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Parkinsonia Biological Control

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Plain English Summary

Parkinsonia aculeata (parkinsonia) is a neotropical shrub/tree species that was introduced in the Australia as an ornamental species and for its potential value as a hedging and fodder plant. It has since spread to occupy over 8000km^2 of the rangelands of northern Australia, and forms dense thickets in floodplains and grasslands, and along water courses and bore drains. It has negative impacts on the pastoral industry and rangeland production systems through limiting pasture growth, restricting stock access to water and impeding mustering. Mechanical and chemical control methods for parkinsonia already exist and are already being effectively used by land managers wherever possible. But these management tactics require repeat application and are not always possible in all parkinsonia infestations (e.g. in difficult terrain or in sensitive riparian environments). Therefore, n this project, two biological control agents approved for release in Australia were mass-reared and distributed widely across northern Australia (incl. QLD, WA and the NT) to assist with the integrated management of parkinsonia. In all in excess of 200,000 of these two moths that are specialised to feed on parkinsonia, and not on other plants, have been released. Over time populations of these insects are anticipated to help keep parkinsonia under control, thereby reducing ongoing control costs and improving rangeland productivity and profitability.

Executive summary

Why was the work done?

Parkinsonia aculeata (parkinsonia) is a neotropical shrub/tree species that was introduced in the Australia as an ornamental species and for its potential value as a hedging and fodder plant. It has since spread to occupy over 8000km^2 of the rangelands of northern Australia, and forms dense thickets in floodplains and grasslands, and along water courses and bore drains. It has negative impacts on the pastoral industry and rangeland production systems through limiting pasture growth, restricting stock access to water and impeding mustering. It also has impacts on the environment through providing refuges for feral animals like pigs, increasing evapotranspiration, contributing to soil erosion, and impacting wildlife habitat. At present widespread prickle bushes like parkinsonia can have control costs between $2-$300/ha/y depending on the density of infestations. Reducing some of these control costs and improving pasture productivity can therefore assist in improving the profitability of rangeland production systems.

Mechanical and chemical control tactics for parkinsonia already exist and are already being effectively used by land managers wherever possible. But these management tactics require repeat application and are not always possible in all parkinsonia infestations (e.g. in difficult terrain or in sensitive riparian environments). Having a landscape-scale self-perpetuating form of control like biological control in these systems may therefore aid in the integrated management of parkinsonia. This was the basis for past projects funded by Meat & Livestock Australia (B.NBP.0366; B.NBP.0620; B.WEE.0134) to identify candidate biological control agents and test their safety, and the current project that focussed on mass rearing and release of the two moths that are feed on parkinsonia but not on other plants.

How was the work done?

Based on detailed tests to demonstrate their safety, CSIRO received approval from the Commonwealth of Australia in 2012 and 2014, to release two closely related leaf-feeding moths, *Eueupithecia cisplatensis* and *Eueupithecia vollonoides* (nicknamed UU1 and UU2 respectively). Building on earlier work on these species, in this project, we (1) developed a detailed understanding of the development of UU1 and UU2 in relation to variations in temperature; (2) undertook bioclimatic modelling to determine where across parkinsonia's infestation in Australia each species is likely to perform best; (3) mass reared and released significant numbers of each species on parkinsonia infestations; (4) monitored establishment of these agents across northern Australia; and (5) worked with a vast network of regional stakeholders to improve awareness of the potential value

of biological control within an integrated management approach for parkinsonia and other rangelands weeds.

What was achieved?

Mass-rearing and widespread releases of agents was achieved through collaborations of CSIRO with key partners in Queensland (Department of Agriculture and Fisheries (QDAF)), Western Australia (Department of Agriculture and Food WA (DAFWA); Pilbara Mesquite Management Group (PMMG); Rangelands NRM WA (RNRMWA)) and the Northern Territory (Northern Territory Department of Land Resources Management (DLRM)). This resulted in the release of over 200,000 UU1 (76 sites; 116 releases) and 75,000 UU2 (24 sites; 37 releases) on parkinsonia infestations across northern Australia. This is in addition to the 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) released as part of an earlier MLA-funded project. The releases in the current project focussed on releasing pupae which are easier to distribute in more remote localities. Field studies have shown that the insects have established at over 50% of the release sites that were monitored and are starting to spread considerable distances (>10km) on their own indicating that they are likely to effectively find parkinsonia plants across the rangelands. In all sites where establishment had occurred defoliation was evident, and over time we anticipate this to translate into impacts on plant health and reproduction that suppress parkinsonia populations. Our previous work has shown that high levels (>50%) of defoliation are possible that such larval damage can impact plant health. The full impacts in the field may take up to a decade to become fully apparent, but the early signs are promising. The science outputs generated by this project on the physiology and bioclimatic modelling of UU1 and UU2 will aid ongoing evaluation of the contribution of these agents to integrated weed management, and also guide similar efforts in future biological control projects.

What industry benefits will arise and what are the results and implications of the work?

The key benefit to the pastoral industry is the presence of biological control as a persistent landscape scale weed management tool in the integrated weed management toolbox for parkinsonia. This will enable land managers to prioritise where in the landscape they can deploy other management tactics (e.g. in areas where the agents have failed to establish for some reason or are easy to access by other control tactics), while biological control is a chronic stressor in areas where it has established. An earlier preliminary cost-benefit analysis indicated that if the impacts of defoliation outlined above are replicated across 50% of the total parkinsonia infestation over the next decade, it could help to reduce current recurring annual weed management costs by 10% (ca $15/ha/y) and improve pasture productivity by $1-2/ha/y. This would translate into a Net Present Value (NPV) of $15.6 million for the investments in the parkinsonia biological control program to date, and a benefit cost ratio (BCR) of 3.44. Future evaluation of agent impacts on parkinsonia populations will be needed to verify these projections.

