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Field establishment of dung beetles in South Australia

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Abstract

Project ERM.0123 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.

In 2014–15 *O. vacca* increased 5-fold at Strathalbyn and 1.5-fold at Port Elliot but failed at Bool Lagoon. Overall, 115 live F_0 females released in October 2014 gave rise to about 240 F_1 females in spring 2015. These beetles bred successfully at Port Elliot and Strathalbyn in spring 2015. In February 2016 the numbers of F_2 females was estimated at 1000+.

B. bubalus (approximately 1300 brood balls) was introduced to field nurseries at all three locations in SA in late spring 2014. These yielded about 10 females (some of which bred) and about 100 third instar larvae (3LL) in diapause. No adults emerged in spring 2015: all live beetles were diapausing 3LL. All broods were exhumed and consolidated at Strathalbyn, where they are expected to produce adults in spring 2016. A second cohort, which falls outside this project, appears to have bred successfully at all three locations in summer 2015–16.

It is expected that both species will be synchronised with southern hemisphere seasonal conditions by spring 2016.

The two species appear to have been saved from extinction in Australia, provided there is ongoing support for the maintenance and breeding program. Widespread distribution of these two species across southern Australia should seriously reduce southern bushfly numbers in spring and lead to increased pasture production and more sustainable farming systems.

April 2015: A pupa of *B. bubalus*

April 2015: An adult *O. vacca*

Executive Summary

Executive Summary

The contract with MLA (Project B.ERM.0214) required reporting progress against seven objectives. Each objective is listed below and progress is briefly reported. This is followed by a more detailed explanation.

1. Have successfully established *Onthophagus vacca* and *Bubas bubalus* to enable field reproduction of beetles

O. vacca has been reared at two of the three test locations from about 115 F_0 laboratory-reared females in spring 2014 to an estimated 1000+ F_2 beetles that are currently (autumn 2016) held in field nurseries as adults in reproductive diapause.

B. bubalus broods (Cohort 1, n=1300) received in Adelaide from CSIRO in late spring 2014 produced about 10 females in spring 2014: some of these bred but no adults emerged from the subsequent broods in spring 2015. The remaining broods from Cohort 1 contained about 100 live third instar larvae (F_0 beetles), which remained as larvae in diapause until the end of the project (1 March 2016). A second cohort of beetles (F_0 adults), which falls outside this project, were sent to Adelaide from CSIRO and appeared to breed successfully in summer of 2015–16. Cohort 2 broods are expected to produce hundreds of F_1 adults in spring 2016, when the 100 F_0 surviving larvae from Cohort 1 (in diapause in March 2016) are also expected to emerge as adults.

2. In conjunction with the producer cooperators of the three sites, undertake monitoring actions and required management of the sites to ensure establishment of beetles

At all three sites (Strathalbyn, Port Elliot and Bool Lagoon) at least two field nurseries were established for each species. During the critical times of the year (spring, summer, autumn) the nurseries were provided with fresh dung each week. The level of beetle activity in the older dung in the nursery was assessed and recorded by the collaborators. The information on seasonal patterns of dung shredding (indicating feeding) and burial (indicating breeding) provided a description of the seasonal changes in the activity of each species. Intensive monitoring was conducted at Strathalbyn and additional large nurseries were constructed to accommodate the expanding beetle breeding program.

3. Provide an assessment of the number of beetles of each species for rearing after one breeding cycle

For *O. vacca*, after one breeding cycle, there were about 240 adult female beetles.

For Cohort 1 of *B. bubalus* (the 1300 MLA-CSIRO broods), after one breeding cycle there were about 20 adult beetles and about 100 diapausing third instar larvae: these latter were displaying a 3-year life cycle. Cohort 2 appeared to breed successfully in spring 2015 and we expect that this activity had given rise to some hundreds of broods by 1 March 2016. These are expected to emerge as adults in spring 2016 and 2017 but the numbers are not known.

4. Report on the emergence behaviour and (based on population estimates) egg production as compared to the European home range, CSIRO lab rearing and previous imported species (e.g. *Bubas bison*)

The laboratory-reared adult F_0 *O. vacca* were introduced to the field cages late in spring 2014. As a consequence the beginning of their breeding activity was delayed by 6–12 weeks and hence extended into late summer. The F_0 beetles gave rise to the next generation (F_1 beetles) which emerged and fed in summer–autumn 2014–15 before entering an adult reproductive diapause and tunnelling into the soil, where they waited until spring 2015 to emerge (as adults) and begin a new cycle.

The spring 2015 emergence of adult F_1 *O. vacca* appears to conform largely to the predicted seasonal pattern based on European data in which the adult beetles emerge from the soil in spring (August) to feed, then breed for several months before dying, and giving rise to a new generation (F_2) that emerges as adults in early summer. However, in contrast to the European data, a proportion of the newly emerged summer F_2 beetles bred soon after emergence as adults (i.e. they did not enter diapause straight away) to produce F_3 broods (in January 2016) before entering diapause in March 2016. These broods had reached the late 3LL/pupal stage by mid-March 2016 and are expected to give rise to adults in autumn 2016. Similar pre-diapause breeding was observed in some *O. vacca* in Canberra.

We expect that the F_3 adults will emerge to breed in spring 2016, thereby demonstrating the possibility of two generations per year in field nurseries in southern Australia.

We anticipate that *O. vacca* will become synchronised with the southern hemisphere seasonal cycle, 6 months out of phase with its European counterpart.

With *O. vacca*, the generation-to-generation increase in numbers achieved in the F_0 to F_1 breeding cycle was about 5-fold at Strathalbyn and 1.5-fold at Port Elliot. This is similar to that achieved in some CSIRO laboratory trials and better than in others, but far less than the reported potential of a 25+-fold increase in numbers (50 broods per female).

The emergence of both cohorts of adult laboratory-reared *B. bubalus* (2014 and 2015) had become partially synchronised with the southern hemispheric conditions, with adult emergence occurring in late spring and breeding being delayed (by an adult non-feeding diapause) until summer. It is expected that the 2016 adult emergence will occur in spring, thus synchronising its seasonal activity with the seasonal weather cycle of southern Australia.

With *B. bubalus*, the generation-to-generation increase in numbers achieved in the F_0 to F_1 breeding cycle is as yet unknown. However, for Cohort 1(from ERM.0123), it appears that the 1300 broods received from Canberra will have produced about 20 adult beetles and about 100 diapausing 3LL over the period winter 2014 to summer 2016. However, a separate breeding program (conducted after termination of Project ERM 0123) produced some hundreds of adults that emerged in Adelaide between August and December 2015.

DBSA field monitoring of *Bubas bison* populations in SA reported a 3- to 5-fold increase per generation. This is far less than the reported potential of 25+-fold

generation-to-generation increases in numbers based on the reported potential fecundity of both *B. bison* and *B. bubalus*.

5. Comment on the success of adaptation of northern hemisphere beetles to southern hemisphere field conditions and make recommendations for importation processes (recognising quarantine requirements).

It appears that the introduction of both species to the field nurseries in SA is likely to have achieved synchronisation of the seasonal phenology in alignment with the southern hemisphere's seasonal weather cycles by spring 2016.

The case for introducing another 25 species to Australia has been addressed in a submission to the Hon Barnaby Joyce (Minister for Agriculture and Water Resources) and the Hon Greg Hunt (Minister for the Environment).

The methodology to achieve high success in the mass rearing of imported beetles in field nurseries needs to be developed with support from MLA. These refined protocols will provide the basis for efficient mass rearing of future importations of dung beetles into Australia.

6. Work with MLA (on behalf of other parties) to devise a delivery plan that can support ongoing rearing and distribution of beetles

A detailed plan to develop mass-rearing conditions (field cage design, beetle density, dung supply, multiple sites to spread the risk of failure, etc.) is being developed by DBSA in association with Creation Care (Strathalbyn). A model in which variables that affect the rate of beetle production is being constructed. This model will enable the sensitivity of the system to various parameters to be probed. The model outputs will indicate the rate at which beetles might be expected to become available for redistribution across southern Australia.

A proposal to identify the key favourable regions for each species has been developed and submitted to MLA (July 2015). We intend that release of starter colonies will initially focus on these preferred regions.

