WESTERN AUSTRALIAN DUNG BEETLE PROJECT FINAL REPORT
PART ONE: IMPORTATION AND REARING OF SPANISH DUNG BEETLES

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1 GENERAL INTRODUCTION

In 1964 the dung beetle program was initiated in Australia by CSIRO and the first dung beetles were released in 1968 (Bornemizza 1976). The major aims of the program were to remove pasture fouling (by dung burial) and the control (by interference competition) of the buffalo fly (*Haemotobia irritans exigua* De Meijere) and the bush fly (*Musca vetustissima* Walker). Both species of fly have an obligate larval stage in dung. Bush flies utilise all types of dung (Pont 1973) and following the settlement by European man and the introduction of cattle, sheep and horses into Australia a new environment was created which did not suit the then present native dung beetle (Scarabaeinae) fauna. This imbalance, caused by an accumulation of dung, led to an increase in the breeding sites of the bush fly. Native dung beetles, mostly found in the natural vegetation of south-western Australia normally feed on the faecal pellets of marsupials, leaving most ungulate dung relatively untouched.

Fifty-two species of dung beetle were released Australia wide by CSIRO between the years 1968 and 1985, of which 23 species are known to have become established between 1 and 10 years after their date of release. In South-western Australia 14 species have been released, of which 7 have become common (Ridsdill-Smith *et al.* 1989). Since 1978 these introduced species have become the dominant members of the dung beetle fauna in pastures in south-western Australia. Between 1990 and 1995 DAWA personnel, observed that 2 species, *Onthophagus binodis* and *O. taurus* have become abundant and widespread along the coastal plain from Perth southwards to Albany. Two other species *Euoniticellus pallipes* and *E. fulvus* are also widespread throughout the southwest but are less abundant. These species were released during the mid to late 1970's. Additionally, some species, released in 1985, have been recently recovered in small numbers; *Onitis caffer* at Bullsbrook (31^{o}42S 115^{o}59E) and *O. aygulus* in the Pinjarra (32^{o}35S 115^{o}55E)and Busselton (33^{o}44S 115^{o}16E) region.

The benefits of dung beetle activity to the livestock industry have been documented extensively in the literature (pasture improvement, Bornemissza (1960), Edwards and Aschenborn (1987), Brussaard and Hijdra (1986), Rougon and Rougon (1983); reduction of gastrointestinal worms, Bryan (1973), Reinecke (1960), Biggane & Gormally (1994); reduction in fly numbers, buffalo fly, Blume *et al.* 1973, Doube 1986, Doube *et al.* 1988, bush fly, Tyndale-Biscoe and Hughes (1969), Ridsdill-Smith and Matthiessen (1988)). Firstly, increasing the breakdown of cattle dung in pastures has reduced the cost of harrowing to the farmer. As well, nutrient recycling by dung burial increases pasture productivity (Bornemissza and Williams 1970, Edwards and Aschenborn 1987). Secondly, gastrointestinal worms spread from dung pats into surrounding grass to reinfect grazing cattle (Durie 1961). Although worm drenches, and in particular the avermectins, have severely reduced the worm load both in bovines and in pastures, most of these drugs cannot be administered to dairy cattle. Thirdly, the buffalo fly and the bush fly which breed in cattle dung are pests of both livestock and humans spreading such diseases as pink eye amongst cattle (Williams *et al.* 1985). The buffalo fly is a biting fly requiring a blood meal before laying their eggs. Biting causes a reduction in meat and milk production (meat, Haufe 1986;

milk, Campbell 1976). The bush fly also pesters livestock, obtaining protein for egg production from around eyes (tears), noses (mucus) or from a wound (blood) (Hughes *et al.* 1972).

The CSIRO Division of Entomology in Western Australia has demonstrated an 88% reduction in bush flies in January in the Busselton region, caused by the activity of introduced dung beetles (Ridsdill-Smith and Matthiessen 1988). Unfortunately, CSIRO ceased dung beetle operations in 1987 and as a consequence dung beetles were not seeded into all potentially suitable areas. In December 1989 a 5 year programme was initiated to originally introduce 3 species, (*Copris hispanus, Bubas bison, Onitis belial)* which later expanded to 4 species (*Bubas bubalus)*, of dung beetles (active from September to early January) from Spain into Western Australia to control bush flies. This was a collaborative programme between CSIRO and the Western Australian Department of Agriculture (DAWA). CSIRO was involved with importing these species from Spain into Australia and the subsequent release of successive batches of eggs (F1) from Australian Animal Health Laboratories (Geelong) (Steinbauer & Wardhaugh, 1993). On receival of F1 eggs or pupae, DAWA was to mass rear and then release (Part 1) adult dung beetles at climatically selected sites in Western Australia.. Part 2 details all the harvested, redistributed and established dung beetle species in WA. Part 3 concerns the ecology of those introduced dung beetles established in WA and their effect on the endemic bush fly population.