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1 Project rationale

Parkinsonia aculeata (parkinsonia) is a neotropical shrub/tree species that was introduced in the Australia as an ornamental species and for its potential value as a hedging and fodder plant. Native to the Americs, parkinsonia is thought to have naturalised in Australia by the late 1800. It has since spread to occupy over 8000 km^2 of the rangelands of northern Australia, and forms dense thickets in floodplains and grasslands, and along water courses and bore drains. It has negative impacts on the pastoral industry and rangeland production systems through limiting pasture growth, restricting stock access to water and impeding mustering (Deveze 2004, van Klinken et al. 2009, van Klinken and Heard 2012). It also has impacts on the environment through providing refuges for feral animals like pigs, increasing evapotranspiration, contributing to soil erosion, and impacting wildlife habitat. At present widespread prickle bushes like parkinsonia can have significant control costs ($2-$300/ha/y) depending on the density of infestations. Mitigating some of these control costs and improving pasture productivity can therefore assist in improving the profitability of rangeland production systems.

Mechanical and chemical control tactics for parkinsonia already exist and are already being effectively used by land managers wherever possible. But these management tactics require repeat application and are not always possible in all parkinsonia infestations (e.g. in difficult terrain or in sensitive riparian environments). Having a landscape-scale self-perpetuating form of control like biological control in these systems may therefore aid in the integrated management of parkinsonia (Deveze 2004, Raghu et al. 2006, van Klinken 2006). This was the basis for past projects funded by Meat & Livestock Australia (B.NBP.0366; B.NBP.0620) to identify candidate biological control agents, and a recent (B.WEE.0134) and current project that focussed on mass rearing and release of the two most recently approved biological control agents (the leaf-feeding moths, *Eueupithecia cisplatensis* and *Eueupithecia vollonoides*) approved for release against parkinsonia in Australia.

The key benefit to the pastoral industry is the presence of biological control as a persistent landscape scale weed management tool in the integrated weed management toolbox for parkinsonia. This will enable land managers to prioritise where in the landscape they can deploy other management tactics (e.g. in areas where the agents have failed to establish for some reason or are easy to access by other control tactics), while biological control is a chronic stressor in areas where it has established. A related benefit is that the network of collaborators forged during the life of this project can be used to further the biological control and integrated management of other similarly widely distributed rangeland weeds.

The overarching aims of this project is the facilitation of establishment of UU1 and UU2 on parkinsonia infestations through (i) development of an advanced system of mass-rearing and field distribution, (ii) spatially optimize field releases across northern Australia, and (iii) establish mass-rearing hubs for supply to pastoralists and NRM groups.

2 Project objectives

This project focused on mass-rearing and release of the two leaf-feeding moths (*E. cisplatensis* and *E. vollonoides*, abbreviated as UU1 and UU2 respectively hereafter) across parkinsonia infestations

spanning Queensland, Northern Territory and Western Australia. Specifically, the project set out to achieve the following objectives:

1- Determine physiological thermal requirements for Eueupithecia species to improve their mass-rearing as Parkinsonia biological control agents, and facilitate field-release processes to improve their potential in bioclimatically suitable areas.

2- Engage a broad coalition of regional NRM bodies, and local land management groups to develop strong local networks for coordinated biological control of Parkinsonia through access to the established mass-rearing hubs for the agents.

3- Provide the best evidence-based on-farm best practice recommendations to integrate biological control into production systems

These objectives were met through the following specific outputs;

- Identify at least 18 field release sites across Queensland, NT and WA and establish mass rearing hubs for insect biological control agent pupae (Output 6a of Rural R&D for Profit project)
- Investigate physiological requirements for life history transitions for both insect biological control agents and publish results in an international journal (Output 6b of Rural R&D for Profit project)
- Release 10,000 pupae of each insect biological control agent across 18 sites in northern Australia and monitor establishment (Output 6c of Rural R&D for Profit project)

3 Method and project locations

The project aims and objectives were met through a combination of scientific research and on-ground release and monitoring of parkinsonia biological control agents. The methods adopted to achieve these activities are outlined below.

3.1 Physiological studies

Understanding the physiology of UU1 and UU2, specifically thermal tolerance, is vital to both advance the efficiency of mass-rearing processes, and also to identify optimal locations for releases in Australia. In order to achieve this, the developmental times of UU1 and UU2 were examined at six constant temperatures (10, 17, 25, 29, 34, 39 ^{o}C) at a fixed relative humidity (60%) and a 12:12h light:dark cycle. Development of the insect species was examined across the three key life-history transitions, viz. Egg to Neonate (E-N), Neonate to Pupa (N-P) and Pupa to Adult (P-A) transitions. For each of these transitions twelve replicates were set-up at each temperature, with twelve individuals per replicate for the N-P and P-A transition, and 20-30 individuals for the E-N transition. Each of the replicates was observed daily to document survivorship of life stages and rates of life-history transitions at each of the temperatures. Development rates in relation to temperature were analysed and modelled using standard statistical approaches (Clarke 1998, Briere et al. 1999).

3.2 Bioclimatic modelling of optimal release sites

We developed ecological niche models using the occurrence records of UU1 and UU2 in its native range to project areas in the invaded range of parkinsonia likely to be climatically suitable for release and optimal establishment of these agents. This bioclimatic modelling of the potential distribution of UU1 and UU2 across northern Australia was undertaken using MaxEnt, a species distribution modelling software package (Elith et al. 2006, Elith et al. 2011).

Extensive surveys of the native range have delimited the native range distribution of UU1 and UU2 to be eastern and northern Argentina (Fig. 1). The bioclimatic characteristics of this native range was described using the first five principal components of the 35 bioclimatic variables, Bio36 - Bio40 (available from http://www.climond.org, Kriticos et al. 2012) were used to model distribution of these insects. Created using principal component analysis, the first five components explains > 90% variance of the 35 bioclimatic variables (Kriticos et al. 2014). Based on a comprehensive modelling approach (Mukherjee and Raghu – in prep), the potential distribution of UU1 and UU2 across Australia was projected and trimmed by the current distribution of parkinsonia to identify sites where these agents are likely to perform best.