7. Describe ongoing management for forty per cent of the harvested beetles, which will be retained by Dung Beetle Solutions Australia

The beetles retained by DBSA will be used as seed stock for mass rearing. They will be provided with *ad lib* dung in field nurseries with low beetle densities at Strathalbyn, Port Elliott and Bool Lagoon. When numbers permit they will be distributed in suitable regions across southern Australia.

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1 Background

MLA funded CSIRO (Project ERM.0123) to import *Onthophagus vacca* and *Bubas bubalus* to fill a gap in dung beetle activity in early spring across southern Australia (Doube and Marshall 2014). The beetles were reared under southern hemisphere conditions (in the laboratory) and their seasonal activity patterns partially synchronised with southern hemisphere weather patterns (Wright et al. 2015). Wright et al. conclude that the major unsolved issue with rearing remains the longevity and fecundity of adults of both species and juvenile mortality among *B. bubalus*.

The literature on the biology and natural history of the two target species has been well summarised by Wright et al. (2015).

In essence, O*. vacca,* a day-flying beetle, is active during spring and early summer, when it shreds and buries fresh cattle and sheep dung in open pasture (Lumaret and Kirk 1987, Mena et al. 1989, Lumbreras et al. 1990). Oviposition in the field takes place over 3–4 months. The *O. vacca* nest has a main vertical tunnel and then secondary tunnels leading to brood chambers (one egg each), with 4–5 brood chambers per dung deposit (Sowig 1996a, 1996b). The larvae develop in the brood chambers and, upon emerging as adults, enter an obligatory diapause until the following spring (Lumaret and Kirk 1991). Three releases of *O. vacca* were made by CSIRO in the ACT in September–October 1980 and another near Toodyay in WA in October 1983 (Tyndale-Biscoe 1996) but none established (Edwards 2007).

B. bubalus is a night-flying univoltine species adapted to grassy pastures and open woodland in the Mediterranean-climate areas of southern Europe, where it buries cattle and horse dung (Kirk and Ridsdill-Smith1986, Lumaret and Kirk 1987, Lumbreras et al. 1990). In southern France, *B. bubalus* emerges in late winter–early spring and, after a brief period of intensive feeding, begins oviposition, which continues for some months. A few adults emerge in autumn and feed before going underground to pass the winter with the larvae, pupae or adults still in the faecal shells (Lumaret and Kirk 1987, 1991). In the cooler areas of Spain, the adult beetles are present in all seasons except summer. Most adults emerge in spring but some also emerge in autumn, and they all begin to breed in late winter–early spring (Lumbreras et al. 1990, 1991). Eggs are laid into 10–15 cm sausage-shaped brood masses up to 30 cm below the surface, generally with one egg at each end. Steinbauer and Wardhaugh (1992) report on imported *B. bubalus* to Australia. Beetles collected in Spain in April (spring in the northern hemisphere) laid an average of 15 eggs per female but, sadly, none of the progeny were released to the field (Steinbauer and Wardhaugh 1992).

These failures to establish field populations of *O. vacca* and *B. bubalus* in the field in Australia emphasise the need to carefully manage the current introductions. With limited beetle numbers arising from the CSIRO rearing program, options were limited. Climate matching has identified priority regions where O*. vacca* and *B. bubalus* are most likely to establish. CSIRO and MLA had an agreed approach for establishment in field conditions. In late spring 2014, in order to minimise geographic risk, beetles were released by Wright et al. (2015) to paddocks in WA and NSW and to field nurseries in SA.

The paddock releases in WA and in NSW were conducted in late spring 2014. Spring that year was unusually dry (Wright et al. 2015 found that *O. vacca* does not breed in dry soil) and so conditions would have been somewhat hostile to the released beetles. They were

released into paddocks with soil types which may not favour survival of *O. vacca* (see Section 5.8) and their environmental care was low (minimal additional feeding). Several hundred adult *O. vacca* and a moderate number of broods were released but it is commonly believed that a release of a thousand or more adults is required to ensure establishment, even under favourable conditions. Large numbers of laboratory-reared broods of *B. bubalus* were released to trenches in the field in late spring 2014 but the proportion of broods that contained live beetles was not assessed. However, a parallel cohort (from the same rearing facility) of 1300 broods was released to the field nurseries in SA in late spring 2014. Only a small proportion of these contained live beetles (a few adults, with the remainder as third instar larvae in diapause). Most of the 3LL were still larvae 18 months later: no adults emerged in spring 2015. If the broods of *B. bubalus* released in WA and NSW were in a similar condition to those sent to South Australia, then only a small number of adult beetles will have emerged in spring 2015 and the remainder will be diapausing 3LL in autumn 2016.

Taking these considerations into account, the establishment of field populations in NSW and WA is at best uncertain and, if they establish, it will be a decade or more before adults can be harvested in numbers sufficient for redistribution. From this we conclude that the high-care environment for the beetles in South Australia provides a far more secure route towards ensuring field establishment than do the field releases in WA and NSW.

The process of establishment of these two species in the field can be divided into two key phases:

- establishment in field conditions
- supporting mass rearing to enable widespread release.

In the first phase of this process (the subject of this report), small numbers of *O. vacca* were sent to the DBSA laboratories in SA in spring 2014, where the beetles were maintained until they were ready to breed. They were then released to field nurseries at three locations in SA, thereby minimising geographic risk. Similarly, a moderate number of laboratory-reared broods of *B. bubalus* were sent to SA and these were also released into different field nurseries at the same three locations.

The project here reports on progress with establishing field nurseries of both species in SA. There are multiple unknowns around the beetles' field emergence pattern in the southern hemisphere. To maximise success with the caged field releases, weekly feeding and monitoring was required. This project provided partial support for the management of three sites in South Australia. A substantial additional contribution (about 80% of the total cost of the project) was provided by Dung Beetle Solutions Australia (DBSA) and Creation Care (Strathalbyn). Our aim was to establish breeding populations of both species in field nurseries and, in association with MLA, to develop an ongoing rearing plan that enables distribution of these two species across southern Australian.

2 Project objectives

By 1 March 2016:

1. Have successfully established *Onthophagus vacca* and *Bubas bubalus* to enable field reproduction of beetles
2. In conjunction with the producer cooperators of the three sites, undertake monitoring actions and required management of the sites to ensure establishment of beetles
3. Provide an assessment of the number of beetles of each species for rearing after one breeding cycle
4. Report on the emergence behaviour and (based on population estimates) egg production as compared to the European home range, CSIRO lab rearing and previous imported species (e.g. *Bubas bison*)
5. Comment on the success of adaptation of northern hemisphere beetles to southern hemisphere field conditions and make recommendations for importation processes (recognising quarantine requirements)
6. Work with MLA (on behalf of other parties) to devise a delivery plan that can support ongoing rearing and distribution of beetles
7. Describe ongoing management for forty per cent of the harvested beetles, which will be retained by Dung Beetle Solutions Australia.

3 Methods

3.1 Origin of breeding stock

The two new species of dung beetles (*Onthophagus vacca* and *Bubas bubalus*) were collected from CSIRO Canberra in spring–early summer 2014 and taken to Adelaide, where they were divided into three subgroups each and allocated to field cages at three locations in South Australia (Strathalbyn, a loamy soil; Port Elliot, a clay-loam soil; Penola (Bool Lagoon), a cracking clay soil). The locations correspond with regions in which these species are expected to prosper, as indicated by climate matching analyses. The field cages are pictured in appendix 2.

The beetles were installed in the field cages shortly after arriving in Adelaide. In the field cages they were fed fresh dung weekly at all three locations. Evidence of beetle activity (dung burial and shredding, and tunnels) was recorded at the same time.

3.2 Field nurseries

Four field nurseries (two for *O. vacca* and two for *B. bubalus*) were established at each of the three locations (Strathalbyn, Port Elliot and Bool Lagoon) (appendix 2).

Each nursery comprised a cut-off rainwater tank about 1 metre high and half filled with local topsoil. A removable lid of beetle-proof mesh was constructed for each field nursery.

At Strathalbyn, an additional six nurseries were constructed to accommodate beetles as successful breeding and emergence led to a requirement to keep various cohorts separate. The cost of these was borne by Creation Care.

3.3 Monitoring

Because the seasonal activity of the beetles in the field was very uncertain (their having recently arrived from the northern hemisphere) it was considered necessary to provide fresh dung on a weekly basis so that any newly emerged beetles would have food available.