2 BRIEF BIOLOGIES OF DUNG BEETLES SELECTED FOR INTRODUCTION AND NOW ESTABLISHED IN WESTERN AUSTRALIA

Biologies of the 4 species of dung *beetles Bubas bison, Copris hispanus, Onitis belial, Bubas bubalus* are detailed in Steinbauer & Wardhaugh (1993). This section describes the biologies of *Bubas bison* and *Copris hispanus* now established in Western Australia.

2.1 *Bubas bison*

Adult emergence begins following the first substantial rains in May. Adult beetles can be found on the soil/pat interface digging shallow tunnels (depth ,10 cm) both directly underneath and around the edges of the pat. The number of adult beetles in pats range from 20 to 320. At this time pats are shredded within 48h.

Adult beetles begin digging deeper tunnels approximately 4 weeks after emergence and this continues from July to early August. With only 10 to 50 beetles per pad and located at depths up to 60 cm in heavy loam soil and 100 cm in sandy soil, beetles are more difficult to harvest at this time. Burial of dung by adult beetles resumes (although less intensive than in May), in mid to late September. Old beetles identified by the complete wearing down of their fore tibial teeth can occasionally be found until late November. Adult beetles have also been found extensively in sheep dung during their active period between autumn and early spring..

B. bison was released at Dardanup (33^{o}30S 116^{o}55E) in 1983, Jingalup (33^{o}53S 117^{o}03E), in 1987 and at Kojonup (33^{o}50S 117^{o}11E) in 1991. Beetles from these 3 releases now cover an area of approximately 720 km^{2}.

2.2 *Copris hispanus*

Newly emerged adult beetles usually appear following the first substantial rains in May. Beetles begin to construct feeding tunnels to a depth of 150 mm which are provisioned with approximately 100 ml of dung stored at the distal end. A lone beetle, rarely two, can be found in these feeding tunnels again usually at the distal end. Adult beetles are rarely found in pats during daylight hours. This phase continues for 6 to 12 weeks.

Following maturation feeding, breeding tunnels appear. These are large and conspicuous and are up to 1000 mm (average is 300 mm) in depth. At the end of the tunnel a breeding chamber is excavated and is provisioned with 450-700 ml of fresh dung. *C. hispanus* becomes increasingly difficult to find from August. By October, a three hour search may locate an unpaired beetle or two from feeding tunnels. At this time, males are usually recovered with their partners, from breeding tunnels or chambers.

C. hispanus was first introduced to Williams (33°10S 116°55E)in 1983 and subsequently in 1986. This species can now be found in low numbers on adjoining properties up to two kilometres from their release point. On these properties adult beetles are usually found in cow dung, but have been observed tunnelling near to and using sheep faecal pellets.

3 IMPORTATION AND REARING

Before the arrival of any eggs and 1st instar larvae consignments from AAHL the DAWA Quarantine Insectary (QI) was set up to both receive these consignments and rear the eggs to adults. A dung media was also necessary to rear beetles.

3.1 Dung for producing suitable brood ball media

A convenient and efficient way of producing brood balls in the QI was desirable. Ideally the dung used to make brood balls was of a high quality. Wardhaugh (*pers comm.*) suggested a moisture content of approximately 40% and its consistency should approximate that of bread dough.

Pasture dung used in the QI was frozen for a minimum of three weeks at -20'C to -30'C. This killed nematodes and arthropod infestations. Another effective way to exterminate these pests was to autoclave the dung. Twenty litre lots of dung containing 40% moisture were autoclaved for a minimum of 30 minutes. The different techniques are described below.

- *Frozen and thawed pasture dung*. Dung was usually stored in 10 lt buckets and kept frozen. Repeated freezing and thawing (with the bucket inverted) allowed for removal of ice and "tea" from the dung. In using this method the dung was prone to sciarid fly infestation. This method was time consuming.
- *Manual removal of excess moisture*. Presses and hydraulic rams have previously been used for producing brood balls (Edwards & Aschenborn; 1988). Apparatus to perform these procedures were not available at DAWA. Instead, moisture was removed by layering pasture dung between thick pads of paper towelling on a sturdy bench. Pressure was applied to the upper surface. The process was repeated until dung of an acceptable consistency was obtained. This method was very messy and also time consuming.
- *Autoclaved dung mixed with pasture dung*. The dung was first allowed to air dry, followed by autoclaving. This was blended with pasture dung (already freeze/thawed once to remove excess water), making it a suitable medium for brood balls.
- *Dung chaff mixed with pasture dung (DCM1)*. The most convenient and efficient method employed for making brood balls used a mix of dung chaff and pasture dung. Dung chaff was prepared by grinding pieces of air dried dung (or dung dried in a soil drying oven) in a grain mill. This chaff was blended with high quality pasture dung. Pasture dung was taken to be approximately 80% water, whereas dung chaff had negligible water content.

Mixing 1 kg of pasture dung with one kg of dung chaff produced a medium with approximately 40% moisture.