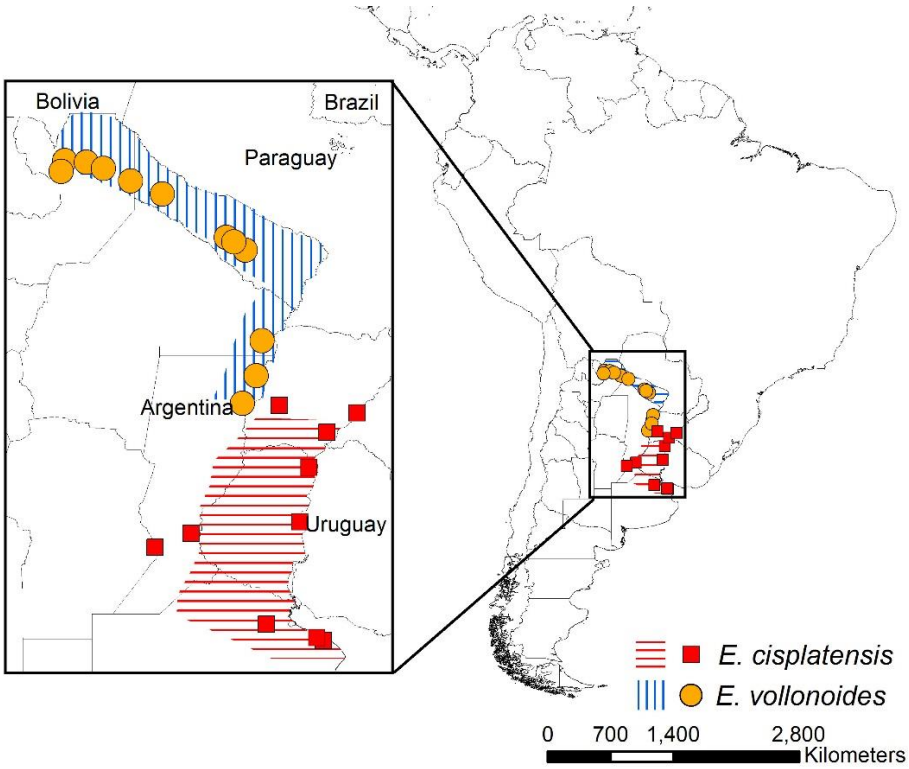

Fig. 1. Distribution of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2) in their native range in S. America.

3.3 Mass-rearing and release of UU1 and UU2

Both UU1 and UU2 were reared at the CSIRO facilities at the Ecosciences Precinct in Brisbane and also at Queensland's Department of Agriculture and Fisheries' Tropical Weeds Research Centre, Charters Towers. In general however, the CSIRO facility in Brisbane served as the mass-rearing hub for UU2, while the QDAF facility in Charters Towers served as the mass-rearing hub for UU1.

Experience based on previous releases suggested that larvae were likely to be more vulnerable to predation by ants and wasps. Releases of pupae enabled UU1 and UU2 to escape such predation, and the adults emerging from puparia are able to find optimal microhabitats within parkinsonia foliage for egg deposition and larval development. Therefore, field releases in this project principally focussed on release of pupae, although on occasion larvae were released as well. When pupae were hand-collected from colony cages (e.g. as was done in Charters Towers for UU1) they were released in the field in a plastic container placed within a pyramid shelter hung on parkinsonia foliage. When larvae were encouraged to pupate by themselves in the rims of cut lips of compostable cups (Fig. 2) (e.g. as was one in Brisbane for UU2), these lids were shipped to collaborators or hand carried to field sites and the cut cup lip was hooked onto branches of parkinsonia plants. When larvae were released, they were transported on sprigs before being transferred onto the vegetation in the field. Release sites were selected on the basis of their ability to serve as nursery sites for the establishment of the agents. In addition to regular at nursery sites, opportunistic releases also occurred during periodic visits to grower properties. The distribution of release sites spanned QLD, WA and the NT.

3.4 Establishment of UU1 and UU2 across northern Australia

All nursery sites were monitored at least once/year during the summer months. Since the larvae are very good at mimicking parkinsonia foliage or thorns, detecting their presence by searching plants is difficult and laborious. The beat-sheet method is a useful monitoring tool for these insects. Beat sheets can either be hand-held or laid on the ground. Up to ten of the healthiest parkinsonia plants close to the release area at a site were randomly selected. A standardized number of beats/tree at each site was used to beat the healthy foliage to dislodge any insects present onto the beat-sheet placed beneath the foliage. The beat-sheet was then examined to record the numbers of UU1/UU2, and the presence of other insects (particularly, predatory insects). The presence of UU1/UU2 after at least one wet season-dry season cycle was determined to be the minimum evidence acceptable to confirm establishment; this time period ensured that the released insects had not only survived the release, but that the local site was able to sustain multiple generations of the insects.

Once populations were recorded as having established, any spread from the original release sites was also monitored using the beat-sheet method (Fig. 2). To detect this spread of these insects, parkinsonia trees were monitored at a sequence of fixed distances (ca 25m from the release area) radiating outwards in different directions from the original release area.

Fig. 2. Shipment, release and monitoring of UU1/UU2 at field sites (a) Shipment of larvae; (b) Shipment of pupae; (c) & (d) Releases of larvae into a parkinsonia "nest"; (e) Setting up a pyramid shelter for release of pupae; (f) Coating the shelter's handle with Tanglefoot™ to prevent ant predation of pupae; (g) Take-away container with pupae placed in pyramidal shelter (with adult UU1 emerging); (h) Cut lip of a compostable cup provided as a p[upation substrate within colony cages showing pupae in groove; (i) hanging of compostable cup lips on parkinsonia branches in the field; (j), (k) & (l) Beat sheet method for detection of dislodged UU1/UU2. Photo credits: (a,c,d,e,f,g,h,I,j,l) – CSIRO; (b,k) – Kelli Pukallus (QDAF).

3.5 Advanced chemical ecology and genetic tools for monitoring field establishment

3.5.1 Chemical ecology: UU1 and UU2 pheromones

Long range species specific pheromones could be used for monitoring and aggregating populations of UU1 and UU2 in the introduced range. The first step in determining if pheromones could be used in this biological control system is to characterise the chemical components of female sex pheromones of the two species. Secondly field assays in areas where these species are known to be established were conducted to see if pheromone extracts were effective at attracting conspecific males.