Further, the impact of the beetles on the dung was monitored in order to provide an index of the developmental phase of the adult beetles, with shredding indicating feeding (but not breeding) and tunnels and dung burial indicating that the beetles were breeding. The amount of dung removed was estimated each week and since the amount that had been added was also known, we could produce a rough measure of the amount of dung buried. Knowing the number of beetles in each nursery, we were able to calculate the approximate amount buried per pair of beetles in a lifetime.

4 Results

4.1 Onthophagus vacca

Adult F_0 *O. vacca* were released to the field cages in October–November 2014 and took several weeks to acclimatise (no activity) before beginning to feed (shredding the dung) and then breed (burying dung and laying eggs in the buried dung). Breeding continued thereafter until January 2015.

Upturned dung pad (left) and soil surface under the pad (right) showing high levels of tunnelling by *O. vacca*, in late January 2015. Photo Greg Dalton

Adults (F_1 beetles) emerged over the period December–March 2015. Egg-to-adult development was considered to take about 8–10 weeks under the conditions in the nurseries. Parents and offspring (recognised by the degree of tibial wear) were present at the same time. Parental beetles died in late summer, while the newly emerged F_1 beetles fed for some time before stopping feeding: it was presumed but not determined that they did not breed during that time. The F_1 adult beetles then entered a non-feeding phase (a non-feeding diapause) and tunnelled into the soil to wait for the following spring, during which time diapause development was completed. In August 2015 these F_1 beetles re-emerged from the soil and fed for some weeks before beginning to breed. Breeding continued from September to early December 2015. The F_2 beetles emerged in December 2015 – January

2016 and fed for some weeks. At Strathalbyn and Port Elliot, an unknown, but substantial, proportion of these individuals bred in January before entering a non-feeding adult reproductive diapause.

Newly emerged (L) and older (R) *O. vacca* showing new and worn digging claws

Canberra-produced broods of *O. vacca* (approximately 150) brought to Adelaide in November 2014 were retained in their original holding material (vermiculite) and placed in permeable bags, which were buried in the soil in field cages at Strathalbyn. Some F_1 beetles emerged in December. In late January 2015 all the broods were exhumed and broken open. Only 16 adults were recovered and added to the local breeding stock. The remainder had died in the early developmental stages (eggs/first instar larvae), before arrival in Adelaide. Some adults began to breed in January.

4.1.1 Strathalbyn

Adult *O. vacca* were installed in two field cages (with 20 and 15 pairs respectively) in late spring 2014. The beetles bred from October 2014 until January 2015. Between January and March the second generation (F_1) emerged and fed (but presumably did not breed), before entering a non-feeding diapause and digging into the soil, where they waited until the following spring before re-emerging. In August 2015 a total of about 350 F_1 beetles were recovered from the breeding pens. This represents a 5-fold increase in numbers, or 10 F_1s per female.

Breeding success of *O. vacca* was low at the other two locations. In order to maximise numbers produced in the next generation most breeding *O. vacca* were concentrated at Strathalbyn. A large breeding pen (3 m x 9 m) was established there in spring 2015 and the beetles were fed 3 times per week at 27 feeding stations within this arena. Judging by the level of dung burial, breeding appeared to be successful. The next generation (F_2 beetles) emerged in December 2015 – January 2016. Some of these beetles were in breeding condition. By March 2016, all appeared to have entered a non-feeding adult reproductive diapause.

There was a low level of contamination of the field cages with another dung beetle, *Onthophagus taurus*, in some instances.

4.1.2 Port Elliot

Adult *O. vacca* were installed in two breeding pens (with 20 pairs each) in late spring 2014. The beetles bred from October 2014 until January 2015. Between January and March the second generation (F_1) emerged and fed (but apparently did not breed) before entering a non-feeding diapause and digging into the soil, where they waited until the following spring before re-emerging, in August 2015. These bred for about one month before being

harvested and transferred to Strathalbyn. The August–September 2015 breeding gave rise to a new generation of F_2 adults at Port Elliot in December 2015.

A fifth field cage was established at Port Elliot in January 2016 to accept the newly emerged F_2 adult beetles. Many of these bred in January 2016 but all surface activity had stopped by the end of February 2016.

An extremely high level of dung burial was observed from December 2014 until March 2015. The species present were not sampled. In September 2015 beetles emerged and began burying much dung. Trapping the beetles in September and October revealed a high level of contamination of the breeding chambers with *O. taurus* and some *Euoniticellus fulvus* despite great care being taken to collect fresh uncontaminated dung. About 120 F_1 *O. vacca* were recovered and over 1000 *O. taurus* were removed at the time of trapping. The poor breeding performance could have been due to competition between *O. taurus* (a contaminant) and the F_1 *O. vacca* in summer, or to the environmental conditions at Port Elliot being less favourable than those at Strathalbyn.

4.1.3 Bool Lagoon

Adult *O. vacca* were installed in two breeding pens (with 20 pairs each). There were 5 feeding stations per pen. The beetles appeared to breed at two adjacent feeding stations in one breeding pen from October 2014 until January 2015. There was minimal evidence of beetle activity at the other 8 feeding stations. No beetles were recovered in spring 2015, despite an intensive trapping program. The explanation for the failure of *O. vacca* at Bool Lagoon is not known, but it is noteworthy that the dung at all 10 feeding stations was strongly colonised by slaters and earthworms, indicating that the environment was not toxic for those species.

It may be that the soil type (a heavy clay) was not suitable for *O. vacca*.

4.2 Bubas bubalus

Two cohorts of *B. bubalus* are considered. Cohort 1 was derived from MLA–CSIRO breeding in autumn–winter 2014 (Project ERM.0123). Cohort 2 was produced by CSIRO in autumn–winter 2015, about 6 months after the work on Project ERM.0123 had ceased.

4.2.1 B. bubalus Cohort 1

Broods of *B. bubalus* (approximately 1300 in 21 boxes of vermiculite) were brought from CSIRO Canberra to Adelaide in November 2014. About 500 of these were natural broods and the remainder were artificial broods: all were produced at CSIRO Canberra in autumn–winter 2014. In Adelaide, the broods were transferred to 21 water-permeable mesh bags (with about half the vermiculite) and the bags were buried 30 cm deep in soil in the field cages at the three test locations in South Australia. Each bag was provided with fresh dung at weekly intervals to ensure that any adults that emerged had food available.

In early summer 2014, a small number of adults (F_0) emerged from two bags of natural broods (one each at Strathalbyn and Port Elliot: about 5–10 beetles each). These were fed at weekly intervals and the beetles buried dung and appeared to breed. However, no F_1 adults emerged in spring 2015, suggesting one of the following:

- that they had failed to breed

- that the broods had died
- that the broods had not yet produced adults (and instead contained 3LL in diapause).

We favour the last explanation and expect adult F_1 beetles to emerge from this field cage (field cage 4) in spring 2016.

During late summer and autumn 2015, the remaining buried broods (about 1200) from all three locations were exhumed, broken open and examined carefully. There were low levels of survival in the broods (range 0%–25% between bags) (Table 1). There was no consistent difference in levels of mortality in natural and artificial broods and bags adjacent to each other in the same field cage displayed widely contrasting levels of mortality, indicating that the cause of the mortality was independent of environmental conditions in the field cages. Careful dissection of all broods revealed that most of the 'dead' broods contained no obvious larval feeding chambers, indicating that mortality had occurred in the egg or early larval instars, before the broods arrived in Adelaide.

Table 1: Summary of *B. bubalus* broods remaining at Strathalbyn in September 2015

Type of brood	Artificial		Natural	
	Number	% of total	Number	% of total
Empty broods	65	32.7	52	28.9
2 LL dead	23	11.6	11	6.1
3 LL dead	24	12.1	39	21.7
3 LL live	43	21.6	42	23.3
Intact faecal shells	13	6.5	32	17.8
Prepupae	0	0.0	0	0.0
Dead adults	5	2.5	2	1.1
Tanned live adults	2	1.0	2	1.1
Brood balls not opened	24	12.1	0	0.0
Total	**199**	**100**	**180**	**100**

Most of the survivors (about one hundred individuals) were third instar larvae at this time and were presumed to be in diapause. The diapausing 3LL (in reconstructed faecal shells) from Port Elliot and Bool Lagoon were collected and the 3LL from all three locations were consolidated into one field cage at Strathalbyn. Examination of a substantial subsample of these broods in spring 2015 indicated that they had remained as 3LL.