- A different DCM2 was used for *B. Bison* and *B. bubalus* in Shipments 19 to 33. This was made with 1 kg of dung chaff blended with 1.3 kg of high quality spring dung. The spring dung was collected from dairy cattle on annual rye/clover pastures and a hay diet. The moisture content of this medium was therefore approximately 45%.

Eggs and 1st instars from the first 3 shipments used hand-rolled brood balls. Subsequent shipments employed brood balls pressed from a mould. Each mould had 144 cells (12 x 12) into which the media was placed, then frozen. Once frozen, the larval cavity was drilled into each dung block. The blocks were ejected from the moulds, bagged and stored in the QI freezer. In this way sufficient brood balls, each measuring 35 mm x 35 mm x 40 mm, could be thawed on demand.

3.2 Quarantine Insectary constant temperature rooms

Constant Temperature Room 4 (CT4) in the QI was set up initially at 24°C and then at 20°C. The CT3 in the QI was set between 24-26^{o}C

3.3 Dung beetle egg and 1st instar handling

Dung beetle eggs once sterilised and released from AAHL they were air freighted to Perth courtesy Ansett Air Freight. All eggs arrived at DAWA QI within 16 hours of their release from AAHL. All eggs and 1st instar larvae of each species were treated as described below.

Tables 1, 2, 4 and 5 detail the number of eggs dispatched to DAWA from AAHL, the percentage hatch and either the number of brood balls or adult beetles released. Most consignments when they arrived at DAWA generally consisted of eggs and 1st instar larvae.

The eggs and 1st instars from Shipment 1 to Shipment 3 arrived in Decor® lunch boxes lined in moist tissue paper. On their arrival at QI, eggs and 1st instars were immediately placed in brood balls. This process was time consuming and many eggs failed to hatch (see Table 1 & 3).

Cardboard forceps were used to transfer eggs and 1st instar larvae to hand-made brood balls. Eggs or 1st instar larvae were transferred to a cavity ($\simeq$ 1 cc) in each brood ball. The cavity was then pinched almost closed.

These brood balls were then housed in Prestige® plastic storage boxes with the lid not fully sealed to allow for gas exchange. A label attached to each box lid allowed for observations to be recorded about the progress of the larva in each brood ball. Only eggs or larvae from the same consignment and of the same species and 'strain' were boxed together. Sixteen brood balls could be housed in

these boxes and were supported to approximately half their height with yellow sand.

The yellow sand had a low level of fines ($\leq$ 5 %) and was baked at 120'C x 10 hr (min) prior to use in QI. When used as a buffer for the brood balls it was brought to a moisture content of 5-7 %.

Eggs and 1st instars from Shipment 4 to Shipment 16 were allowed to hatch in their shipping (Decor®) boxes. Each lunch box was lined with a 150 mm slab of dung and vermiculite, or autoclaved dung, with small depressions where eggs were placed. Eggs were held in situ with moist tissue paper. First instars only were transferred to brood ball cavities and the cavity was pinched almost closed.

B. bison and *B. bubalus* eggs and 1st instars from Shipment 17 to Shipment 33 were dispatched in Nunc® trays. The bottom of each cell in the Nunc® tray was lined with moist tissue paper and an egg was placed in each cell. A dung/dung chaff medium (DCM2) was sprinkled into each tray cell, sufficient to just cover each egg. The Nunc tray lid was replaced and the entire tray gently inverted. Eggs would usually adhere to the moistened tissue bed on which they were placed, whereas the DCM2 from each cell would rest on the inner surface of the tray lid. Occasionally, before hatching the Nunc® trays would be infected by an unknown fungus. The effect of this on hatch rate is unknown.
On hatching in the Nunc cell, the larvae would fall onto the rearing medium and commence feeding. Tray cells containing viable larvae (i.e. hatchlings that commenced feeding) could be easily detected. Viable larvae were transferred to brood balls at approximately the stage of late first to early second instar. Brood ball cavities were enlarged to house these larger larvae. Approximately 1 ml of DCM2 was placed onto the cavity floor in each brood ball before inserting the larvae. The cavity was then pinched almost closed.

Third instars of *B. bison, B. bubalus* and *O. belial* in these hand-made brood balls sometimes broke out and ended up on the sand. If these larvae were not detected quickly, they died. Errant larvae, detected in time, often survived. However, time and resources were wasted replacing them and repairing their brood balls. Furthermore, covering all but the top of brood balls with sand did not prevent larvae exiting through the top of their brood balls. This technique was also not used because it was necessary to observe the whole brood ball on a daily basis for sciarid fly infestations.

Due to this persistent problem of larvae breaking out of their brood balls a different method was employed using plastic cups. The plastic cups were half filled with DCM1. A larva was placed into a depression in the DCM1 and the lid containing multiple pin holes was replaced. Once feeding had commenced, the larva quickly established a feeding cavity. At this point, the cup was almost filled with fresh DCM1 and the lid replaced.

