016)

The beetles that gave rise to the estimate of several hundred *B. bubalus* larvae in February 2016 (Project B.ERM.0214 final report) were the progeny of cohort 2 brought to Adelaide from Canberra in early spring 2015. The nursery arrangements and breeding performance of *B. bubalus* for the period April to December 2016 are listed here and discussed in the discussion. The key points are:

- Adult F0 *B. bubalus* breeding activity had largely ceased by mid-summer 2015–2016 (Project B.ERM.0214) (before this reporting period).
- F1 larvae (in the soil) were developing during autumn–winter 2016.
- Post-diapause F1 adult beetles began emerging in July 2016 and emergence continued for 6–10 weeks.
- Newly emerged F1 beetles commenced breeding soon after emergence.
- One new *B. bubalus* tunnel nursery was constructed during this review period (Figure 1).
- In spring 2016 newly emerged beetles were trapped, counted, sexed and transferred to the new tunnel nurseries (n=1,331 beetles) and one other tunnel nursery (n=162). In addition, 165 beetles were allocated to the 'insurance' locations.

- Breeding continued for at least several months after emergence in the tunnel nurseries.
- The F0 to F1 (2015–2016) generational change (about 600 to 1,658 beetles) comprised a 2.8-fold increase in numbers.
- Project B.ERM.0216, reported in this final report, () began on 1 December 2016. At that time the nurseries contained the 'tail end' of the breeding F1 adults (that emerged in spring 2016 and bred, producing the F2 generation) and an unknown number of larvae underground derived from the spring 2016 breeding event and the spring 2015 breeding event (a diapause-controlled 2-year life cycle).
- F1 beetles were transferred to small field nurseries at each of the two minor test (insurance) sites in South Australia (n=64 at each site). Some breeding occurred at both sites.
- A third 'insurance' test site was established at Wilsons Inlet in Western Australia with one loam and one sandy soil nursery (n=37 beetles). Beetles appeared to breed in both soil types. Cattle destroyed the cages after some months and the trial was abandoned.

1.2 Beetle stocks in spring 2016

A total of 240 adults and 150 broods of *Onthophagus vacca*, and 1,200 broods of *Bubas bubalus* (cohort 1), were made available by MLA via CSIRO (November 2014). A further 350 adults and 300 faecal shells of *B. bubalus* (cohort 2) were made available the following year (August 2015) (Project B.ERM.0214). The estimated numbers of *Onthophagus vacca* and *Bubas bubalus* that comprised MLA assets in spring 2016 are presented in Table 1.

Table 1: Stocks of *O. vacca* and *B. bubalus* (spring 2016)

	Onthophagus vacca	***Bubas bubalus***
Strathalbyn (total stocks)	3349	995
Beetles transferred to:		
Port Elliot	50	64
Bool Lagoon	50	64
Wilson Inlet	60	37
Net assets for breeding at Strathalbyn	**3189**	**830**

2 Project objectives

By the end of spring 2017 (1 December) DBSI will:

- implement the mass rearing actions in field nurseries for *Onthophagus vacca* and *Bubas bubalus* to enable significant increase in reproduction and survival towards a field release (complete or partial) in spring 2017.
- outline a refined release plan based on prevailing opportunities (emerged beetles, potential partners).

3 Methodology

Project ERM.1102 (19 September 2017 – 15 February 2018), titled 'The release, and monitoring the establishment of, *Onthophagus vacca* and *Bubas bubalus* in high-care field rearing environment in southern Australia', ran concurrently with Project B.ERM.0216. Milestone reports 1 and 2 for Project ERM.1102 were submitted on Friday 22 Dec 2017. Both projects relied on the same rearing facilities.

3.1 Maintenance and feeding

Newly emerged *O. vacca* adults were fed in the field nurseries from December 2016 to April 2017. By April all of the beetles had ceased feeding activity 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) (Figures 1 and 2). 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.

The dung collection, beetle feeding and activity monitoring have continued and follow the procedures outlined in Project B.ERM.0214. Monitoring of the spring emergence began for both species in July 2017. Newly emerged beetles were counted and transferred to new nurseries.

Figure 1: V-nurseries (galvanised tanks) in the background with an S-nursery (in the foreground). The superstructures (Figure 2) were added to the S-nurseries in 2016.

Figure 2: Tunnel nurseries (S-nurseries) constructed for the two new species

4 Results

4.1 *Onthophagus vacca*

As indicated below there were three sets of emergences of *O. vacca* in the reported year: a summer 2016–2017 emergence (the product of spring 2016 breeding), an autumn emergence (the product of summer 2017 breeding) (see section 4.1.1) and a spring emergence (the post-diapause product of spring 2016 breeding) (see section 4.1.2).

The large nursery S-9 was partitioned into S-9 North (a 7-square-metre surface area) and S-9 South (a 20-square-metre surface area). The autumn 2017-emerged beetles were accumulated in a large container with soil and dung and, about 4 weeks after the first emergence, were all transferred to S-9 North and provided with *ad lib* fresh dung. They fed there, entered diapause and went underground for some time, and then emerged again in spring 2017. This spring re-emergence of the autumn 2017-emerged beetles was retained in S-9 North and fed fresh dung.

The spring 2017 emergence of *O. vacca* derived from the spring 2016 breeding event (which took place in large and small nurseries) were transferred to S-9 South as they emerged.

4.1.1 Autumn 2017-emerged beetles

The *O. vacca* that emerged in autumn 2017 were harvested in spring and sent to Western Australia for mass rearing.

A total of 1,706 adult beetles were collected in May–June (autumn emergence at Strathalbyn). They were held in a nursery separately from the other *O. vacca* and fed fresh dung, much of which was buried. They did not breed in the 4 weeks following emergence, whereafter they entered diapause and went underground. They emerged again in spring at the same time as the other *O. vacca* (that is, the spring 2016 emergence derived from the spring 2016 breeding event) and were given *ad lib* fresh dung but they did not begin to breed until about 4–6 weeks had elapsed. At this stage they were trapped and sent to WA. Only 377 beetles (22% recovery) were recovered and we do not know what happened to the beetles that were not recovered: presumably they died but why is not known. The causes of this important mortality deserve attention.

The autumn emergence was clearly a distinct emergence of new beetles (Figure 3) and was not part of a general 'slow leakage' of adult beetles to the surface of the nursery throughout autumn, as has been observed in other situations (Patrick Gleeson, pers. comm.). It seems likely that the autumn emergence arose from a breeding event in February 2017. Whether the parents of the autumn-emerged beetles died or entered a non-breeding diapause is not known.