From this we conclude that most of the surviving live *B. bubalus* (100+) in the broods produced in Canberra in autumn–winter 2014 had become diapausing 3LL by spring 2014, remained in diapause over the 2014–15 summer and were still in diapause in spring–early summer 2015. We anticipate that these beetles will emerge in spring 2016 as adults synchronised to southern hemisphere conditions.

These data suggest that in some circumstances *B. bubalus* can exhibit a three-year life cycle, with the 3LL remaining in diapause for over two years.

4.2.2 B. bubalus Cohort 2

MLA Project ERM.0123 to import two new species to Australia was completed by December 2014. However, after that time, CSIRO staff member Patrick Gleeson continued laboratory rearing a small number of *B. bubalus*, using knowledge gained over the previous 2 years. This effort resulted in a batch of adults and broods which were transferred to Adelaide in August 2015.

The shipment (collected from Canberra on 7 August 2015) comprised a total of 300 faecal shells, 350 live adults and 11 dead adults.

The faecal shells were monitored at weekly intervals for the next 4 months, over which time, through natural emergence, they produced over 250 adult beetles. By mid-December 2015 the remaining small number of entire faecal shells were broken open and inspected. These contained mostly dead adults, and a few diapausing 3LL. Overall there were low levels of mortality.

Adult beetles were held in boxes with fresh horse dung in the laboratory in Adelaide until they showed an inclination to feed and bury the dung. This took 4–8 weeks: During the early weeks the beetles secreted themselves just under or in the dung and showed no inclination to feed. Once the beetles had become obviously active (in October–November), they were transferred to field cages and supplied with fresh cattle dung at weekly intervals.

The majority of the beetles were located in one large (3 m x 9 m) field nursery at Strathalbyn but four smaller field nurseries (each with 5–10 pairs of beetles) were also established at Port Elliot and at Bool Lagoon. In all nurseries at all locations, the beetles initially fed on the dung (as evidenced by dung shredding) and then buried much dung in late spring–early summer. After that time little dung was shredded or buried and dead beetles were observed on the soil surface in some of the nurseries.

5 Discussion

5.1 Establishing breeding colonies in field nurseries

O. vacca was successfully established in field nurseries at two locations (Strathalbyn and Port Elliot). A small number of *O. vacca* adults were placed in field nurseries at three locations in South Australia in late spring 2014 (35–40 pairs per location).The beetles were most productive one location (Strathalbyn) and so most of the breeding in spring 2015 was consolidated there, with about 240 pairs established in a large field nursery with 27 feeding stations, but a small cohort (about 50 pairs) was also set up in a new nursery at Port Elliot.

B. bubalus appeared to be breeding (i.e. there was considerable dung burial) in field nurseries in spring 2015 at all three locations. However, the field nurseries are yet to produce any F_1 beetles and so breeding in the field has not yet been confirmed. The small number of adults produced in spring 2014 were the product of laboratory rearing and, although they appeared to breed in summer 2014–15, no F_1 progeny emerged in spring 2015, suggesting that, in this cohort, either the larvae died in the broods or diapause had delayed emergence by one year. Examination of exhumed broods in late summer 2014–15 revealed large, healthy third instar larvae, suggesting that larval mortality was unlikely to be the cause of the absence of newly emerged adults in spring 2015.

There were two cohorts of *B. bubalus*. Cohort 1 was derived from MLA–CSIRO breeding in autumn–winter 2014 (Project ERM.0123) and Cohort 2 was produced at CSIRO in autumn–winter 2015, about 6 months after the work on Project ERM.0123 had ceased.

Cohort 1: About 1300 *B. bubalus* broods were produced in Canberra in autumn–winter–spring 2014 and were transferred to Adelaide in early summer 2014. Broods were placed in mesh bags in soil in field cages at three locations in South Australia in November 2014. Sampling in summer–autumn 2015 indicated that most (70% to 100% depending on the batch) of the occupants of the broods had died as eggs or young larvae before arriving in Adelaide. There were no obvious differences in levels of survival between the three test locations or between natural and artificial broods. In summary, by March 2016, the 1300 broods from CSIRO Canberra had given rise to about 20 adults and 100+ diapausing third instar larvae, now consolidated at one location (Strathalbyn). Adult emergence in spring 2016 (comprising F_0 and F_1 beetles) is anticipated, but is far from guaranteed. F_0 emergence in spring 2016 would demonstrate the presence of a 3-year life cycle in that cohort of beetles.

Cohort 2: In August 2015, about 350 adult beetles and about 300 broods were transferred to Adelaide. Most broods gave rise to adults during spring 2015. Adults were fed in the laboratory until they appeared to be ready to feed and breed (a period of 4–8 weeks) and then introduced to field nurseries and supplied with fresh dung at weekly intervals. They appear to be breeding in the field nurseries at all three field locations.

Cohort 1 contrasts strongly with Cohort 2 in that the latter:

- suffered little juvenile mortality
- produced only a small proportion of diapausing 3LL
- produced a majority of healthy adults that (after an initial non-feeding phase in early spring) appeared ready to feed and breed (lay eggs) in spring 2015.

There was no obvious difference in the way in which Cohorts 1 and 2 were treated in the laboratory in Canberra (P. Gleeson, pers. comm.).

5.2 Financing and managing beetle production in 2015–2016

During winter 2015, well before the expected spring emergence of the two new species, an extensive proposal for beetle management in spring and summer 2015–16, and beyond, was prepared by DBSA. It was submitted to MLA on 22 July 2015 (appendix 1) and contains a recommended option for managing the MLA component of the two species as well as three appendices (namely, relevant data from the 2012–14 CSIRO mass rearing program, transition of *B. bubalus* from laboratory to the mass rearing facility in the field and transition of *O. vacca* from laboratory to the mass rearing facility in the field).

In July 2015 DBSA estimated that about 500 pairs of *O. vacca* and 135 pairs of *B. bubalus* would be available in spring 2015. MLA and DBSA had joint ownership of the spring emergence in the ration of 60:40 respectively. This estimate of numbers proved to be optimistic, with about 240 pairs of *O. vacca* emerging and no *B. bubalus* emerging (due to a persistent larval diapause). Adult *B. bubalus* (possibly 100 beetles) from Cohort 1 are expected to emerge in spring 2016.

The fate of the MLA component needed to be resolved in winter 2015. Suggested options proposed in July 2015 for these beetles (appendix 1) were:

- to deliver them (140 pairs of *O. vacca* and 50 diapausing 3LL of *B. bubalus*) to MLA for distribution at their discretion
- to allocate them to a DBSA-managed experimental program designed to improve mass rearing procedures and to determine the suitability of a range of environments across southern Australia.

DBSA recommended the latter option to MLA.

Between 22 July and 27 October 2015 there was a series of 13 emails between MLA and DBSA concerning progress with the current and proposed new projects (appendix 1), but there was no specific direction from MLA regarding how we were to manage the MLA component of the two species.

We note that in winter and spring of 2015 there was substantial reorganisation occurring within MLA and that management were under considerable pressure, in an uncertain environment, and with seriously contracting budgets. No doubt this uncertainty contributed to the lack of direction provided by MLA for the management of their component of the dung beetle progeny. Fortunately DBSA and Creation Care were in a position to initiate, at their own expense, strategies to accommodate the expanding dung beetle breeding program.

In spring 2015 over 400 *O. vacca* emerged. DBSA had received no definitive response from MLA about what to do with the MLA component of those beetles. In addition, some hundreds of adult *B. bubalus* emerged from Cohort 2.

The emails in appendix 1 outline the attempts by DBSA to elicit instructions about the fate of the MLA component of the two species. None was forthcoming and so we needed to act independently.

In order to optimise the probability of the two species establishing in Australia, DBSA and Creation Care designed and built a new set of field nurseries to contain these beetles under favourable conditions. Creation Care provided the capital for their construction (labour, land and materials). Both DBSA and Creation Care managements provided extensive *pro bono* support for this expanded project.

The end result is that we expect 1000+ (possibly up to 5000) pairs of each species to be available for mass rearing in spring 2016. This project urgently needs support from MLA. Land needs to be leased and dung production facilities and mass rearing nurseries need to be developed by the end of July 2016, ready for the beetle emergence expected in August 2016.

5.3 Numbers to initiate mass rearing in spring 2016

The breeding success (F_1 to F_{2+3}: spring 2015 to spring 2016) of *O. vacca* will not be known until spring 2016 but we estimate that there will be between 1000 and 5000 pairs available.