To characterise female pheromones of the two moths, pupae of each species were kept in individual vials until they emerged as adults. Adult females were observed overnight until they began extruding the pheromone gland at the end of the abdomen (also known as 'calling'). Sex pheromone glands were then extruded by applying slight pressure to the female's abdominal tip to force eversion of the ovipositor, and were excised with small scissors and immersed in chromatographically pure n-hexane for 15 min. The n-hexane extracts were transferred and pooled in a clean conical glass vial and kept in a freezer at -10°C if not used immediately. Extracts were concentrated under a gentle stream of pure Nitrogen before analysis, which contained material from 5 moths.

Pheromone extracts were analysed on a Gas Chromatograph - Mass Spectrophotometer (GC-MS). Field assays with extracts were undertaken to assay attractiveness to UU1 and UU2. We adapted the methods of Gibb et al. (2007, 2008) for this work.

3.5.2 Molecular diagnostics: genetic identification of UU1 and UU2

As indicated earlier, UU1 and UU2 are not easily distinguishable using morphology and definitive identification is only possible through dissection, characterization and comparisons of the morphology of genitalia. To simplify the identification process we developed molecular diagnostic tools based on the mitochondrial marker CO1 and the ribosomal marker 28S; these markers have been identified as having potential to discriminate between animal species (Hebert et al. 2003, Hebert et al. 2010).

To characterize CO1 and 28S markers, DNA was extracted from individuals of UU1 and UU2 stored at -20°C following procedure described in Brookes et al. (2015). Briefly, either 3 legs or head + thorax (based on DNA concentration) were homogenized in lysis buffer along with proteinase K and digested overnight at 55°C followed by RNAse treatment to limit RNA contamination. Binding buffer and ethanol were then added to bind DNA into a spin column and the spin column was washed using wash buffer. Finally, the bound DNA was eluted using elution buffer, quality checked and quantified. PCR amplification of CO1 and 28S genes were carried out using primers HCOLep(F)-LCO(R) for CO1 and A335(F)-S3660(R) for 28S following PCR conditions described in Brookes et al. (2017), and the genes were sequenced using Sanger sequencing method (Sanger et al. 1977). These sequences were examined for differences between UU1 and UU2.

4 Results

4.1 Physiological studies

Thermal physiology studies revealed key differences between UU1 and UU2 in terms of their thermal tolerance. These differences were particularly evident at the egg-neonate transition. Eggs of UU1 developed most rapidly into neonates at 29 °C (7.36 ± 0.75 days; mean ± SE) and slowest at 17°C (16.01 ± 0.24 days) (Fig. 3). No UU1 egg development took place at 10 or 39 °C. In contrast, eggs of UU2 developed far more rapidly UU1; eggs of UU2 developed most rapidly at 34 °C (3.07 ± 1.06 days) and most slowly at 17 °C (12.14 ± 0.62 days) (Fig. 3).

The differences between UU1 and UU2 for the other two transitions were not as marked but, in general UU2 developed faster than UU1 (Fig. 3).

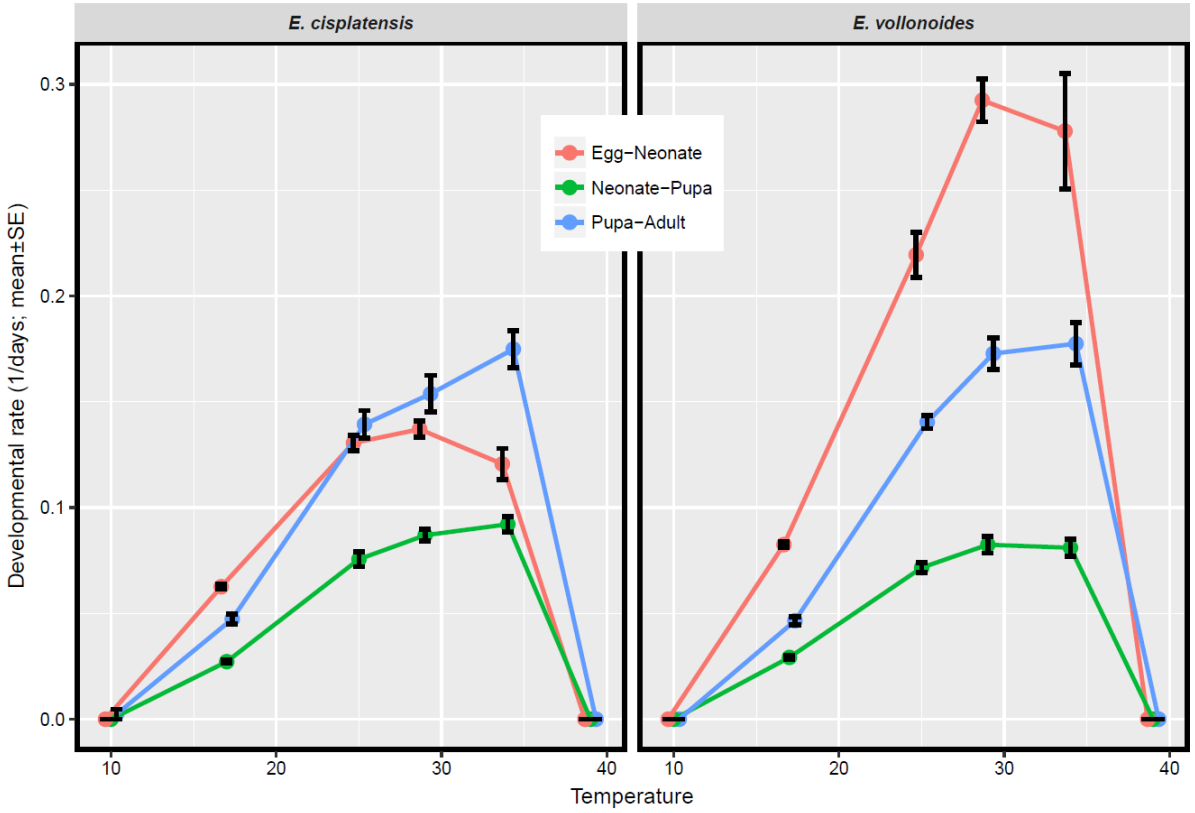

Fig. 3. Temperature (°C) dependent development rate (mean ± SE) for the different life history transitions of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2). N=12 replicates/temperature for Egg-Neonate and Pupa-Adult transitions and N=6-14 replicates/temperature for Neonate-Pupa transition. The number of initial individuals/replicate was 20 eggs, 12 neonates and 12 pupae for the Egg-Neonate, Neonate-Pupa and Pupa-Adult transitions, respectively. Development was studied at six temperatures (10, 17, 25, 29, 34, 39 °C); the depictions on the plots are marginally staggered to facilitate comparisons between the transitions for each species.