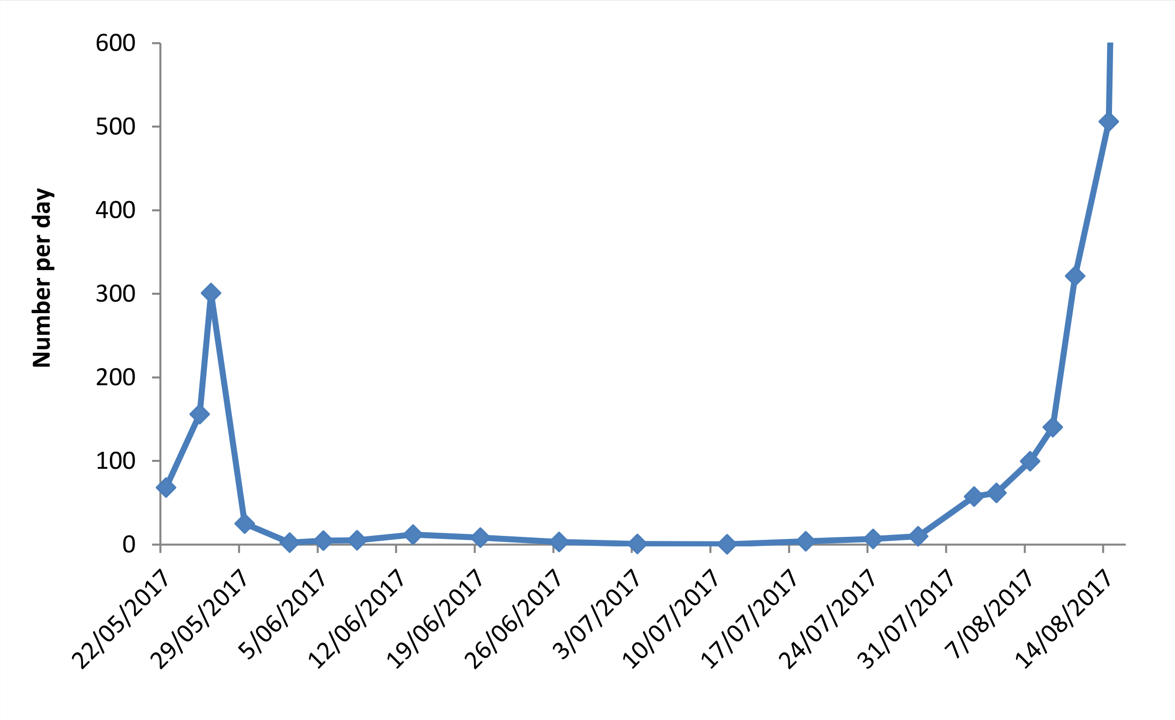

Figure 3: The pattern of emergence of the autumn-emerged adult *O. vacca* and the beginning of the emergence of the spring beetles. This graph covers only four months of the year and does not report the late spring 2017 emergence of the next generation.

4.1.2 Spring-emerged beetles

Most of the spring–summer 2016-emerged *O. vacca* (derived from the spring 2016 breeding event) (egg to adult taking about 2 months) remained in diapause from late summer 2017 until August 2017, when most re-emerged over about 6 weeks: they began breeding soon after this emergence. Initially (until 13 September) the newly re-emerged beetles were trapped, counted and transferred to a new nursery (S-9 South) where they were provided with a regular supply of fresh dung.

The total emergence from the S (n=3,814) and V (n=1,608) nurseries in spring 2016 (28 July to 25 November) was 5,422 F2 beetles.

The total emergence from the S (n=11,183) and V (n=5,612) nurseries in spring 2017 (28 July to 25 November) was 16,795 F3 beetles. This represents a 3.0-fold increase from the F2 to the F3 generation. The project component of this was 10,077 F3 beetles. In addition there were 1,706 autumn-emerged F4 beetles that were derived from summer (February 2017) breeding by an unknown number of F3 *O. vacca*.

The F2 to F3 (plus autumn F4) (2016–2017) generation change (5,581 F2 beetles to 16,795 F3 plus 1,706 F4 beetles) comprises a 3.3-fold increase in numbers.

By 11 September 2017 a total of 7,984 *O. vacca* had emerged and these were all held in the 20 square metres of nursery S-9 South. Our current view is that a total of about 3,000 beetles in a 27-square-metre nursery (about 110 per square metre) is near the upper limit of an appropriate density. Nursery S-9 South had nearly four times that density (about 400 per square metre).

Over the period 12 September to 13 October a total of 8,811 adult beetles were placed in these large storage containers.

The remaining project component of the spring 2017 emergence was dispersed amongst the F3 beetles in the overcrowded S-9 South nursery, the F3 beetles that had not yet emerged from the spring 2017 breeding event and been trapped, and the 1,706 F4 adults derived from the autumn emergence. With hindsight (November 2017), we calculated that the remaining portion amounted to 1,266 beetles.

In mid-September the storage containers were partially emptied in order to retrieve 1,100 adults (for the WA field release) and 119 beetles for the WA breeding program. These were taken to WA on 19 September. The putative total remaining in the storage containers was then 7,592 beetles.

In early October 2017, 1,600 adults were extracted from the storage containers, and dispatched to WA on 4 October, leaving a putative 5,992 beetles in the storage containers.

The remaining beetles in the containers were harvested on 12 October 2017 but only 3,000 adults were recovered. The cause of death of the other 2,992 beetles is not known. The 3,000 beetles were ready for dispatch to Armidale. Unfortunately the nursery at Armidale was not complete at that time and so the beetles were stored for a time at Strathalbyn in other containers of soil with dung. On 26 October a total of 2,700 beetles were recovered from their holding containers and dispatched to Armidale, where on 27 October 2,453 live healthy adults were recovered and formed the basis of the NSW breeding program.

By the time Armidale was ready to receive adult beetles (that is, in late October 2017, 3 months after beetles began emerging and breeding) beetle activity in nursery S-9 South (where most of the remaining F3 and F4 beetles were housed) had become minimal and it was not possible to harvest any remaining beetles. It is likely that there was considerable mortality (associated with overcrowding) in S-9 South.

The substantial losses that occurred during storage in the large containers of soil (supplied with *ad lib* fresh dung) and in the F4 beetles in nursery S-9 North (only 22% recovery) is a great concern. It appears that survival was severely compromised by storage both in the large containers (separate from the nurseries) and in the nurseries. This problem needs urgent attention.

The type of nursery in which the beetles were maintained appeared to have a substantial influence on the chronological pattern of spring emergence in 2017 but not in 2016 (Figures 4 and 5). In 2017 the emergence of adult beetles from the V-nurseries (galvanised above-ground tanks (Figure 1) was delayed by about 5 weeks compared with the emergence from the S-nurseries (Figure 2) but there was no difference in 2016 and both nurseries produced emergence patterns similar to that produced in the S-nurseries in 2017. The reason for these differences is not known.