One problem with the plastic cup method was that they retained moisture. These conditions resulted in an unsuitable environment for larvae to develop and pupate. This may be solved in future by using unwaxed paper cups.

3.4 Dung Beetle Rearing

3.4.1 *Bubas bison*

Table 1: Summary of periodic shipments of *Bubas bison* to WA. This table details the hatch rate and the number of broods released.

Shipment number	Date	No. of eggs received	% Hatch at DAWA	1st Instar	No. broods released
Consignment 1					
1	28/05/90	467	48.4	226	0
2	16/06/90	658	64.0	421	0
3	26/06/90	575	38.6	222	0
5	11/07/90	532	11.8	62	0
9	14/08/90	280	57.5	161	11
10	28/08/90	121	19.0	23	16
11	06/09/90	44	27.3	12	11
	Total	2677		1127	38
Consignment 2					
14	07/02/91	51	0.0	0	0
15	12/03/91	204	45.1	92	0
16	26/03/91	754	9.5	72	0
17	10/04/91	666	11.0	73	3
18	24/04/91	558	22.8	127	7
19	10/05/91	358	41.3	148	10
20	28/05/91	269	58.3	159	43
21	17/06/91	55	60.2	33	22
22	02/07/91	74	70.3	52	16
24	17/07/91	479	50.7	243	40
26	29/07/91	106	47.2	50	31
27	01/08/91	30	23.3	12	11
28	19/08/91	50	2.0	1	0
29	29/08/91	22	4.6	1	0
30	04/09/91	8	12.5	1	0
	Total	3684		1064	183

Four shipments of eggs, (a total of 560 eggs) were also dispatched to Canberra (Steinbauer and Wardhaugh 1993).

Instead of releasing adult dung beetles, brood masses were buried at a number of localities. At each locality brood masses were buried to a depth of 30 cm

Brood balls buried at Cowaramup 38
Brood balls buried at Blue Hills 120

At DAWA 63 brood balls were buried in cages. Six walk-in cages were constructed on a base of hardwood each 2 m x 2 m x 1 m and filled with 2 m^3 of

red brown sandy loam. A metal tubular frame was built over (2 m) each cage and covered in 3 mm^2 plastic mesh. The base and sides were also lined with plastic mesh. Each cage was serviced by a zippered flap (door).

Sixty three brood masses of *B. bison* were removed from QI on 11.12.92, and buried to a depth of 600 mm inside one-cage. On 15th February 1993 a further 120 brood masses of *B. bison* were sent from J. Feehan (CSIRO, Canberra) and also buried to a depth of 600 mm inside a cage.
On emergence (see Table 2) all adult beetles were fed *ad lib* on high quality annual or irrigated pasture dung

Table 2 Total number of *B.bison* emerged from QI and Canberra releases into external walk in cages at DAWA

1993	**m**	**f**
May/ June	18	20
August	0	2
September	0	1
TOTAL	18	23

1994	**m**	**f**
May/June	70	70
August	1	4
TOTAL	71	74

1995	**m**	**f**
April	8	16
May	228	246
June	9	9
July	12	10
August	2	0
TOTAL	259	265

3.4.2 *Onitis belial*

Table 3: Summary of periodic shipments of *Onitis belial* to WA. This table details the hatch rate and the number of adult dung beetles produced.

Shipment number	Date	No. of eggs received	% Hatch at DAWA	1st instar	Pupa	Adult beetles
Consignment 1						
1	28/05/90	175	33.7	59	1	0
2	16/06/90	172	57	98	2	0
3	26/06/90	135	53.3	72	2	1m
4	03/07/90	152	29.6	45	2	0
5	11/07/90	11	0.0	0	0	0
6	17/07/90	86	30.2	26	4	0
7	25/07/90	125	6.4	8	0	0
8	08/08/90	46	13	6	0	0
9	14/08/90	11	0.0	0	0	0
	Total	861		314	11	1
Consignment 2						
20	28/05/91	337	3.6	12	0	0
21	17/06/91	132	0.0	0	0	0
22	02/07/91	24	54.1	13	7	2m2f
23	04/07/91	47	40.4	19	5	1m1f
24	17/07/91	99	10.1	10	5	2f
25	25/07/91	32	0.0	0	0	0
29	29/08/91	9	0.0	0	0	0
30	04/09/91	12	0.0	0	0	0
33	03/10/91	2	100	2	0	0
	Total	694		56	17	8

The beetle from consignment 1 died. However, 17 pupae in brood balls from consignment 2 were removed from the QI before it became a high security facility (February 1992). Pupae were set in plastic cups and sealed with self-sealing lids. A pin hole was made in each lid for aeration. Each pupa was effectively isolated from other pupae, and to a certain extent, environmental contamination. Each was checked routinely for changes in appearance.

It was observed previously that the beetle derived from shipment 3, maintained at 25°C eclosed with disfigured elytra. As a consequence a different temperature regimen was employed. Larvae were reared at 25°C, but 10-14 days following pupation when pupa commenced changing from a normal butter colour to a pale caramel colour, they were transferred to a Constant Temperature cabinet at 17°C $\pm$ 1°C.