Similarly, the breeding success of *B. bubalus* will not be known until spring 2016 but we anticipate that about 100 Cohort 1 adults will emerge in spring 2016. In addition some adults from Cohort 2 may emerge in spring 2016 while others (those in diapause) are likely to

emerge in spring 2017. We estimate that there will be between 1000 and 5000 pairs available, but there is great uncertainty around these estimates.

Obviously the numbers that emerge in spring 2016 will have a huge once-off effect on the numbers of each species achieved in the following years.

5.4 Number of generations per year

5.4.1 O. vacca

The literature (Wright et al. 2015, Sowig 1996a, 1996b) suggests that *O. vacca* has an obligate adult reproductive diapause that inhibits feeding and breeding during autumn and winter and thus that it has only one generation per year in the field. However, in the laboratory, two generations per year have been achieved by abbreviating the 'vernalisation' process, which reactivates the diapausing adult beetles (MLA Project ERM.0123).

Our data for 2015–16 clearly show that at Port Elliot and Strathalbyn early-breeding (August) F_2 *O. vacca* gave rise to a new generation (F_3 beetles), some of which bred in early summer, and that these F_3 beetles stopped breeding and entered reproductive diapause in late summer. Monitoring the development of the broods produced by the F_3 beetles in January 2016 indicates that by mid-March 2016 all broods contained either mature third instar larvae, or pupae. There is no suggestion of a larval diapause in *O. vacca* and so we expect these broods to give rise to adults in about April–May 2016. If they produce breeding adults, a third generation in the one year may be possible.

These observations clearly demonstrate that, in the field nurseries in 2015–16, *O. vacca* achieved two generations per year. The proportion of F_3 adults that bred is not known. Similarly, it is not known whether two generations per year in the field nurseries was an aberration or is a regular feature of the seasonal biology of the beetle in South Australia. These matters will be carefully monitored over the next year, provided that the rearing program continues.

5.4.2 B. bubalus

The data for Cohort 1 (2014–16) clearly show that a small number of adults displayed a 1-year life cycle while the majority remained as diapausing third instar larvae in March 2016. Provided these beetles emerge as adults in spring 2016, they will have displayed a three-year life cycle. In marked contrast, the majority of Cohort 2 displayed a one-year life cycle, with only a small proportion entering larval diapause.

There were no obvious differences in the laboratory rearing of Cohorts 1 and 2 in the CSIRO Canberra laboratories (P. Gleeson, pers. comm.). The factor(s) that induce diapause are not known.

However, from these data we can conclude that *B. bubalus* can express a 1-, 2- or 3-year life cycle which is regulated through a diapause in the third instar larva. This parallels the seasonal biology of *B. bison* in southern Australia.

In addition, there appears to be an adult reproductive diapause in *B. bubalus* which is expressed immediately following natural emergence from the faecal shell, and which delays the beginning of feeding and breeding by 1–2 months. Again, the same phenomenon has been observed in *B. bison*.

5.5 Generation-to-generation increase in numbers

5.5.1 O. vacca

The F_0 to F_1 increase in numbers of *O. vacca* (spring 2014 to spring 2015) was about 5-fold at Strathalbyn and 1.5-fold at Port Elliot. This is at least equivalent to the best achieved in the Canberra laboratories.

Data for the F_1 to F_2 increase in numbers in the field nurseries will be available in spring 2016 but will be somewhat compromised because there was a degree of breeding of F_2 beetles in summer 2015–16. We presume that the progeny of this summer breeding (F_3 beetles) will emerge in spring 2016, and so the spring emergence of 2016 will be a mixture of F_2 and F_3 beetles. This will provide a year-on-year estimate of increased numbers.

The level of reproduction currently observed in the field nurseries is far lower than can be expected if natural fecundity is fully expressed and if there is a high level of brood survival. Improving the expressed fecundity and brood survival of *O. vacca* is a vital aspect of improving rearing efficiency. We should be aiming for a 25-fold increase per generation.

Preliminary estimates of the numbers of *O. vacca* emerging in spring 2016 range from 1000 to about 5000 females (Table 2) with a generation-to-generation increase of 5-fold and 20-fold respectively.

Table 2: Preliminary estimate of numbers of adult pairs of *Onthophagus vacca*

	Spring 2014	**Spring 2015**	**Spring 2015**	**Spring 2016**	**Spring 2016**
	F_0* set up	**F_1 emergence**	**F_1 set up**	**F_2 X 5-fold**	**F_2 X 20-fold**
Strathalbyn	36	200	240	1000	4800
Port Elliot	40	60	20	100	400
Bool Lagoon	40	0	0	0	0

* F_0 = number of adults from Canberra in October 2014

5.5.2 B. bubalus

No data are yet available on the generation-to-generation increase in numbers of *B. bubalus* in the field nurseries. Cohort 1 (about 1300 broods) is likely, at best, to produce about 100 adults (Table 3).

Table 3: Preliminary estimate of numbers of third instar larvae of Cohort 1 *Bubas bubalus* in spring 2015, likely to emerge in spring 2016 (all at Strathalbyn)

Location	**Brood type**	**Broods to Adelaide**	**F_0 adult emergence**	**3LL in spring 2015**
Strathalbyn	Natural broods	213	a few	About 100 pooled over all sites
	Artificial broods	287	none	
Port Elliot	Natural broods	202	a few	
	Artificial broods	226	none	
Bool Lagoon	Artificial broods	395	none	

In contrast, Cohort 2 appears to be breeding well at all three locations, although the actual numbers breeding, their fecundity and brood survival rates are, as yet, unknown. The initial

population of breeding beetles in spring 2015 is considered to be in the order of 300 females, pooled across the three locations. If we assume that breeding occurs equally at all three locations the population in spring 2016 will include in the range of 1200 to 5000 females, with a generation-to-generation increase of 5-fold and 20-fold respectively.

5.6 Synchronisation

In 2014 *O. vacca* adults were released to the field nurseries in late spring and took some weeks after release to begin to feed and then to breed. This delayed their seasonal activity by some months (i.e. they were breeding during summer rather than in spring) but the following year (2015) the emergence of adults began in August and continued over the next month or so. From this we conclude that *O. vacca* in the field nurseries at Strathalbyn and Port Elliot had largely synchronised its seasonal breeding cycle with the weather patterns of the southern hemisphere.

In spring 2014 a few female *B. bubalus* emerged from Cohort 1 and appeared to breed but no F_1 beetles emerged in spring 2015. The progeny of these few beetles may have remained as third instar larvae in diapause during 2015 and, if so, are likely to emerge in spring 2016. About 100 third instar larvae from Cohort 1 have remained in diapause from winter 2014 to summer 2015–16. These are expected to emerge as adults in spring 2016.

In spring 2015 Cohort 2, with 600 *B. bubalus*, arrived in Adelaide. This cohort comprised a mixture of adult beetles and broods.

The adults were provided with fresh dung each week but took 4–8 weeks to enter a feeding phase. This was followed by a breeding phase. At that time they were introduced to field nurseries where they bred in late spring and early summer 2015 (some months later than expected in spring-breeding beetles). The broods were held at room temperature in a laboratory in Adelaide and were checked regularly over the next 3 months and the newly emerged adults set up with fresh dung each week. Adults emerged steadily during late spring and early summer, and these also took some time to enter a feeding phase before breeding. They were released to field nurseries and bred during summer.

We do not know whether the F_1 beetles from Cohort 2 will enter larval diapause. Those that do will delay their emergence for one year (emerging in spring 2017), while the others we expect to emerge in spring 2016.

In conclusion, the synchronisation of *B. bubalus* to the southern hemisphere is not yet assured but it is likely that there will be an emergence of adults in spring 2016, and such beetles will have achieved synchronisation.

5.7 Beetle density in field nurseries

Field nurseries (4 square metres each) established on the Fleurieu Peninsula in 2002 and 2003 in association with the Fleurieu Beef Group yielded very few F_1 beetles (Doube and Marshall 2014): We now attribute this to the use of high densities of beetles and large quantities of dung in a small area, the burial of which generated a moist environment in the vicinity of the underground larvae during spring. Other studies with *B. bison* and *Onitis caffer* have shown that moist conditions when larvae are growing rapidly create a hostile environment and few larvae survive (Doube and Marshall 2014).

Great care is therefore needed when choosing densities of beetles for the mass rearing nurseries. We must eliminate larval mortality due to hostile moist subsoil during spring. We emphasise that there is no sound information on which to base a decision about density, and so our choice for 2015 has been very conservative.