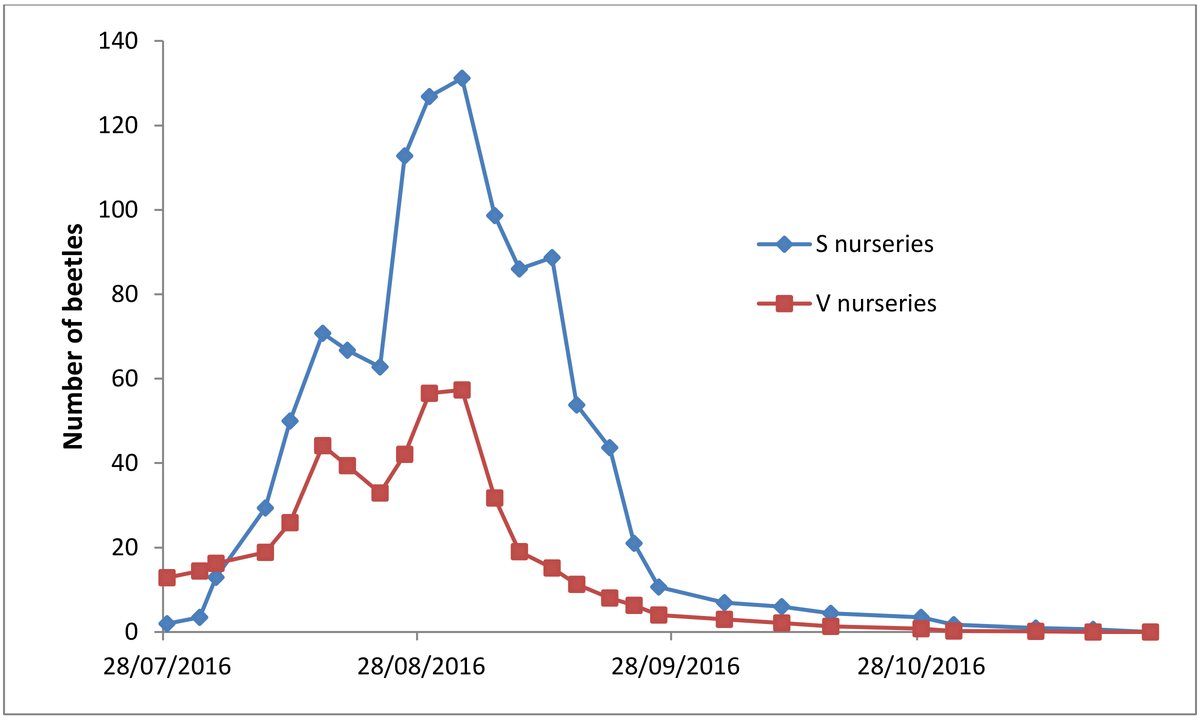

Figure 4: ***O. vacca*** **emergence patterns from V- and S-nurseries in spring 2016. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 3 to 5 days.**

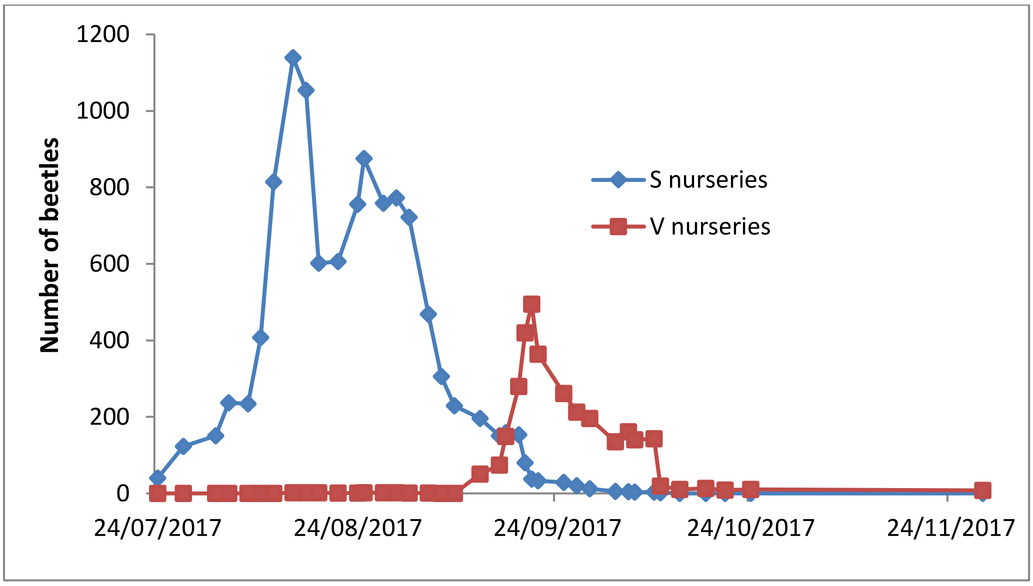

Figure 5: ***O. vacca*** **emergence patterns from V- and S-nurseries in spring 2017. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 3 to 5 days.**

Of these spring-bred 2016 *O. vacca*, a total of 1,100 was released in the field in WA in spring 2017 (reported in the milestone 1 report for Project ERM.1102). The remaining beetles were held and fed in a large nursery from emergence until they were trapped for dispatch to NSW and WA. These beetles were dispatched via airport-to-airport delivery to Armidale and WA. Beetles were healthy on arrival in both locations.

4.1.3 Releases to the field and to mass rearing

A program for the release of the *O. vacca* and *B. bubalus* to 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.

Beetles for Armidale, NSW

The beetles that were to be supplied to Armidale were harvested and 3,000 *O. vacca* were collected and ready for dispatch on 12 October 2017. 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 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 2 weeks at Armidale until the construction of the nursery was completed (Figure 6), 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 6: The nursery at Armidale

Beetles for Mandurah, WA

Beetles were provided to the WA breeding hub on 22 September, 4 October and 18 October 2017. An account of the supply of dung beetles to the Western Australia breeding hub is provided in Table 2.

Table 2: 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	Contact person
1: B. Doube	Spring Cohort 3 dead in transit	*O. vacca*	119	20 September 2017	In washed river sand B. Doube delivered to K. Dawson. Held overnight for collection by J. Allen.	B. Doube/ K. Dawson
2: G. Dalton	Spring Cohort 12 dead in transit	*O. vacca*	1600	4 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth, collected by J. Allen	G. Dalton
3: G. Dalton	Autumn Cohort 8 dead in transit	*O. vacca*	370	18 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth, collected by J. Allen	G. Dalton
3 G. Dalton consignment	Aug. 2017 eclosion 5 dead in transit	*B. bubalus*	185	18 October 2017	In washed river sand Qantas Air Freight, Adelaide to Perth, collected by J. Allen	G. Dalton

4.2 *Bubas bubalus*

The spring 2017 emergence of *B. bubalus* began in August 2017 and continued for about 8 weeks (Figure 8). Newly emerged beetles were trapped, counted and transferred to a new nursery, where they were provided with a regular supply of fresh dung.

The initial phase of the emergence in 2017 was delayed by several weeks compared with that observed in 2016 (Figures 7 and 8). It appears that in 2017 there were two peaks of emergence, one in late August and one in early October (Figure 8). There is no obvious explanation for the separation of the spring emergence into two peaks.

The number of beetles that emerged in spring 2017 was considerably lower than expected based upon the observation that large quantities of dung were buried over many weeks in spring 2016. This suggests that a substantial amount of breeding in *B. bubalus* occurred in spring 2016. The low number emerging in spring 2017 suggests that either a high proportion of the 2016 breeding event had entered diapause or that there was an unknown and high immature mortality factor at work.