Analyses of developmental thresholds revealed another key difference between UU1 and UU2 at the egg to neonate transition. The developmental zero (D_0; the critical minimum temperature below which development is arrested) was 6.78°C for UU1 eggs, while it was much higher at 9.57°C for UU2

(Fig. 4). There was little difference between the two species in terms of the developmental thresholds for the other two life history transitions (Fig. 4). Once the developmental thresholds were met, in general UU2 needed fewer degree days to complete its development than UU1 across all life history transitions (Fig. 4).

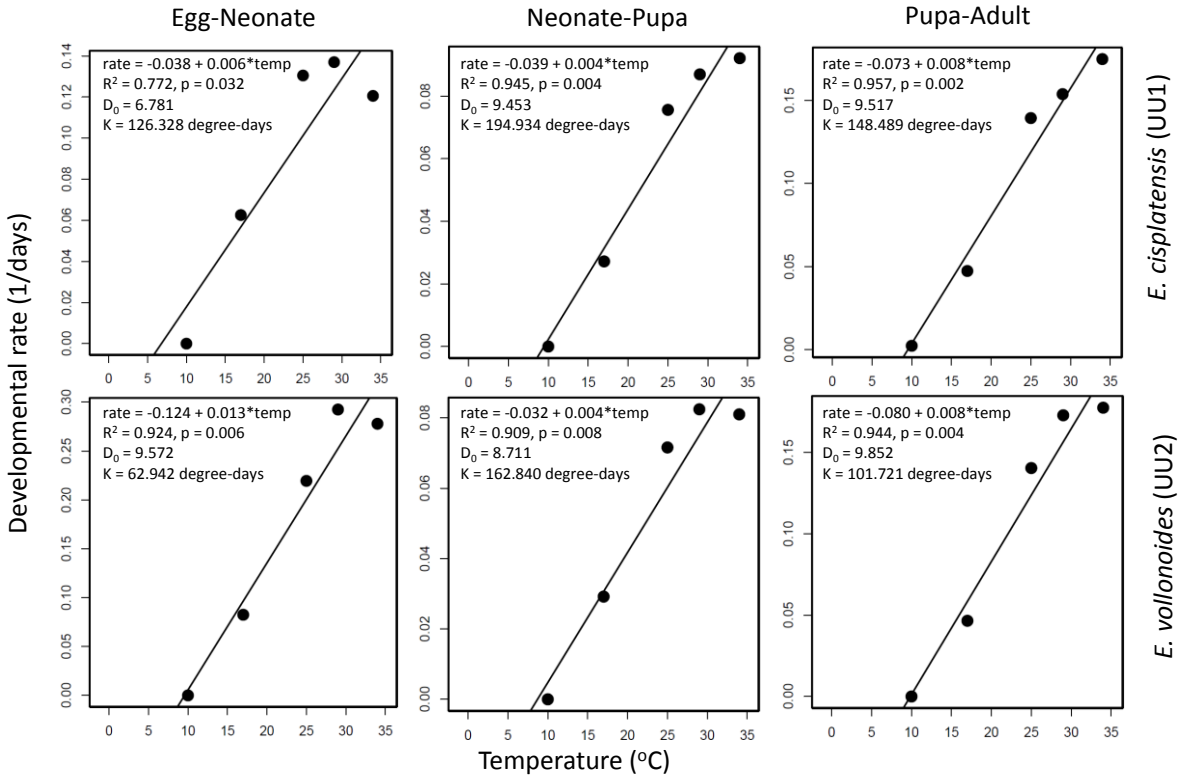

Fig. 4. Estimation of developmental thresholds and degree days required to complete life history transitions, by regression on development rates (1/duration of transition) on temperature (°C) for the linear portion of the developmental curve. The top row represents *E. cisplatensis* (UU1) while the bottom row represents *E. vollonoides* (UU2). The transitions represented by each of the columns of graphs are indicated above the top panel. Note differences in y-axis scale between graphs.

4.2 Bioclimatic modelling of optimal release sites

Through a process of iterative model-fitting in MaxEnt we identified areas within the current extent of parkinsonia in Australia that are a good fit for UU1 and UU2 based on where they occur in their native range. These modelling efforts projected that southeast Queensland, parts of southwestern Queensland and parts of northern New South Wales and eastern parts of South Australia within known occurrences of Parkinsonia were highly suitable for UU1 (Fig. 5). Parts of southwestern Queensland and the savannahs bordering the arid zone and the zone of seasonable rainfall across northern Australia were also moderately suitable (Fig. 5). In contrast to UU1, the projected distribution of UU2 in Australia was generally wider and include northern parts of Queensland, southern Northern Territory, as well as parts of Western Australia were most suitable for *E. vollonoides* (Fig. 6).