For the *O. vacca* field nurseries in spring 2015 we used one feeding station per square metre with 8–10 pairs of beetles per station. Casual observations during the breeding season strongly suggest that far higher densities may be possible. For the *B. bubalus* field nurseries in spring 2015 we used a similar density of beetles per feeding station.

Beetle density experiments using field nurseries are essential if we are to use the nurseries efficiently and yet without compromising survival. This needs to be tested in spring 2016. For example, if the density of *O. vacca* can be increased from 10 to 50 pairs per square metre there will be a huge impact on the rate at which we can increase beetle populations and fewer nurseries will be needed for the same end result.

5.8 Effect of soil type on breeding and survival

There is a substantial body of literature that clearly shows that some species of dung beetles have strong soil-type preferences. For example in Australia *Bubas bison* cannot survive on deep sand but prospers in loam and clay soils (Doube and Marshall 2014). Similarly the summer-active beetles *Onthophagus vacca* and *Onthophagus binodis* can be extremely abundant on light soils (thousands per dung pad) but are much less abundant on clay and loamy soils (Doube et al. 1991, Doube and Marshall 2014.)

It is possible that a soil type preference may explain the failure of *O. vacca* to breed at Bool Lagoon on clay soil. The seasonal biology of *O. vacca* gives some credence to the idea that it may survive well in sandy soils but not in hard-setting clays. Adult beetles emerge in spring (when soils are moist and soft) and breed for several months before dying. The next generation emerges in early summer, feeds for some time, and then tunnels into the soil to pass autumn and winter underground. If the soil in summer is dry and hard, the newly formed adults may be prevented from digging to the surface and thus become trapped underground. Similarly, once adult beetles have fed, they need to burrow into the soil to find a safe haven for the autumn and winter. The hard summer clay soils may prevent this.

The three test sites represent different climates but also a gradient in soil type. Breeding success of *O. vacca* was correlated with soil type (high in the loam, moderate in the clay-loam and minimal in the clay), suggesting a link to soil type. Further, Sowig (1996a, 1996b) consistently used sand as the medium in which to rear *O. vacca*. In 2015 all new *O. vacca* nurseries were provisioned with a light sandy loam, in which they appeared to do well. Clearly we require an experiment with *O. vacca* to separate the effects of climate and soil type at the three test locations.

In spring 2015 in the *O. vacca* nurseries at Port Elliot, far fewer adults emerged than was expected on the basis of the amount of dung burial observed during summer 2014–15. Contamination with *O. taurus* was likely to have caused part of this dung burial, but we considered that the hard spring soil may have prevented the spring beetles from tunnelling to the surface and so trapped them underground. In late spring the equivalent of 50 mm of rainfall was added to one nursery and this made the soil damp and soft. Nevertheless, very few additional beetles came to the soil surface, suggesting that soil hardness had not confined them underground.

B. bubalus appears to feed and bury dung effectively in association with each of the four soil types(sandy loam, loam, clay-loam, clay) but data on the breeding success in these soils will not be available until spring 2016 (no diapause) or spring 2017 (diapause delaying emergence for 12 months).

5.9 Spreading the risk of failure

Although the breeding stock of both species appears to have increased significantly, they are far from present in numbers that ensure their continued existence in Australia. In order to spread the risk of failure it is essential that both species be reared at the three test locations in 2016 and 2017.

5.10 Contamination of the mass rearing nurseries

Although great care was taken in collecting the dung for use in the 2015 mass rearing program, there was a significant level of cross-species contamination in a number of the field nurseries. In particular the day-flying summer beetles *Onthophagus taurus* and *Euoniticellus fulvus* began to appear and breed in some field nurseries. At Port Elliot the numbers of *O. taurus* that were reared in the nurseries greatly outnumbered the number of *O. vacca*, and created distortions in the data on the use of dung by *O. vacca*.

The message from this is that we need to be assured that there are no contaminant species in the dung used for mass rearing. CSIRO used thawed frozen dung that was collected and stored in large quantities. However, we do not have such freezing facilities and the quantities of dung that will be required for mass rearing in future years run into tens of tonnes per year, and so installing and using a freezing facility would be time consuming and expensive.

The possibility of developing a custom-built beetle-proof stall system to house stalled cattle needs to be considered. In addition we need to seriously consider the possibility of developing artificial dung that can satisfy the needs of adult and larval beetles.

5.11 Dung supply

Mass rearing of large numbers of beetles will require a substantial supply of fresh dung from August to late summer each year. In 2015, despite great care in collecting dung, contamination with other species was a significant problem, and so beetle-free dung needs to be assured. This can be achieved using a purpose-built facility or possibly by developing an artificial dung which can be mass produced at will. In any case, storage facilities for several 100 kilograms of dung will be required.

5.12 Modelling mass rearing

Optimal rearing of the two species requires:

- that each species expresses close to its maximum fecundity (possibly 50–100 eggs per female)
- high levels of survival of the immatures to the adult stage
- regulation of the life cycle such that each generation is completed in minimum time.

If these conditions were optimised, large numbers of beetles could be reared (for redistribution) quickly. In contrast, with the current modest levels of success, it will take some years before widespread distribution is feasible

A model that evaluates the sensitivity of a series of key factors on the success of the beetle rearing program is being constructed but does not form part of this project.

6 Conclusions and recommendations

6.1 Conclusions

- Both species can be reared successfully in high-care field nurseries.
- Current generation-to-generation increases are low compared with what might be achieved.
- The seasonal breeding cycle of both species will have become largely synchronised with the southern hemisphere seasonal cycle by spring 2016.
- *O. vacca* exhibits a non-feeding reproductive diapause in autumn and winter.
- In 2015–16 *O. vacca* produced two generations, each with a breeding cycle.
- *B. bubalus* has a facultative third instar larval diapause.
- *B. bubalus* can exhibit a one- or two or three- year life cycle.
- With support from MLA to refine the mass rearing procedures, both species could begin to be distributed across southern Australia in 2 years' time.
- Using mass rearing facilities, widespread distribution of the two species across southern Australia could be largely complete within a decade, compared with 3 decades as has occurred with previous introduced species.

6.2 Recommendations

- That the field breeding program be supported by MLA for a further 4 years
- That breeding of both species be continued at the three test locations
- That the breeding success of the beetles be increased substantially. This can be achieved by modifying the beetle management in response to information derived from experiments that examine:
    - the effect on survival of beetle density in field nurseries
    - the effect of soil type on breeding and survival
    - the effect of dung quality on adult fecundity
- That the number of generations per year be maximised so as to reduce generation times
- That a model examining the effects of a range of variables on the projected number of beetles available for distribution be constructed.

7 Key messages

- Both species are being successfully reared in field nurseries in South Australia.
- The distribution of *Onthophagus vacca* and *Bubas bubalus* across southern Australia depends on developing productive large-scale rearing facilities.
- The effects of soil type and of crowding on breeding and survival are key factors in developing efficient mass rearing facilities.
- MLA support for the next phase of the project is crucial.

8 Acknowledgements

MLA is acknowledged for its support for this project, providing about 20% of the costs incurred. The remaining support was provided by Creation Care (CC, Strathalbyn), which provided substantial capital for nurseries, labour and land, as well as *pro bono* scientific and technical support, and by Dung Beetle Solutions Australia, which provided extensive support for the three field programs as well as scientific guidance, technical support and analysis and reporting of the results. We thank Dr Jane Wright, Ms Freya Robinson and Mr Patrick Gleeson for breeding the beetles used in SA and for technical advice. Patrick is also thanked for breeding Cohort 2 of *B. bubalus*, a key contribution to its survival in Australia. Greg Dalton and James Ryan (CC) are acknowledged for their thoughtful and innovative contributions to the program. Dr Kerry DeGaris is thanked for her careful monitoring and Peter DeGaris for hosting the Bool Lagoon study site. Mark and Philip Higgins are thanked for their conscientious attention to detail in monitoring and their considered involvement in the program. Loene Doube is thanked for her careful editing and support.

9 References

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

Doube, BM, Macqueen, A, Ridsdill-Smith, TJ and Weir, TA 1991, 'Native and introduced dung beetles in Australia', in *Dung beetle ecology*, eds I Hanski and Y Cambefort, Princeton University Press, Princeton, New Jersey, pp 253–278.