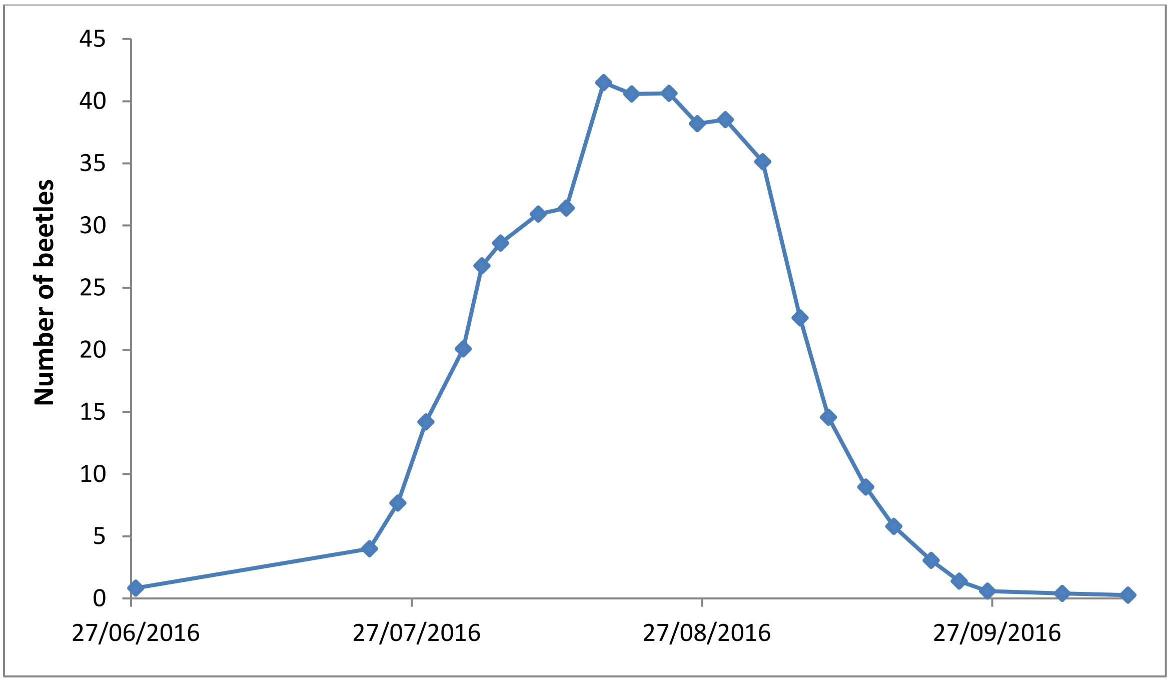

Figure 7: ***B. bubalus*** **emergence patterns in late winter – spring 2016. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 3 to 5 days.**

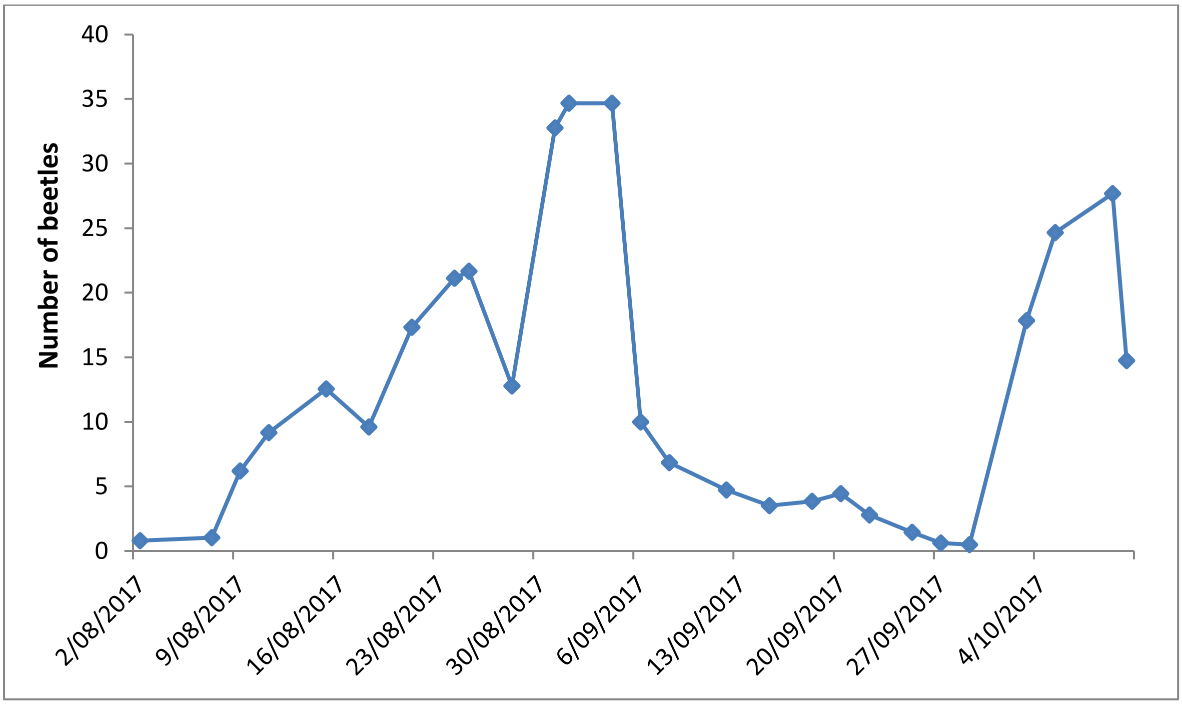

Figure 8: ***B. bubalus*** **emergence in spring 2017. Data are the 3-point average of the estimated number of beetles emerging per day. Sampling intervals ranged from 1 to 5 days.**

The decision in late September 2017 to transfer the breeding of *B. bubalus* to Western Australia meant that we needed to harvest a component of the breeding *B. bubalus* and ship them to WA.

Traps were set regularly over a number of weeks and all the *B. bubalus* that could be trapped (N=185, Table 2) 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 nursery. A recent observation (21 December

2017, J Allen pers. comm.) of a mature third instar *B. bubalus* larva indicates that successful breeding is under way in WA.

4.3 Release plan

Protocols for release and monitoring the two new species were drafted in September 2017 and the field release plan involved partners in Western Australia, South Australia, Tasmania, Victoria and New South Wales. We recommend that the future release plan follow the protocol for release and monitoring already outlined, but note that the number of starter colonies to be released in future years will depend upon breeding success in WA and NSW and on the number retained as breeding stock.

5 Discussion

The two project objectives are marginally different from the three issues identified in the milestone requirements. Overall the project objectives and the milestone requirements cover largely the same territory, namely that, as specified in the project objectives, by the end of spring 2017 (1 December) DBSI will:

- implement the mass rearing actions in field nurseries for *Onthophagus vacca* and *Bubas bubalus* to enable significant increase in reproduction and survival towards a field release (complete or partial) in spring 2017.
- outline a refined release plan based on prevailing opportunities (emerged beetles, potential partners).

Both project objectives have been met. A release and monitoring plan is outlined in Appendix 1.

There was a substantial increase in the numbers of *O. vacca* from spring 2016 to spring 2017. One field release (1,100 beetles) was conducted in Manjimup, WA. Mass rearing of *O. vacca* was transferred from Armidale to Charles Sturt University (Wagga Wagga, NSW).

In the following discussion we consider the life cycles of the two species as commonly accepted and how they varied in our mass rearing facilities.