In March, 1992 another eight adult beetles had emerged. The eight beetles were set in a constant temperature room at Light 14 hr; 24'C: Dark 10 hr; 18'C. Feeding was *ad lib* with high quality pasture dung. One pair of beetles and two trios (2f and 1m) were placed into separate plastic buckets with fitted mesh

screen lids. The rectangular buckets measured 300 mm x 210 mm x 210 mm. They were filled to 2/3 their depth with red-brown loam. Feeding was *ad lib* with high quality pasture dung. No breeding occurred under these conditions.

Four beetles died using this method. Two males and two females remained. The remaining beetles were paired and placed into separate plastic buckets with fitted mesh screen lids. The rectangular buckets measured 300 mm x 210 mm x210 mm. They were filled with to 2/3 their depth with red-brown loam. Feeding was *ad lib* with high quality pasture dung.

In July 1992 at 26'C constant temperature the two pairs of *O. belial* commenced breeding.

3.4.3 *Copris hispanus*

Table 4: Summary of periodic shipments of *Copris hispanus* to WA. This table details the hatch rate and the number of adult dung beetles produced.

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Shipment number	Date	No. of eggs received	% Hatch at DAWA	1st instar	Pupa	Adult beetles
Consignment 1						
3	26/06/90	14	57.1	8	4	2m2f
9	14/08/90	13	38.5	5	0	0
11	06/09/90	4	0.0	0	0	0
12	09/10/90	7	0.0	0	0	0
13		3	0.0	0	0	0
	Total	41		13	4	4
Consignment 2						
16	26/03/91	3	0.0	0	0	0
17	10/04/91	9	11.1	1	0	0
18	24/04/91	60	6.7	4	0	0
19	10/05/91	32	6.2	2	0	0
20	28/05/91	48	8.3	4	0	0
21	17/06/91	38	7.9	3	0	0
23	04/07/91	23	17.4	4	0	0
	Total	210		18	4	0

From shipment 3, 2 male and 2 female adult beetles emerged. These beetles produced 6 brood balls from which 5 larvae survived for a period of 10 days. Both adults and larvae died.

3.4.4 *Bubas bubalus*

Table 5: Summary of periodic shipments of *Bubas bubalus* to WA. This table details the hatch rate and the number of adult dung beetles produced.

Shipment number	Date	No. of eggs received	% Hatch at DAWA	1st instar	Pupa	Adult beetles
19	10/05/91	51	7.8	4	1	0
20	28/05/91	52	28.9	15	0	0
21	17/06/91	6	0.0	0	0	0
25	25/07/91	120	21.7	25	4	1
27	01/08/91	59	20.3	12	0	0
28	19/08/91	96	3.1	3	1	0
29	29/08/91	80	6.2	5	0	0
30	04/09/91	80	7.5	6	1	1
31	16/09/91	87	6.9	6	0	0
33	03/10/91	53	26.4	14	0	0
	Total	684		90	7	2

Five shipments of eggs (292 eggs) were dispatched to Canberra. None survived to the adult stage. Only 2 survived to the adult stage in the DAWA shipments but both died within 14 days.

4 QUARANTINE INSECTARY (QI)

The QI was upgraded to a high security facility when the remaining Spanish Dung Beetles were transferred from the Australian Animal Health Laboratories (AAHL). The protocol for using the DAWA QI is detailed below.

4.1 Objectives

- To have remaining adult dung beetles transferred to DAWA from AAHL.
- Upgrade the "level of quarantine" existing in the Quarantine Insectary at DAWA.

4.2 Background

The Dung Beetle Programme was a 5 year joint venture between DAWA and CSIRO. The CSIRO component of the project was the importation of adult dung beetles from Spain into AAHL in Geelong. The eggs produced by these beetles were harvested, surface sterilised and sent to DAWA (QI) where the eggs were reared through to adults. These individuals were then released into climatically (CLIMEX) matched sites. The contract concerning AAHL finished at the end of January 1992.

Unfortunately, the adult beetles following a brief egg laying period when they first arrived at AAHL (March 1991) did not lay eggs again for the next 11 months. One reason might have been that the beetles phenology was still synchronised with northern hemisphere conditions and the beetles would recommence egg laying in March 1992. It was hoped that egg harvesting would then continue for about 2-3 months. Alternatively, the beetles may not produce eggs again. Nonetheless, if the beetles had of remained in AAHL then they would have been sacrificed. The solution was to transfer the beetles to the DAWA QI facility and then induce the females to lay eggs. The consignment (Table 6) arrived on 15th March 1992 and in April 1992 only *C. hispanus* beetles began producing eggs and egg extraction continued for the next 8 months.

Table 6: Consignment of Spanish Dung Beetles sent from AAHL to DAWA.