Edwards, P 2007, *Introduced dung beetles in Australia 1967–2007: current status and future directions*, Landcare Australia.

Kirk, AA and Ridsdill-Smith TJ 1986, 'Dung beetle distribution patterns in the Iberian Peninsula', *Entomophaga* 32, pp 183–190.

Lumaret, JP and Kirk, AA 1987, 'Ecology of dung beetles in the French Mediterranean Region (Coleoptera: Scarabaeidae)', *Acta Zoologica Mexicana* 24, pp 1–55.

Lumaret, JP and Kirk, AA 1991, 'South temperate dung beetles', in *Dung beetle ecology*, eds I Hanski and Y Cambefort, Princeton University Press, Princeton, New Jersey, pp 97–115.

Lumbreras, CJ, Galante, E and Mena, J 1990, 'An ecological study of the dung beetle *Bubas bubalus* (Oliver, 1811) (Col. Scarabaeidae)', *Acta Zoologica Mexicana*, 37: 1–23.

Lumbreras, CJ, Galante, E and Mena, J 1991, 'Ovarian condition as an indicator of the phenology of *Bubas bubalus* (Coleoptera: Scarabaeidae)', *Annals Entomological Society of America* 84 (2), pp 190–194.

Mena, J, Galante, E and Lumbreras, CJ 1989, 'Daily flight activity of Scarabaeidae and Geotrupidae (Col.) and analysis of the factors determining this activity, *Ecología Mediterranea* 15 (1–2), pp. 69–80.

Sowig, P 1996a, 'Brood care in the dung beetle *Onthophagus vacca* (Coleoptera: Scarabaeidae): the effect of soil moisture on time budget, nest structure, and reproductive success', *Ecography* 19, pp. 254–258.

Sowig, P 1996b, 'Duration and benefits of biparental brood care in the dung beetle, *Onthophagus vacca* (Coleoptera: Scarabaeidae)', *Ecological Entomology* 21, pp. 81–86.

Steinbauer, MJ and Wardhaugh, KG 1992, *Western Australian Dung Beetle Project,* final report for WADA, CSIRO Entomology, Canberra.

Wright, J, Gleeson, P and Robinson, F 2015, *Importation of 2 winter-spring active dung beetles for southern Australia,* final report for MLA project ERM.0123, Meat & Livestock Australia Limited, North Sydney.

10 Appendices

10.1 Appendix 1: Emails concerning project extension and fate of MLA beetles

Appendix 1 comprises emails that document:

- the proposed development of a new project to support the extension of Project ERM.0124 (saving the species from extinction) to a 4-year mass rearing program
- requests for direction regarding the fate of the MLA beetles under the control of DBSA in spring 2015.

Key components of emails are highlighted in bold red type.

10.1.1 Email 1, 22 July 2015: DBSA submission to MLA

From: Bernard Doube
Sent: Wednesday, 22 July 2015 8:18 PM
To: Cameron Allan
Cc: Loene Doube
Subject: What to do next: the two new species

Dear Cameron,

Please find attached a document which suggests how we might take the 'two new species program' to the next phase.

As we both know, CSIRO field releases of both species occurred in WA and NSW in spring 2014. These, provided that they establish, are unlikely to yield harvestable beetle until about 2024.

Further, a portion of the CSIRO beetles are now in 'transition to the field' in a captive breeding program in South Australia. Efficient field-based mass rearing procedures are being developed and appear to be successful.

Our current estimates are that about 500 pairs of *Onthophagus vacca* and 135 pairs of *Bubas bubalus* will be available in spring 2015. MLA and DBSA have joint ownership of the spring emergence in the ration of 60:40 respectively.

The DBSA beetles from spring 2015 will be returned to the captive breeding program in South Australia.

Our predictions (assuming support from MLA) indicate that we could have distributed over 500,000 of each species across southern Australia by the time that harvest of the CSIRO-released field populations becomes a possibility in 2024.

The options for the MLA beetles in spring 2015 are:

- to deliver them to MLA for distribution at their discretion, or
- **to allocate them to a DBSA-managed experimental program** designed to improve mass rearing procedures and to determine the suitability of a range of environments across southern Australia.

We recommend the latter course of action for the MLA beetles.

The attached document contains a proposal that details one option for managing the MLA component of the two species and three appendices:

- information about the 2012–2014 CSIRO mass rearing program
- transition of *B. bubalus* from laboratory to the mass rearing facility in the field
- transition of *O. vacca* from laboratory to the mass rearing facility in the field.

Support from MLA will most likely achieve the following:

- establish both species in a series of locations across southern Australia
- distribute the MLA component of the beetle livestock to producers across southern Australia

- reveal the environmental requirements of both species
- indicate the optimum density for mass rearing the beetles in field cages
- ensure that we can produce many thousands of beetles annually.

We look forward to your response to this proposal.

Best wishes

Bernard

10.1.2 Email 2, 30 July: brief MLA response to DBSA

From: Cameron Allan
Sent: Thursday, 30 July 2015 5:41 AM
To: Bernard Doube
Cc: Loene Doube
Subject: RE: What to do next: the two new species

Hello Bernard,

Thankyou for this note – quite a bit to digest just based on your introduction to the document below.

I have not yet read the document, when could “relocation” occur ?

Thanks

Cameron

10.1.3 Email 3, 2 August: response from DBSA

From: Loene Doube
Date: 2 August 2015 12:03:53 pm ACST
To: Cameron Allan <callan@mla.com.au>
Subject: Fwd: What to do next: the two new species

Dear Cameron

Thanks for you brief email, The scheduling is outlined in document one:In brief

We propose that the MLA beetles be set up in field cages I spring 2015

Beetles from mass rearing will supply a further but expanded field cage program across Southern Australia in 2016 spring

So a MLA funded field cage program across Southern Australia in 2015 and 2016 during spring

This will see farm releases of viable colonies across Southern Australia in spring of 2017, two years from now

Best wishes

Bernard

10.1.4 Email 4, 20 August: DBSA requests a response from MLA

From: Bernard Doube
Sent: Thursday, 20 August 2015 9:37 PM
To: Cameron Allan
Cc: Loene Doube
Subject: FW: What to do next: the two new species

Dear Cameron,

We await a response from you regarding the fate of the MLA spring beetles.

Best wishes

Bern

10.1.5 Email 5, 20 August: brief response from MLA

From: Cameron Allan
Sent: Thursday, 20 August 2015 10:02 PM
To: Bernard Doube
Cc: Loene Doube
Subject: RE: What to do next: the two new species

Hello Bernard,

Thanks you for the options paper. A key issue is funds – reduced budget and merger of actions with the restructure in MLA..

Can you please comment on my **30 July email (attached)**.

Thanks

Cameron

10.1.6 Email 6, 20 August: response from DBSA

From: Bernard Doube
Sent: Thursday, 20 August 2015 10:56 PM
To: 'Cameron Allan'
Cc: Loene Doube; Greg Dalton
Subject: RE: What to do next: the two new species

Thanks Cameron,

I replied to your email of 30 July on 2 August, , on Loene's phone. See the email string below my email of 20th Aug. for details.

The text of your email **email of 30 July** stated

'Hello Bernard,

Thank you for this note – quite a bit to digest just based on your introduction to the document below. I have not yet read the document, when could "relocation" occur ? Thanks'

Dear Cameron,

What do you mean by 'relocation'?

If you mean putting them in field cages in a series of interstate locations, then we propose that (with MLA assistance) this should happen this year, and again next year. Please refer to my options paper and the covering email.

If you mean releasing full starter colonies to the field/paddock environment, then this is likely to occur in spring of 2017, about a decade earlier than by field releases. Again, please refer to my options paper and the covering email.

We look forward to your response.

Please read the options paper. That is the only way we can move forward effectively. Spring is upon us.

Best wishes

Bern

10.1.7 Email 7, 31 August: DBSA requests response from MLA

From: Bernard Doube
Sent: Monday, 31 August 2015 11:36 AM
To: Cameron Allan
Cc: Loene Doube; Greg Dalton
Subject: Two new species

Dear Cameron,

Spring is upon us and we need to act now to ensure that the two new species have the best chance of establishing in Australia.