5.1 *Onthophagus vacca*

A stylised diagram of the life cycle of *O. vacca* is presented in Figure 9. This indicates that there is one generation per year which is regulated by a non-feeding, non-breeding adult reproductive diapause that holds the adult beetles underground during autumn and winter. The overlapping of generations was observed in spring 2017 (Figure 10).

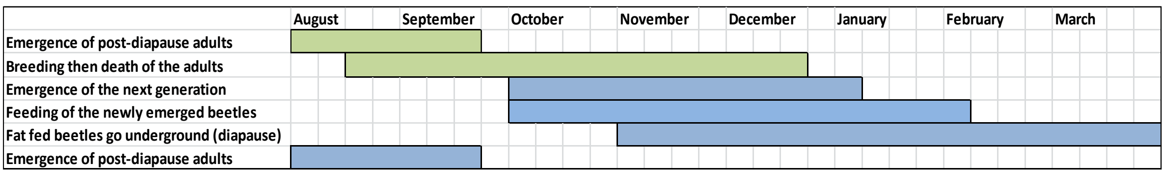

Figure 9: The conventional life cycle of *O. vacca* in the field

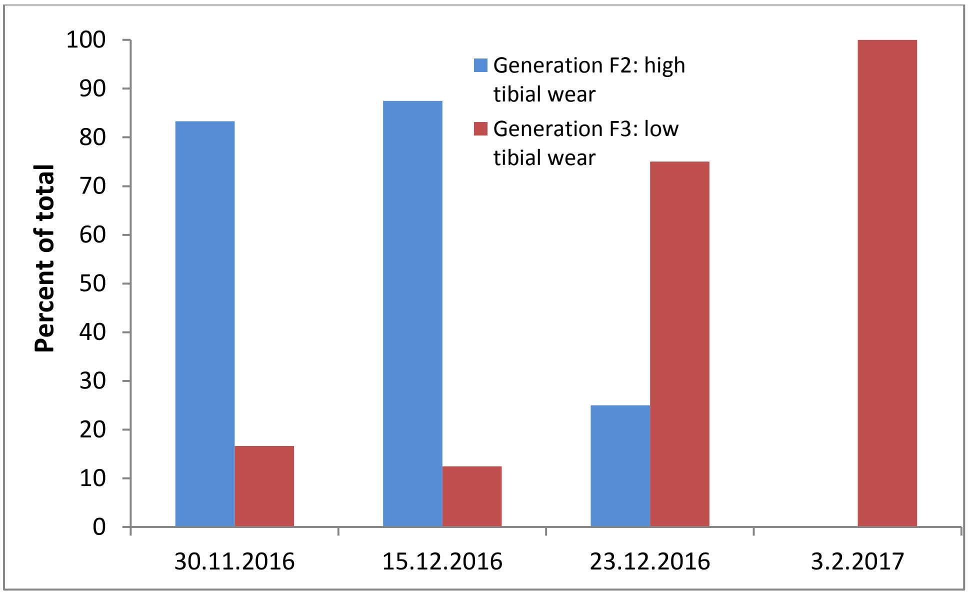

Figure 10: Overlapping generations in spring 2017 at Strathalbyn as indicated by the degree of tibial wear in four samples of beetles taken from the mass rearing facilities (minimal tibial wear= newly emerged beetles: worn tibia = old beetles)

Our observation that a cohort (n=1,706) of adult beetles emerged in autumn 2017 (Figure 3) and the corresponding observation that egg to adult takes about two months indicate that there had been a separate and additional breeding event in about February 2017 that produced the new F4 adult emergence in May 2017 (autumn). We do not know whether the F3 adults that produced the autumn cohort died following breeding (as usually occurs) or entered a reproductive diapause and then emerged again the following spring.

The reality of a second generation within 12 months raises the possibility that there could be strains of *O. vacca* that could have multiple generations per year. This would greatly increase our capacity to produce beetles for widespread mass release across southern Australia but raises the concern that such multivoltine genotypes may not be those required to close the spring gap in dung beetle activity in southern Australia.

The generation-to-generation rate of increase in population size of *O. vacca* was about 6-fold in 2015, about 12-fold in 2016 but only about 3-fold in 2017. The reason for the reduced reproductive success is not known, but the interaction between environmental conditions during each year and the nursery conditions may provide some clues. The year 2017 was wetter than 2016 and the winter was cooler (Figures 11 and 12), and these factors could have influenced the survival of *O. vacca*. These issues deserve further attention.

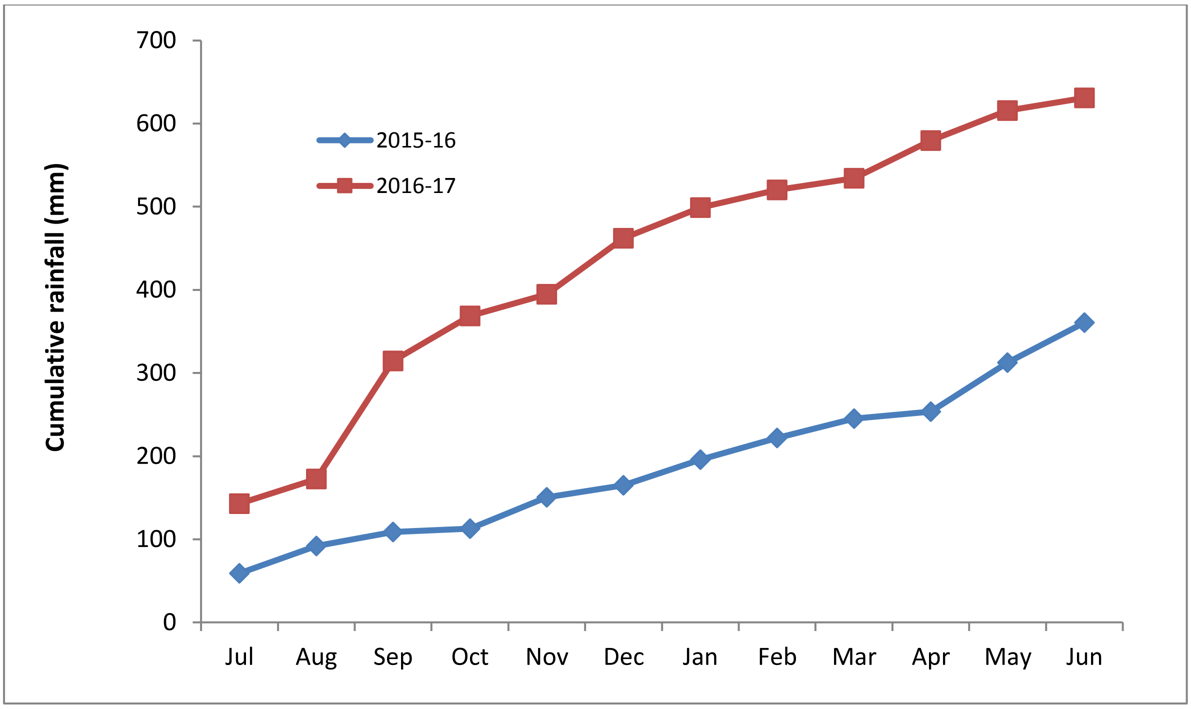

Figure 11: Cumulative rainfall at the Strathalbyn racecourse
Source: Bureau of Meteorology