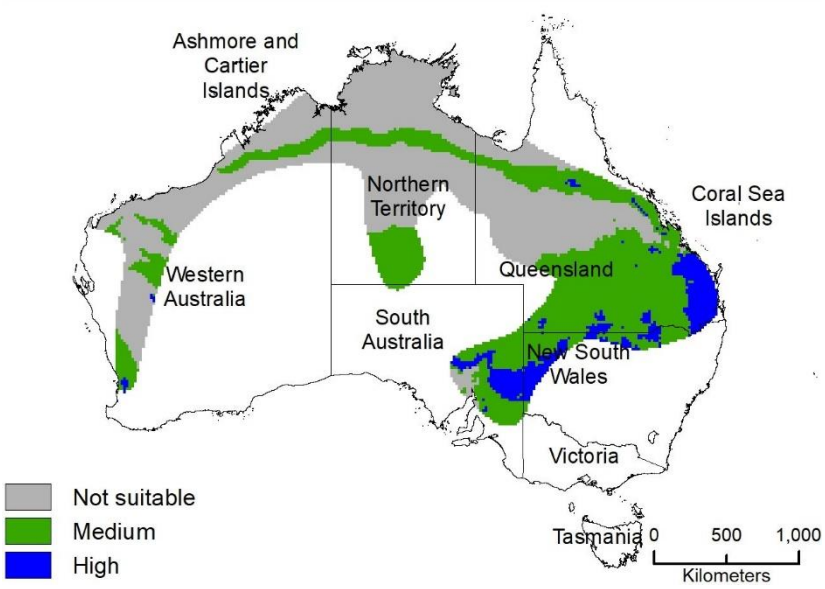

Fig. 5. Bioclimatic suitability of the current Australian extent or parkinsonia (region shaded in grey, as captured by a minimum convex polygon of distribution records) for *E. cisplatensis* (UU1) as revealed by species distribution modelling using MaxEnt. Suitability is defined as "Medium" when less than 50% of the iterative models predicted a particular grid cell on the map was suitable for the species, while it was defined as "High" if more than 50% of the models predicted a regional was suitable. Projections are at 1-degree grid resolution.

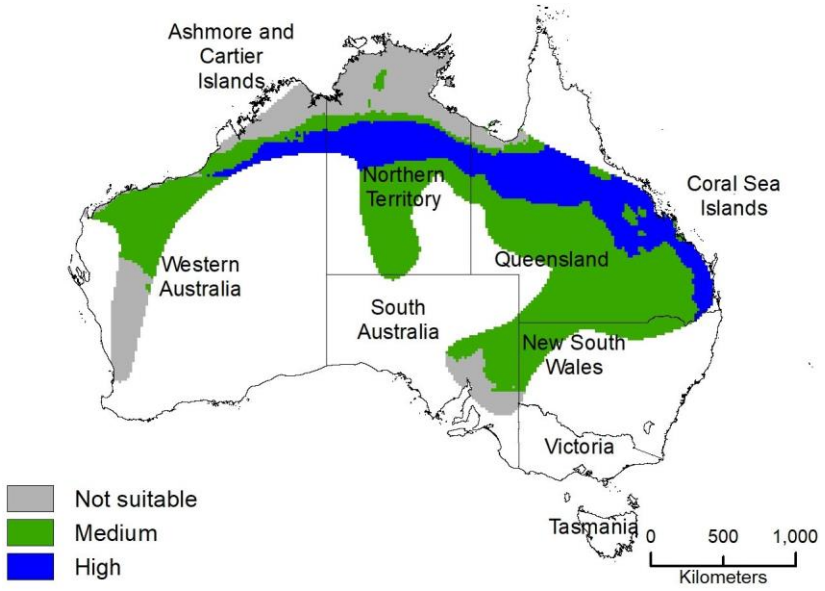

Fig. 6. Bioclimatic suitability of the current Australian extent or parkinsonia (region shaded in grey, as captured by a minimum convex polygon of distribution records) for *E. vollonoides* (UU2) as revealed by species distribution modelling using MaxEnt. Suitability is defined as "Medium" when less than 50% of the iterative models predicted a particular grid cell on the map was suitable for the species, while it was defined as "High" if more than 50% of the models predicted a regional was suitable. Projections are at 1-degree grid resolution.

4.2.1 Mass-rearing and release protocols for UU1 and UU2

Rearing at both locations was done under optimal environmental conditions for the plant and the two insect species. Colonies of these insects were maintained as follows. Eggs laid by female moths were maintained in the laboratory until neonates hatched; these were then transferred onto the leaves of parkinsonia plants growing in cages in an air-conditioned greenhouse (ca 25-28^{o}C; 50-60% RH). After completion of their development through larval and pupal stages, newly emerged adults were collected daily from colony cages and paired with adults emerging from different cages (to ensure an adequate mix of their genetic diversity and limit the likelihood of any negative inbreeding effects). These mating pairs were confined in plastic containers (17 x 11 x 5 cm) to ensure mating and oviposition. These containers were lined with moistened power towels to maintain a high level of humidity to prevent desiccation of eggs laid. Neonates from eggs laid by newly mated females were initially lab-reared prior to being transferred to rearing cages in the glasshouse or laboratory in anticipation of field release of pupae.

Lab rearing involved maintaining the eggs in the plastic containers in a lab environment (25-28^{o}C; 50-60% RH), after removing the adults that were confined in the container for mating. Upon egg-hatch, neonates were presented with healthy sprigs of parkinsonia leaves as food; fresh sprigs of leaves were supplemented regularly to ensure that a density of up to 200 larvae could be maintained in each container. As the larvae progressed to the second instar stage (typically, within one week) they were transferred into the leaves on parkinsonia plants growing in colony cages in the glasshouse or laboratory. For UU1 from Charters Towers pupae were collected from glasshouse cages using featherweight forceps and stored in plastic containers for distribution in the field (Fig. 2). For UU2 from Brisbane, as larvae begin to progress through the late instars, the cut lip of a compostable cup was placed in the cage adjacent to the plant materials being consumed by the larvae (Fig. 2). This is to provide a pupation substrate and take advantage of the natural tendency of the larvae to pupate in the constrictions of the lip of the cup. The compostable nature of the cup lid enables us to minimise the impact of lab materials/waste being left behind in the field (Fig. 2).

4.3 Releases and establishment of UU1 and UU2 across northern Australia

Releases in excess of the 10,000 pupae of each species across 18 sites in northern Australia have been achieved over the duration of the project (Table 1).

Table 1. Summary of release numbers of UU1 and UU2 over the course of the current project.