SPECIES	TOTAL	STAGE	
		Adult	Larvae
C. hispanus	1113	1110	3
B. bubalus	26	24	2
B. bison	20	18	2
O. belial	67	0	67

4.3 Cattle dung

Rearing of dung beetles requires sand and cow dung.

Cattle dung was supplied from spring annual and/or irrigated pastures. Dung was stored in the freezer (between -20°C and -30°C) in 10 litre buckets which were double lined with plastic bags. This freezing procedure was required to kill nematode and arthropod contaminants. Sufficient dung was carried into the high security area of the Quarantine Insectary by staff as they entered through the shower bay. Surplus buckets of dung were stored in either the QI freezer or the refrigerator which were both housed in the fumigation chamber.

4.4 Possible diseases carried by beetles

These beetles collected in Spain come from areas known to have

a) African Swine Fever
b) African Horse Sickness
c) Foot and Mouth

4.5 Quarantine facility

The QI was capable of becoming a high security facility as many quarantine requirements were already installed. These included

A. HEPA or Absolute filters (negative pressure)
B. Shower out facilities
C. sewerage system, sealed septic tanks.
D. fumigation chamber
E. autoclave system for sterilisation
F. incinerator/oven

The only addition to the QI was a dunk tank used for the passing out of eggs and small items to the outside (see Building Protocol).

The facility was monitored on a regular basis (3 monthly) by a Quarantine Veterinary Officer.

4.6 Disposal

1. Following extraction of eggs from brood masses, the eggs were surface sterilised in a 1% formalin solution in a dunk tank system and passed to the outside of the facility.
2. Adult beetles following their reproductive life were sacrificed.
3. All solid animal material was either autoclaved or incinerated within secure area.
4. The residual dung was incinerated and the sand was steam sterilised before disposal.

4.7 Protocol: Building

Please see highlighted notes on QI plans attached (Fig. 1)

1. rooms (CT 1 and CT 2) along south side of corridor were locked. The doorways were sealed.
2. Toilet door was locked and sealed. The toilet was locked to prevent its use while the QI was in a state of high security. Monthly, the toilet bowl reservoir was topped with water and stericide to manufacturer's specifications to maintain the water trap
3. Floor wastes in CT 4 and CT 3 were plugged and sealed with silicon filler and capped with 2 mm aluminium plate. Each aluminium plate was fixed to the concrete floor with four screws. This measure was required because the floor wastes empty directly into a number of independent soak wells.
4. All garments were packaged into autoclave bags, autoclaved, washed and dried in the preparation laboratory.
5. Formalin bombs were installed for fumigation purposes. Fumigation of equipment such as microscopes, was performed in the fumigation chamber using formaldehyde. This gas was generated by adding 50 ml of 40% formaldehyde to 25 gm of potassium permanganate. All equipment was cleaned to remove soilage prior to fumigation. Fumigation was commenced in the evenings and continued automatically overnight. The following day, the chamber was evacuated and equipment was removed. Gas masks and gloves were supplied and utilised by staff involved in formaldehyde fumigation.
6. A Dunk Tank was installed for sterilising objects being passed out of high security quarantine. It was located between the change clean and dirty rooms. The dunk tank was constructed in accordance with specifications provided by AAHL and cost approx. $1,000. All joins were stainless steel welded and dyeline tested. Hospital grade stainless steel plate (1 mm) plus stainless steel bolts were used throughout the construction. Fixing laminated glass and silicon sealant cost a further $200. The laminated glass window was secured by a mantling frame, bolted in place using silicon sealant as gaskets on both faces of the glass. The entire dunk tank panel was secured into the door recess with a silicone sealant gasket and bolted into the door frame with stainless steel bolts and loxons. The dunk tank was filled with glutaraldehyde solution to manufacturer's specifications bringing the solution surface within 100 mm of the dunk tank brim (approximately 90 litres). The glutaraldehyde reservoir was maintained at a minimum 100 mm above the bottom line of the dunk tank baffle. The reservoir was drained and recharged at six month intervals. This period was possible as only unsoiled items passed through the solution, thereby extending its useful life. The glutaraldehyde waste was disposed of in the high security sink in the Preparation Room.
7. Sewerage treatment: The two septic tanks on the north side of the building have sealed bottoms and run off into a leach drain. Prior to becoming a secure area the leach drain was blocked off and the tanks were evacuated. The only waste was shower and sink water. **No Solids**. In this way all waste water was stored in the two inter-connected septic tanks for decontamination and disposal by an authorised waste disposal contractor. Existing septic tanks were pumped as above and waste material was treated with chlorine. An alternative (this was not used) was to bypass the septic system and connect into the Isolation House

sewerage treatment plant which mixes and recycles sewerage adding metered amounts of chlorine. This is located 50 m from the QI.