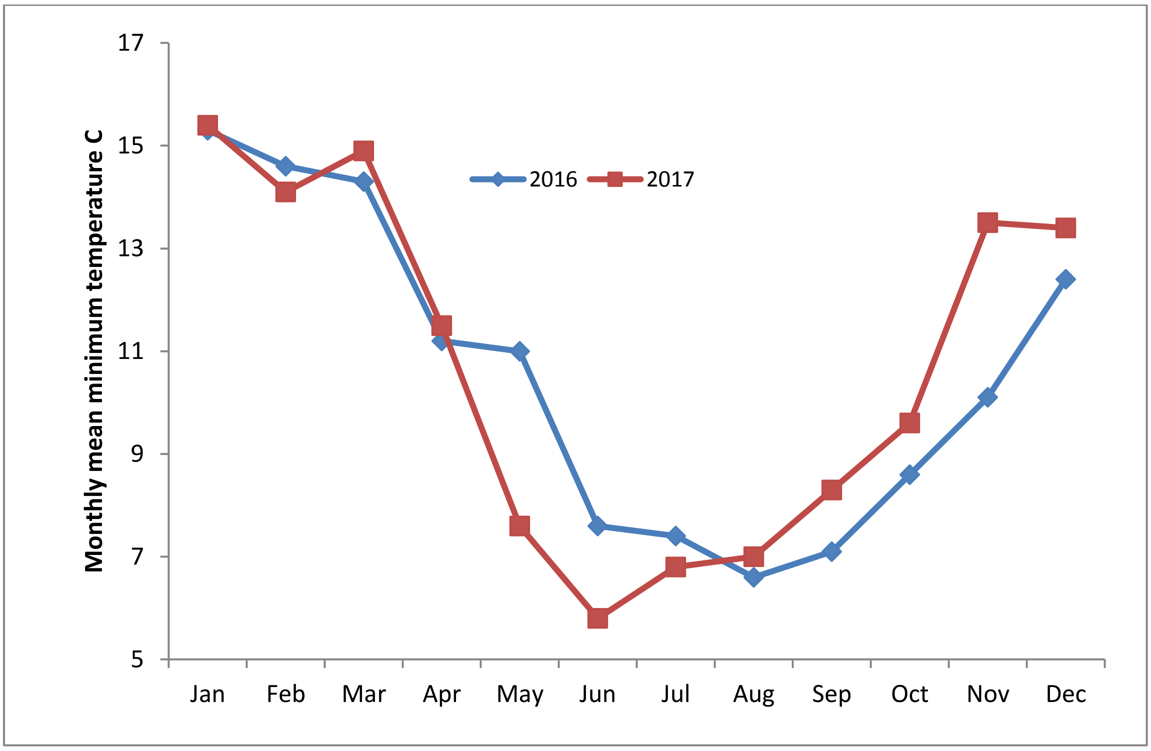

Figure 12: Monthly mean minimum temperatures at the Strathalbyn racecourse
Source: Bureau of Meteorology

5.1.1 Discussion summary: *Onthophagus vacca*

The beetles that gave rise to the estimated several thousand *O. vacca* in February 2017 were the progeny of the beetles that bred in spring 2016 in a large nursery. The key points concerning the history and rearing of *O. vacca* at Strathalbyn are:

- The F0 to F1 (spring 2014–2015) generation change (65 to 443 beetles) comprised a 6.8-fold increase in total numbers.
- The F1 *O. vacca* bred in spring 2015 (Project B.ERM.0214), giving rise to numerous F2 *O. vacca* in late spring–summer 2015. Egg-to-adult development was considered to take 6–8 weeks. These beetles were not trapped (hence numbers were not known) but they were fed and remained active for weeks after emerging (Project B.ERM.0214). Adult F2 *O. vacca* feeding activity ceased in late summer–autumn 2016, when they then tunnelled into the soil as non-feeding, non-breeding diapausing adult beetles.
- Post-diapause F2 adult beetles emergence began in August 2016 and continued over a period of about 6–8 weeks. These newly emerged F2 adults (n=5,421)
- were trapped and counted, and removed to a separate nursery, where they were fed fresh dung. In addition, 160 beetles were sent to the three 'insurance' locations (two in SA, one in WA). Newly emerged F2 beetles commenced breeding in the new nurseries soon after emergence and transfer.
- The F1 to F2 (2015–2016) generation change (443 F1 to 5,581 F2 beetles) comprised a 12.6-fold increase in total numbers.
- The F2 beetles bred in spring, giving rise to the F3 generation in spring–early-summer 2016. The F3 generation of adults began emerging in spring 2016 while the F2 parental stock were still breeding (overlapping generations) and the generations were not separated.
- Most F2 beetles had stopped breeding and died by December 2016 but the F3 beetles fed voraciously in early summer and most appeared not to breed. The F3 beetles had stopped feeding by March 2017 and most tunnelled into the soil as non-feeding, non-breeding diapausing adults. Some bred in February.
- The post-diapause F3 adult beetles in the S-nurseries began to emerge in August 2017 (parallel seasonal timing with that of the 2016 emergence) and continued for three months, with contrasting patterns in the S- and V-nurseries in 2017 but not in 2016 (Figures 4 and 5).
- The F2 to F3 (and autumn F4) (2016–2017) generation change (5,581 F2 beetles to 16,795 F3 and 1,706 F4 beetles) comprised a 3.3-fold increase in numbers.
- The initial plan was to release the F3 beetles to the field but altered priorities meant that only one starter colony of 1,100 beetles was released. These beetles were collected in a storage chamber with *ad lib* dung before they were dispatched.
- The remaining beetles were retained for mass rearing. These beetles were dispatched via airport-to-airport delivery to Armidale (n=2,453) and WA (n=2,089). Beetles were healthy on arrival at both locations.
- There were substantial delays between harvesting the F3 beetles and their dispatch to WA and NSW. Beetles were held in temporary accommodation during those delay times. The numbers

recovered (by sieving the soil) after storage were substantially lower than the numbers put in the storage containers. This suggests that significant mortality occurred during these periods of storage but recovery may have also been made difficult by their cryptic colouration and so some individuals might have been overlooked, despite great care being taken.

- It appears that a small proportion of the adult F3 beetles bred in February and gave rise to an F4 generation in autumn 2017 (n= 1,706 adults). These were hungry and fed on fresh dung but did not breed. They were maintained separately from the F3 beetles. They emerged in spring 2017 at about the same time as the F3 beetles but did not breed for about 4 weeks. Eventually they were trapped out and those that were recovered (n=377) were transferred to the mass rearing facilities in WA. The reason for the substantial mortality as adults in the period May to August is not known.

5.2 *Bubas bubalus*

A stylised diagram of the life cycle of *B. bubalus* is presented in Figure 13. This indicates that there can be a maximum of one generation per year and the beetle may express a 1-, 2- or 3-year life cycle which is regulated by a non-feeding, third instar larval diapause that holds the larval beetles underground for about 6, 18 or 30 months. Overlapping generations have been observed.