Species	State	No. Sites	No. Releases	Total pupae released	Total larvae released
E. cisplatensis (UU1)	QLD	70	109	131,172	68,918
	WA	3	4	13,574	0
	NT	3	3	8,406	0
	TOTAL	**76**	**116**	**153,152**	**68,918**
E. vollonoides (UU2)	QLD	15	21	19,576	25,600
	WA	4	10	5,887	19,800
	NT	4	6	5,285	950
	TOTAL	**23**	**37**	**30,748**	**46,350**

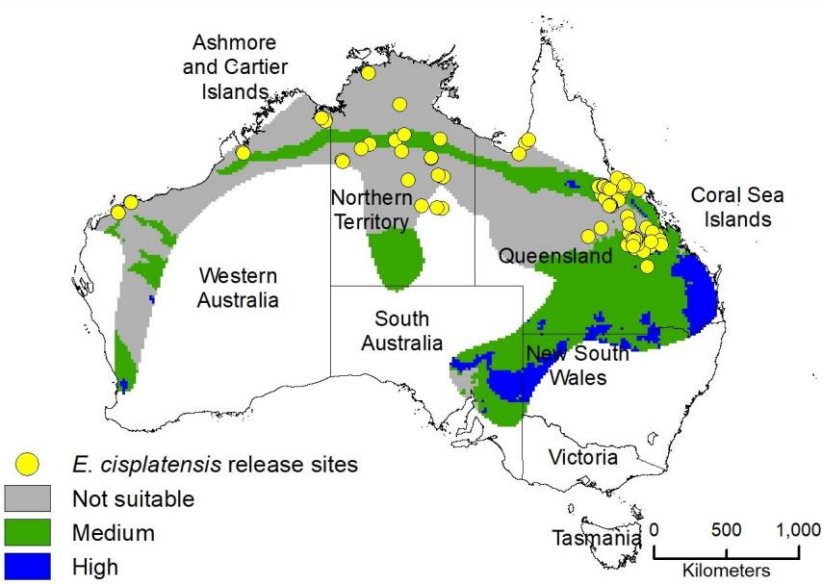

Fig. 7. Distribution of release sites of *E. cisplatensis* (UU1) in relation to its bioclimatic suitability. See caption of Fig. 5 for definition of suitability.

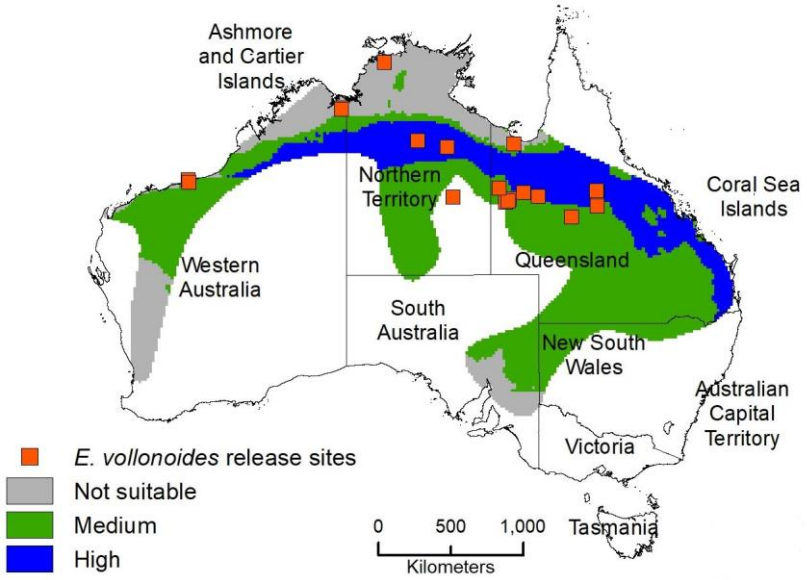

Fig. 8. Distribution of release sites of *E. vollonoides* (UU2) in relation to its bioclimatic suitability. See caption of Fig. 6 for definition of suitability.

To date 65% of the 54 sites where UU1 has been surveyed post-release have resulted in the establishment of self-sustaining populations. The establishment rate for UU2 sites is 39% across the 18 sites that were monitored post-release. At some sites the natural spread of these agents has been impressive with spread of up to 15 km and 32km from the nearest release sites recorded for UU1 and UU2 respectively. Since some of release sites have only recently received agents, it is premature to determine establishment (formally defined as persistence and detection of populations through one wet season and one dry season). These releases are in addition to the over 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) from a previous MLA-funded project.

4.4 Advanced chemical ecology and genetic tools for monitoring field establishment

4.4.1 Chemical ecology: UU1 and UU2 pheromones

Pheromone extracts were analysed on a Gas Chromatograph - Mass Spectrophotometer (GC-MS). This found that UU1 consistently had 8 major peaks while UU2 had 3 (Table 2, Fig. 9). The 3 chemical components present in UU2 extracts were the same as those present in UU1 (Table 2). Pheromone lures were then created by impregnating rubber septa with 200 µl of the extract of a single female moth gland.