8. Waste dung, and solid and semi-solid wastes were heat treated at for a minimum of 120°C x 10 hours in the Quarantine Insectary sterilising oven. Two heat resistant plastic bags were used to contain each parcel of the waste.
9. After treatment the bagged, cooked waste was removed from the low security side of the oven and left for collection at a designated site immediately adjacent the Quarantine Insectary. Security and garbage collection staff were notified of the collection requirements. They ensured that treated wastes were incinerated in the industrial, gas-fired DAWA incinerator.
10. The 2 Quarantine rearing rooms and corridor were maintained under negative pressure and hepa filtering
11. Alarm systems were linked to DAWA Security Office in case of mechanical breakdowns

4.8 Protocol: Entry and exit of Quarantine Insectary

Entry

1. From outside QI enter through shower facility into change clean room. Change into underwear
2. Wash feet in footbath containing chlorine (Halomid) between corridor and Lock 2.
3. Enter Lock 2 change into overalls, cap, socks and gloves
4. Enter lock 1 put shoes on
5. Enter rearing rooms

Exit

1. Enter lock 1 remove shoes
2. Enter lock 2 remove overalls, cap, socks and gloves.
3. Enter into corridor through footbath.
4. Enter change clean room. Remove underwear
5. Shower for 3 minutes, wash hair
6. Exit into dirty room.

Exit of eggs from QI

The procedure was identical to that used in AAHL

1. Eggs were recovered from brood masses and refrigerated at 10^{o} C before dunking procedure.
2. Eggs were housed in cassettes and placed in dunking bag and washed in detergent
3. Eggs were immersed in 1% formaldehyde in screw top jar (3 minutes)
4. Jar were placed into dunk tank and held in gluteraldehyde (part of 3 minute period in formaldehyde). Recovered on outside of secure area.
5. Eggs were washed in 10 water baths for 15 sec each.
6. Eggs were set up in quarantine rearing rooms to hatch. The rearing of F1 took place in conditions set out in the original protocol.

Only *C. hispanus* eggs were produced and removed from the quarantine insectary. The practice followed was that employed by AAHL (Steinbauer & Wardhaugh 1993).

Some changes were made as follows

1. the vessel used to convey the eggs through the dunk tanks was a perspex cylinder, 60 mm x 50 mm diameter. The cylinder's bottom was replaced with cheesecloth. A 50 mm hole was drilled into the cylinder's lid. The lid was then used to secure a second film of cheesecloth. Eggs were inserted into this egg vessel.
2. Eggs were allowed to reach ambient temperature (18-20^{o}C)and placed into the egg vessel which was in turn placed into a screw capped jar containing 1% formaldehyde solution. This jar was gently agitated within the dunk tank's glutaraldehyde reservoir maintained at ambient temperature. The process continued for a minimum of three minutes.
3. Eggs were passed out to the dirty room. The egg vessel was quickly removed and the eggs bobbed gently up and down in a series of 10 rinses in RO water at ambient temperature.
4. Gas masks and gloves were supplied, and utilised by staff involved in formaldehyde/glutaraldehyde procedures surrounding egg sterilisation.

<u>Exit of pupae from QI</u>

1. Only applies to *C. hispanus.*
2. Procedure was as indicated in Request for approval to use a new technique for the release to WA of dung beetles from the AAHL insectary.

No pupae were removed from the DAWA QI.

5 CULTURE NOTES FOR DUNG BEETLES IN HIGH SECURITY QUARANTINE

5.1 Quarantine Insectary rearing rooms

When adult beetles were first received into the QI they were housed in 10 litre buckets of moist sandy loam. Each bucket held approximately 20 beetles (males and females were housed separately) for 8 weeks. Buckets were checked daily and feeding was *ad lib,* generally 1 litre of high quality dung per bucket per week (previously frozen and thawed). Only *C. hispanus* adults produced eggs.

5.2.*Copris hispanus*

Adult beetles were paired-up and housed in 2 litre plastic ice cream containers with approximately 40 one millimetre perforations in each lid for aeration. A 2 centimetre layer of sterilised sandy loam was placed in each container. This absorbed any excess moisture. The rearing room was set at 24 hr dark phase / 25^{o}C.

Each pair was fed weekly with a food ball of 200 ml, containing 65% moisture. The pair generally took a few weeks to adjust. During this time they recommenced feeding which was easily observed by tunnelling and shredding of the food ball. During this phase food balls were replaced weekly.

The next phase began when the beetles commenced 'walking' (a track appears) around their food ball. The food ball was left alone at this stage.

The last phase began whereby the ball was formed and smoothed over. Brood ball production and egg laying followed within 7 days. Each brood ball containing a single egg was carved from the ball every 24 hours. Each brood ball was removed and set up in boxes containing 20-30 brood balls. These were kept ventilated.
By 'harvesting' the brood balls from each breeding pair the female continued to lay eggs. Some females produced up to 30 brood balls instead of the 4-6 brood balls that are normally produced. When males were in short supply they could be removed from their breeding partner and placed with a spare female. This caused the new females to form brood balls and lay eggs.