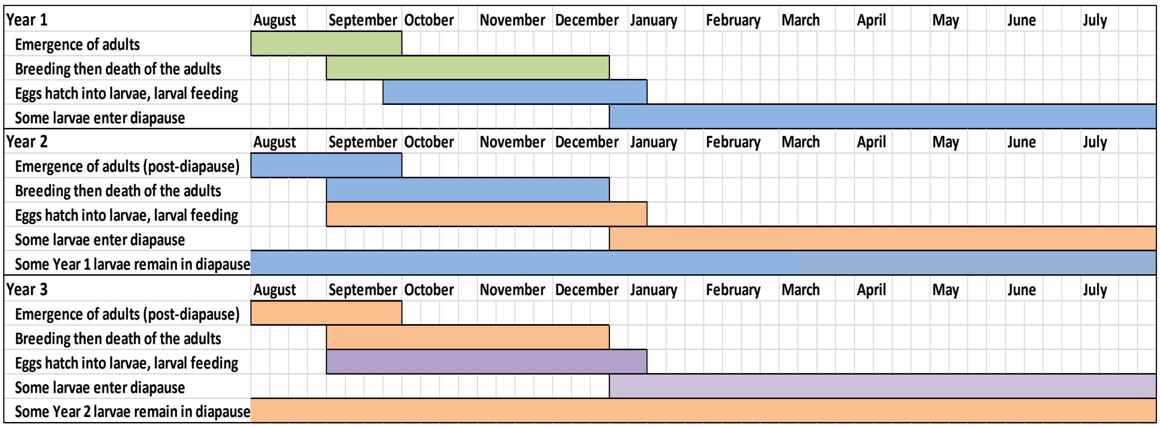

Figure 13: Life cycle of *B. bubalus* in the field

In spring 2016 new beetles began to emerge in August (Figure 7) and were trapped, counted, sexed and transferred to the new tunnel (S) nurseries (n=1,331 beetles) and one other tunnel nursery (n=162, total n=1,493). In addition, 165 beetles were allocated to the 'insurance' locations. The F0 to F1 (2015–2016) generational change (about 600 to 1,658 beetles) comprised a 2.8-fold increase in numbers but a proportion of the F1 generation remained in the soil in larval diapause, of which a few (about 30) emerged in spring 2017.

The one-year cycle F2 beetles began emerging in Strathalbyn in August 2017 and over 8 weeks 2,250 beetles emerged. There were two apparent emergences, one in early spring and one in late spring (Figure 8), in contrast with the single emergence in 2016 (Figure 7). We have no explanation for the bimodal emergence pattern in 2017.

The newly emerged beetles were trapped and counted and transferred to a new large nursery where they were fed *ad lib* fresh dung. They began burying much dung soon after being placed in the new nursery. Adult beetles will feed for a few weeks following emergence in spring and then begin breeding. Eggs will be produced from September until the adult beetles die. Death of the early-emerged beetles (August) may occur in November–December in the same year but it is likely that the October-emerged beetles will continue breeding for a month or so into the new year.

The temporal emergence and breeding patterns of *B. bubalus* are likely to vary considerably across southern Australia, and we expect that they will be influenced primarily by rainfall (soil moisture) and soil temperature, with the breeding extending into the summer months in the cooler, wetter regions. This prediction needs to be tested in association with the monitoring of beetle establishment.

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 may stay underground as third instar larvae for a further 12 months – a three-year life cycle. This delayed development 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 rainfall and with temperature, with cold conditions inducing and increasing the incidence of diapause. This needs to be monitored at each *B. bubalus* release site. A protocol for this evaluation is outlined in Appendix 1.

5.2.1 Discussion summary: *Bubas bubalus*

The key points in the history of *B. bubalus* in relation to South Australia are:

- Cohort 1 (sent to Adelaide as broods in spring 2014) produced about 10 adults in spring 2014, none in spring 2015 and 15 in spring 2016 (a 3-year life cycle).
- The beetles that gave rise to the estimate of several hundred *B. bubalus* larvae in February 2016 (Project B.ERM.0214 final report) were the progeny of cohort 2, brought to Adelaide from Canberra in spring 2015.
- Adult F0 *B. bubalus* breeding at Strathalbyn ceased in mid-summer 2015–2016 (Project B.ERM.0214) and the F1 larvae (in the soil) developed in autumn–winter 2016.
- Post-diapause F1 adult beetles began emerging in July 2016 and emergence took place over a period of about 6–10 weeks: they commenced breeding soon after emergence.
- Newly emerged beetles were trapped, counted, sexed and transferred to the new tunnel nurseries (n=1,331 beetles) and one other tunnel nursery (n=162, total n=1,493). In addition, 165 beetles were allocated to the ‘insurance’ locations.
- The F0 to F1 (2015–2016) generational change (about 600 to 1,658 beetles) comprised a 2.8-fold increase in numbers but a proportion of the F1 generation remained in the soil in larval diapause and emerged in spring 2017.
- The one-year cycle F2 beetles (n=2,250 beetles) began emerging in Strathalbyn in August 2017. There were two apparent emergences, one in early spring and one in late spring (Figure 8). We have no explanation for the bimodal emergence pattern.

- The F1 to F2 (2016–2017) generational change (about 1,658 to 2,250 beetles) comprised a 1.4-fold increase in numbers but a proportion of the F2 generation remains in the soil in larval diapause and may emerge in spring 2018.
- The decision in late September to transfer the breeding of *B. bubalus* to Western Australia meant that we needed to harvest a component of the breeding *B. bubalus* and ship them to WA. All the *B. bubalus* that could be trapped (N=185, Table 2) were transferred to WA on 18 October 2017.

5.3 Field releases

A program for the release of the *O. vacca* and *B. bubalus* to field situations across southern Australia in September 2017 was developed and approved. However, a subsequent decision to retain breeding stock for mass rearing altered these plans and one field release occurred (19 September 2017).

The *O. vacca* beetles at Strathalbyn had been destined for field releases but on Wednesday 27 September 2017, however a revised plan to mass rear beetles at Armidale, NSW and Mandurah, WA was developed. New field nurseries were commissioned at Armidale and new nurseries were activated in WA to receive the spring-active beetles from Strathalbyn. Future releases to the field will be entirely dependent upon the breeding success achieved at CSU, NSW and at Mandurah, WA.

5.4 Danger points in the dung beetle life cycle

There are a number of situations during the life cycle of the beetles where unfavourable conditions may jeopardise the number of broods produced per female, and the survival of the broods and the subsequent adults. These situations are:

- newly emerged F0 adults in spring
- F1 adults in late spring–early summer
- brood production and survival.