This is where we stand at the moment:

- Both species are currently at the beginning of their spring emergence
- The spring management of these beetles must be in place by mid to late September
- Our current estimates are that about 500 pairs of *Onthophagus vacca* and about 135 pairs of *Bubas bubalus* will be available for spring breeding. The background to these estimates is detailed in the attached proposal, of which you have a copy already.
- 60% of these are the property of the MLA
- We will use our share for mass rearing in South Australia
- What would you suggest be done with the MLA share of the beetles?
- The attached proposal for environmental testing and distribution of the beetles meets the MLA goal
- We will be unable to maintain the MLA beetles without financial support
- We do not have the financial resources to carry out the interstate testing of the environmental testing of the species

So where do we go from here?

We look forward to your prompt response.

Best wishes

Bern

10.1.8 Email 8, 30 September: DBSA requests response from MLA

From: Bernard Doube
Sent: Wednesday, 30 September 2015 12:17 PM
To: Cameron Allan
Cc: Loene Doube; Greg Dalton
Subject: FW: Update on Bubas bubalus from the MLA stock to Adelaide in November 2014

Dear Cameron,

Welcome back to the land of toil.

In the email appended below is an account of where we are up to with *Bubas bubalus*. It is possible that the *B. bubalus* (n= 85 see Table below) that are still in their 3LL stage will emerge as adults later in spring, as it warms up. They have the appearance of *Bubas bison* that are about to pupate (empty guts and lots of yellow fat body).

The relevant information is

- in the Table below
- in the email below

We need to act soon to decide the fate of the 60% of the *O. vacca* and *B. bubalus* that belong to the MLA.

O. vacca is beginning to become quite active at Strath but not so at Port Elliot (very cold still) or Bool Lagoon.

Hot weather next week will speed thing up.

Table: Summary for all remaining broods at Strathalbyn September 2015 *B. bubalus*

	Artificial		Natural	
Type of brood	**Number**	**% of total**	**Number**	**% of total**
Empty broods	65	32.7	52	28.9
2 LL dead	23	11.6	11	6.1
3 LL dead	24	12.1	39	21.7
3 LL live	43	21.6	42	23.3
Intact faecal shells	13	6.5	32	17.8
Prepupae	0	0.0	0	0.0
Adults dead	5	2.5	2	1.1
Tanned live adults	2	1.0	2	1.1
Total brood balls not opened	24	12.1	0	0.0
Total	199	100	180	100

Best wishes

Bern

10.1.9 Email 9, 16 October: DBSA requests response from MLA

From: Bernard Doube
Sent: Friday, 16 October 2015 8:48 PM
To: Cameron Allan
Cc: Loene Doube; Greg Dalton
Subject: FW: Update on Bubas bubalus from the MLA stock to Adelaide in November 2014

Dear Cameron,

Below is for your information. We need to act soon to decide the fate of the 60% of the *O. vacca* that belong to the MLA. The beetle is beginning to become quite active at Strath but not so much at Port Elliot (very cold still) or Bool Lagoon. Hot weather next week will speed thing up.

It is possible that the many *Bubas bubalus* that are still in their 3LL stage at present (n= 85 see below) will emerge as adults later in spring as it warms up. But they may remain in diapause for another year?????

Best wishes

Bern

10.1.10 Email 10, 20 October: Telephone link organised

Email not included.

10.1.11 Email 11, 26 October: DBSA requests response from MLA

From: Bernard Doube
Sent: Monday, 26 October 2015 11:46 PM
To: Cameron Allan
Cc: Loene Doube
Subject: Milestone report

Dear Cameron,

The next Milestone report is due on 1 November 2015. This will be a brief report as detailed below. (extracted from proposal)

Brief report (4 page max) to MLA including: - commentary on beetle emergence behaviour - preliminary estimate of population numbers - recommendations for any corrective actions - outline of a potential rearing program including potential populations	01-Nov-2015

My previous correspondence with you has provided a detailed account of progress to date and some estimates of future numbers (attached) for both species.

As we discussed on the phone recently, there is contamination of the *Onthophagus vacca* rearing facilities at Port Elliot with *Onthophagus taurus*. And we are harvesting low numbers of *O. vacca* from that facility. I consider that it is possible that there may be many more present but they are currently immobilised by hard dryish soil. It may be that 50 mm of rain will release them. If it does release them, then we will need to try the same trick at Strathalbyn and Bool Lagoon.

I plan that the watering of one field cage will happen this week. Beetle emergence in response to that moist soil (if it occurs) is likely to occur over the next week or so, maybe sooner.

At present we have about 400 *O. vacca*. If they are released when the soil becomes moist, and summer 2015 activity is reflected in these numbers, we may have double or triple our current numbers (a big if).

The adult *Bubas bubalus* are now in field rearing cages and appear to be beginning to tunnel. Breeding is likely to begin in a few weeks.

The 18 month-old larvae of *B. bubalus* are currently in their third larval instar: they may be in diapause or developing into adults. We will know which in about a month's time.

In 6 weeks' time we will be in a far better position to assess the numbers and activity of both species.

In view of this information, I request that the due date for the next Milestone report be delayed by 6 weeks, until the 13th December. The basic report is largely completed but there are a number of uncertainties that would be resolved if the report were delayed by 6 weeks. This will make for better planning of future activities.

I look forward to your response to this suggestion.

Best wishes

Bernard

10.1.12 Email 12, 27 October: DBSA summarises MLA discussions

From: Bernard Doube
Sent: Tuesday, 27 October 2015 12:29 AM
To: Cameron Allan
Cc: Loene Doube; Greg Dalton
Subject: Dung beetle project proposal

Dear Cameron,

A very useful discussion the other day. Thanks for making time in your busy schedule.

A brief reminder: Can you please send to project form that we discussed along with a few dot points.

My reading of the outcome was broadly as follows: you dot points may be rather similar

- Both new species are recognised as being in very vulnerable
- Our primary objective is to secure viable population of *Onthophagus vacca* and *Bubas bubalus*
- My view is that the field releases in WA and NSW (a few hundred beetles) are unlikely to succeed
- The high care program in SA is possibly the only hope for the two species in Australia, this time round
- The focus will therefore be on high care facilities in SA initially, not interstate, and especially at Strathalbyn where technical developments, success and high care have been outstanding

- We are to prepare a 4 year project plan to cement the establishment and dispersal of the two new species
- We would put this proposal in a project form supplied by you along with costings
- We would cost two option: a bare bones option and one that allowed experimental development to fast track the process
- There are key issues associated with mass rearing that need experimental attention (eg density, soil type, dung quality)
- The number of generations per year is a crucial issue
  O. vacca may be induced to breed twice a year:
  B. bubalus needs to be managed so that it does not enter diapause in the 3 LL stage (which imposes a 2-year life cycle)
- For both species fecundity and brood survival are the key issues
  For example, the CSIRO Factsheet indicates that at female *O. vacca* might produce 90+ broods in a lifetime. We managed about 14 successful broods (=F1 progeny) per female at Strathalbyn in 2014-15. Clearly there is great capacity for improvement. This requires careful experimental analysis. Improving survival from 14 to 50 broods per female will have an enormous impact on the rate of increase of the captive population.
- Interstate environmental evaluations will follow in subsequent years
- This proposal would be independent of the Rural Profit Project round

We look forward to developing the project with you.

Best wishes

Bern

10.1.13 Email 13, 27 October: MLA responds to DBSA

From: Cameron Allan
Sent: Tuesday, 27 October 2015 3:54 AM
To: Bernard Doube
Cc: Loene Doube; Greg Dalton
Subject: RE: Dung beetle project proposal

Hello Bernard,

Sorry, I thought I had closed the loop.

There are some standard guidelines / comments that have been left in the template – please read and delete, also I have inserted some text.

The primary concern is to ensure success in establishment, and so increase in numbers of OV and BB, to enable re-distribution.

This is a "caretaker role" overseeing the establishment phase, less so research on adaptation zone etc, and providing feedback to MLA on the population and when redistribution may be appropriate.

Please consider a 4 year project with 2 reports annually (progress/ annual). Each annual report year would be a Stop/Go point with recommendations for action stated in the milestone. The annual report is an assessment of how establishment and reproduction is going, towards providing a recommendation of next steps. As numbers increase that may be for redistribution or related work.

As indicated in the hookup, this needs to be a simple low cost maintenance project.

Thanks

Cameron

10.2 Appendix 2: Establishing the field cages

10.2.1 Strathalbyn

10.2.2 Port Elliot

Philip Higgins at Port Elliot

10.2.3 Bool Lagoon

Peter De Garis at the Bool Lagoon site