During the first month (April) of egg laying no eggs were sterilized and passed out of the QI resulting in 420 larvae at various stages of development. From these only 13 adult beetles emerged (Table 7). These beetles were paired with remaining beetles from AAHL.

During May 1992 eggs were harvested and held until the mouth parts of the embryo were observed. These eggs were then sterilized and passed out of the QI. Eggs were placed in CT rooms at 22 to 26^{o}C. All pupae were transferred to 16-18^{o}C when they became caramel coloured.

Table 7: Details of eggs extracted out of DAWA QI and their subsequent survival. Eggs were reared at a range of temperatures.

Date	Eggs out	Instars 1st	2nd	3rd	Pupae	Adult	Temp °C
2.5.92	63	9	6	0	0	0	22-24
14.5.92	92	9	3	3	3	1m	22-24
2.6.92	43	24	6	6	3	1m	22-24
12.6.92	66	17	13	5	2	1m	22-24
26.6.92	62	41	4	4	2	1f	22-24
3.7.92	37	19	3	3	3	3m	26
20.7.92	21	15	14	14	14	5m4f	26
20.8.92	15	3	3	3	3	1m1f	20
26.8.92	10	2	0	0	0	0	25
4.9.92	7	3	0	0	0	0	26
24.9.92	10	0	0	0	0	0	26
8.10.92	27	3	0	0	0	0	26
TOTAL	453	145	52	38	30	12m6f	

By 20.11.92 breeding activity had slowed and only eggs that showed signs of development were sterilized out. Beetles that had not laid or made brood balls after one month were stored in sand in buckets of the same sex at 16°C-18°C in darkness and fed *ad lib*. By 31.12.92 all breeding had ceased.

The 18 adults were placed in external walk in cages. In June 1994 30 *C. hispanus* adults (18m and 12f)emerged from the external cage and they were released into the Gingin area. This will be monitored over the next 12 months.

The adult beetles in the QI were paired once again after 6 weeks (4.2.93) at 16°C-18°C beetles and breeding commenced. Low numbers of eggs were produced, few were viable and only 8 were sterilized out. All 8 eggs hatched but none survived to the adult stage.

6 SUMMARY AND CONCLUSIONS

The Western Australian Dung Beetle - Bush Fly project was a joint venture between the Western Australian Department of Agriculture (DAWA) and CSIRO Division of Entomology. Its main aim was to facilitate the establishment of four species of spring/autumn active dung beetles (*Bubas bison, Copris hispanus, Onitis belial, Bubas bubalus)* in Western Australia.

A total of 8,831 dung beetle eggs in 32 shipments were received by DAWA from AAHL (Table 8). Survival to the adult stage was low with a total of 15 adult dung beetles emerging. This is comparable with Steinbauer and Wardhaugh (1995) who demonstrated that 100% of eggs of *B. bison* and *O. belial* treated with formaldehyde did not survive to the adult stage. Furthermore, their study had a similar hatch rate for eggs of *O. belial* (22.2%), but a much higher hatch rate for eggs of *B. bison* (100%) following treatment with formaldehyde (Table 8).

Table 8: Summary table of 32 shipments of eggs received by DAWA. This includes mean hatch and survival to adult. Only survival to broods was recorded for *B. bison.*

Species	Eggs received	Mean % hatch	% Survival to adult
B. bison	6,361	32.9 ± 4.8	3.5 (broods)
O. belial	1,535	23.9 ± 6.6	0.58
C. hispanus	251	12.8 ± 5.1	1.6
B. bubalus	684	12.8 ± 1.0	0.29
TOTAL	8,831		

The only major difference between the two studies is that eggs once released from AAHL were transported to DAWA by air freight and this may account for some of the reduction in egg hatching. In many cases eggs in shipment containers had been displaced. Unfortunately, cargo such as this seems to be extremely sensitive to both handling and temperature/pressure changes. In future projects, the rearing of dung beetles from egg to adult should be conducted at the one quarantine facility.

Following the transfer of the 4 species of dung beetles from AAHL to DAWA, only *C. hispanus* produced eggs with 4% surviving to the adult stage. This slightly better performance in survival to adult stage may be accounted for by only sterilising those eggs containing embryos with visible mouth parts.

The technical information supplied in this report are a useful set of guidelines for any future importation of live insect pests for research or biological control agents for release into Western Australia.

7 ACKNOWLEDGEMENTS

The authors wish to thank James Ridsdill-Smith, John Matthiesson and David Cook for all their co-operation throughout this project. Many people, too numerous to name, participated in this project at both a technical level and on a volunteer basis. Three people that deserve special mention are Grant MacDonald, Brett Scourse and Wayne Morris for their the technical assistance throughout this project. We would also like to thank the technical staff at CSIRO Floreat for helping out with field work.

The co-operation between CSIRO staff at AAHL and DAWA staff was a useful partnership with exchange of staff and reciprocal visits to each facility throughout the project.

Finally, without all the support and infrastrucure of DAWA, especially the Entomology Section, such a project could not have been completed.

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