Conditions that may lead to unfavourable environments and hence lower than maximal brood production and survival are:

- The **supply of appropriate dung** may be insufficient to allow the expression of their maximal reproductive potential. A regular supply of fresh, moist dung is required.
- **Dung quality** may be low. Dung from stock grazing green pasture is best.
- **Dung type**. Sheep and cattle dung appears to be acceptable. Horse dung may be acceptable.
- **Dung contamination** with chemicals. The effects of gut-worm treatments of cattle and sheep on *O. vacca* and *B. bubalus* have not been established but it is reasonable to assume that they will be killed by most of the macrocyclic lactones, so it is essential to source dung from stock that have not been treated with parasiticides for the previous 6 weeks at least.
- **Soil type/soil moisture**. The soil may not allow the beetles to construct appropriate tunnels (being too soft or too hard). The interactions between soil type, soil moisture and incident rainfall patterns, along with the impact of moist dung (for breeding and feeding) on burial, may create soil environments that are hostile to the breeding adults or to brood production and

survival. For example, low (22%) spring recovery of the autumn-emerged F1 *O. vacca* may be due to anyone of a number of factors, but high soil moisture is a prime candidate.

All of these factors need to be considered when evaluating the performance of future field releases.

5.5 Breeding success at Strathalbyn

5.5.1 Onthophagus vacca

The generation-to-generation increase in numerical abundance in the Strathalbyn nurseries was 6.8-fold for F0 to F1, 12.6-fold for F1 to F2 and 3.0-fold for F2 to F3. In addition there was a summer breeding event in the F3 beetles that produced an autumn-emerged cohort of F4 beetles. Over that 3-year period the total number of *O. vacca* at Strathalbyn has increased from 76 adults to 16,795.

While this is commendable, we point out strongly that if the generation-to-generation increase had been 15-fold each year, the total emergence in spring 2017 would have been in the order of 240,000 beetles (or 570,000 beetles with a 20-fold increase), rather than the achieved 16,795 beetles.

This marked contrast illustrates the priority need to understand and resolve the constraints on beetle breeding in the nursery environment. We consider that in ideal conditions a generation-to-generation increase of 20-fold is quite feasible. 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.

Over the past four years successive requests to investigate these parameters have not been supported. Observe above the consequences of those decisions.

Similarly, the poor results from the CSIRO mass rearing in quarantine may be in large part attributed to a lack of understanding of the physical requirements of the larval and adult beetles.

We also consider that emphasis on the fecundity of *O. vacca* (one of the main arguments for importing the Moroccan strain) is misplaced because the importance of potential changes in fecundity is minor in relation to the importance of improved adult and larval survival. For example, a 30% increase in the fecundity of the beetles that arrived at Strathalbyn would have increased the 2017 number from the current 17,000 to about 37,000 beetles. In contrast, increasing the generation-to-generation survival to 15-fold would have increased the 2017 number from the current 17,000 to about 240,000 beetles.

5.5.2 Bubas bubalus

The generation-to-generation increase in numerical abundance in the Strathalbyn nurseries was 2.8-fold for F0 to F1 and 1.4-fold for F1 to F2.

We point out that a 20-fold increase per generation would have yielded over 200,000 F2 beetles in spring 2017 with one generation per year (no diapause) instead of the current crop of 2,250 adults.

The issues raised in the above discussion apply not only to *O. vacca* and *B. bubalus*, but also to all new species that are to be brought to Australia. Serious attention must be given to the constraints limiting reproductive success of dung beetles in the laboratory and in field nurseries.

6 Conclusions/recommendations

- We conclude that mass rearing of both species in field nurseries can generate significant increases in numbers over successive generations.
- We conclude that there are a range of factors that appear to reduce the generation-to-generation increase in numbers, which has ranged from 3-fold to 12-fold for *O. vacca* and from 1.4-fold to 2.8-fold for *B. bubalus*. The factors responsible for these variations are not known. Both species are considered to have a potential of a 20-fold increase per generation.
- We strongly recommend that experimental studies on the reproductive performance and survival of the immature dung beetles of both species be undertaken to develop rearing protocols that can consistently produce a 20-fold generation-to-generation increase in numbers in field nurseries.
- We recommend that studies be undertaken to capitalise on the observation that a component of the *O. vacca* population can have two generations per year, thereby potentially doubling the rate of increase of the species.
- We recommend that *B. bubalus* diapause be examined with a view to providing environmental conditions that allow all individuals to express 1-year life cycle, and so avoiding the expression of 2- and 3- year life cycles.

7 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).

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 *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

One of the determined and reliable dung beetle enthusiasts has successfully introduced *Bubas bison* and *Copris hispanus* to his property. He initiated feeding biochar to cattle so that the dung burial would increase soil carbon levels. He has co-authored a refereed journal article on dung beetles and biochar.

A second determined and reliable dung beetle enthusiast who has successfully introduced *Bubas bison* to his property on Kangaroo Island. He has provided daily trap catch data for *B. bison* for one year and his data are presented in the book *Dung down under*. *B. bison* has become extremely abundant on his property and it is expected that *O. vacca* and *B. bubalus* will both prosper there.

A third determined and reliable dung beetle enthusiast has successfully introduced *Bubas bison* to her property in the Adelaide Hills.

A Landcare member who over the past 5 years has introduced *B. bison* to his property, and has worked with DBSI and the Mitta Valley Landcare Group to introduce *B. bison* throughout the district, where it has prospered.

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

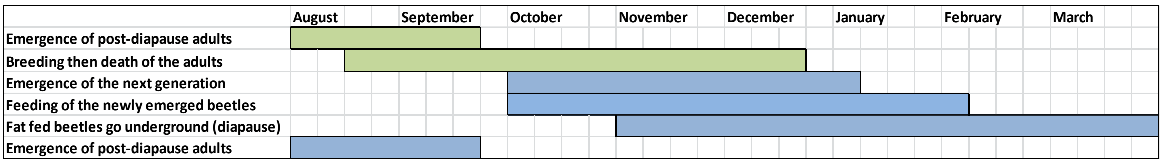

Figure 1: Life cycle of *O. vacca* in the field

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.

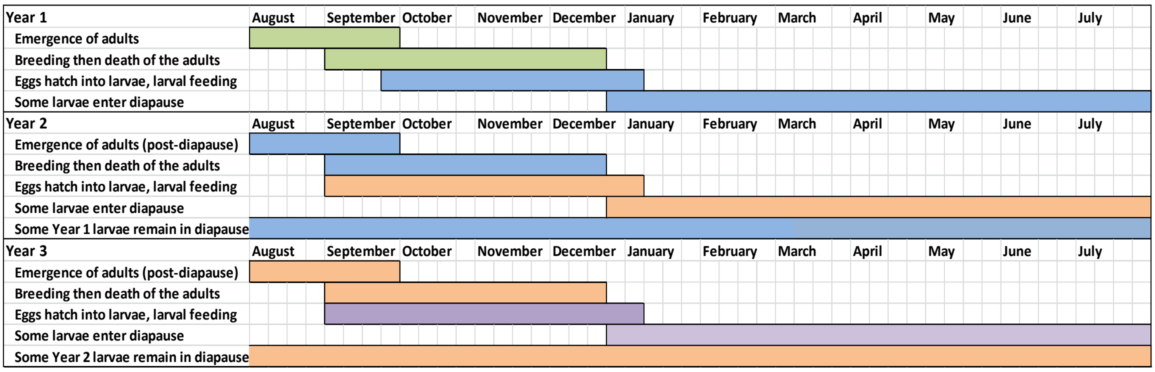

Figure 2: Life cycle of *B. bubalus* in the field

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) (that is, 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				