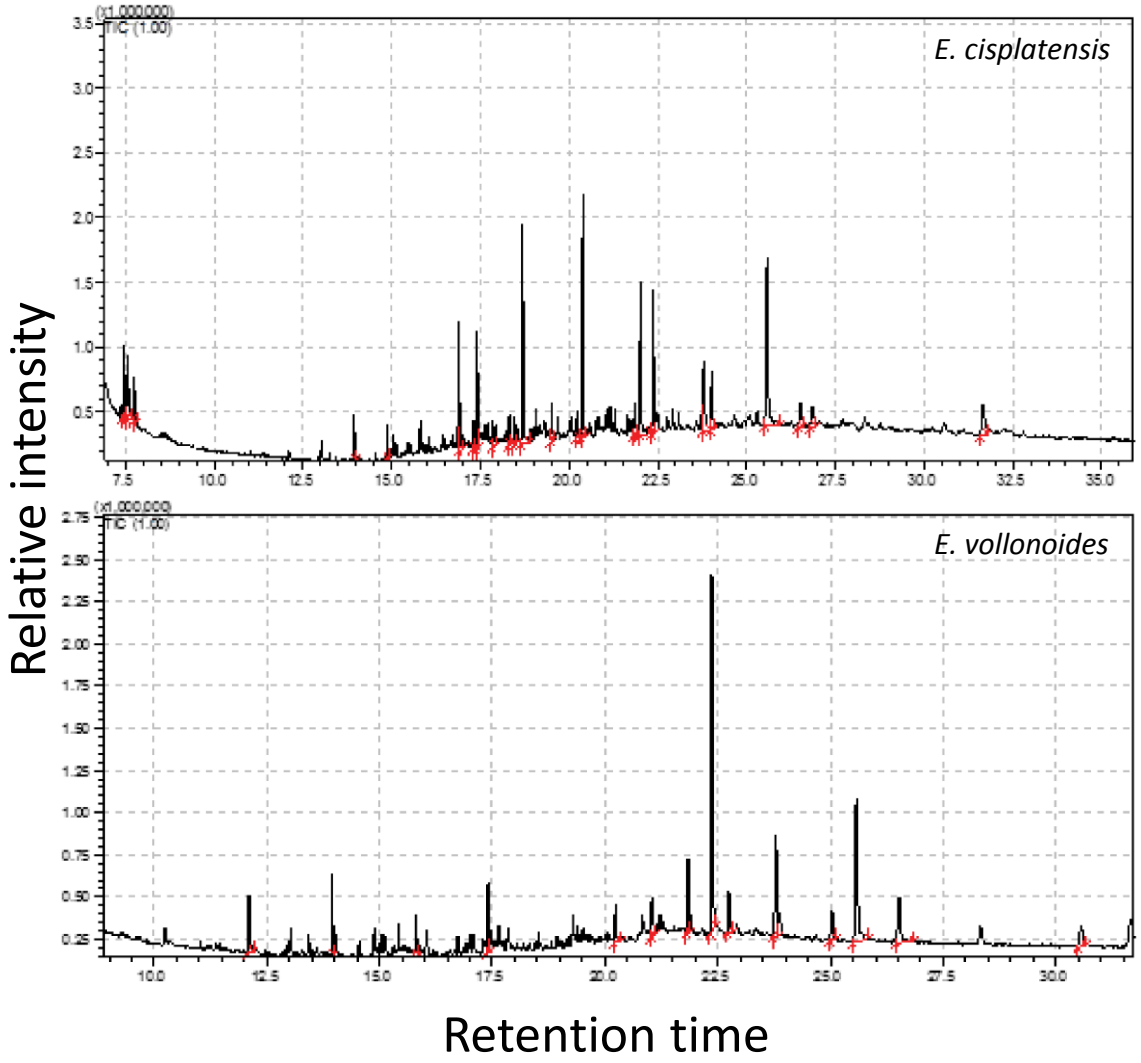

Fig. 9. Chromatogram of pheromone profiles of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2)

Table 2. Likely chemical composition of extracts from female pheromone glands of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2) that correspond with peaks in the chromatogram.

Retention time	**Putative chemicals**	
	Eueupithecia cisplatensis (UU1)	*Eueupithecia vollonoides* (UU2)
18.690	Z3Z6Z9-OCTADECATRIENE/ Z3Z6Z9-EICOSATRIENE	
20.383	Z3Z6Z9-NONADECATRIENE/ Z3Z6Z9-EICOSATRIENE	
21.844	Z3Z6Z9-NONADECATRIENE	
22.011	Z3Z6Z9-EICOSATRIENE	
22.368	Z3Z6-9,10-EPO-EICOSATRIENE	Z3Z6-9,10-EPO-EICOSATRIENE
23.794	Z3Z6Z9-HENEICOSANETRIENE	Z3Z6Z9-HENEICOSANETRIENE
24.021	Z3Z6-9,10-EPO-HENEICOSANETRIENE	
25.579	Z3Z6-9,10-EPO-OCTADECATRIENE	Z3Z6-9,10-EPO-OCTADECATRIENE

4.4.2 Molecular diagnostics: genetic identification of UU1 and UU2

The sequence information for CO1 and 28S of UU1 and UU2 are presented in table 1. Analyses of these sequences using 4 to 6 individuals of UU1 and UU2 respectively revealed that the CO1 marker was unique for UU1 and UU2 (Fig. 10a), whereas for 28S, sharing of both UU1 and UU2 haplotypes was observed in a couple of individuals (Fig. 10b). Additional replications and analyses are underway using all insect life stages.

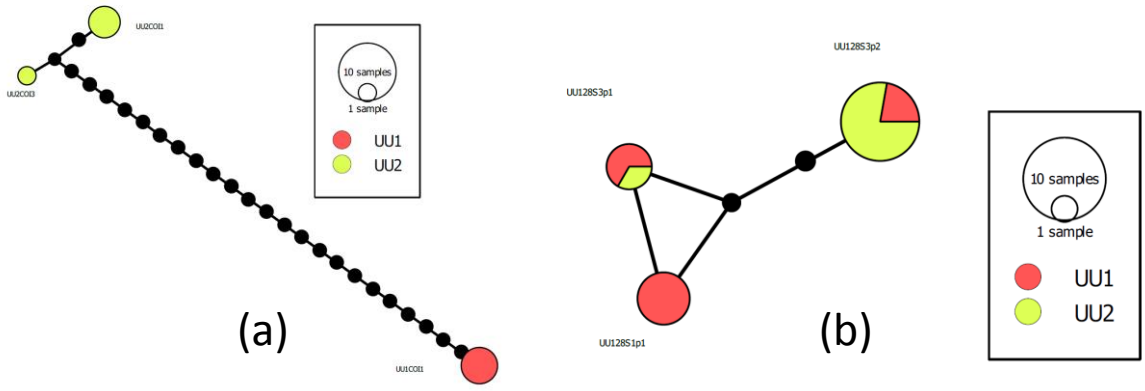

Fig. 10. Preliminary haplotype network for (a) CO1 and (b) 28S markers of UU1 and UU2

Table 3. Nucleotide sequence information for CO1 and 28S markers for UU1 and UU2

Species	Marker	Nucleotide sequence
E. cisplatensis	CO1	CAAAAAATCAGAATAAATGTTGATAAAGAATTGGGTCTCCTCCACCAGCAGGGTCGAAAAATGATGTATTTAAATTTCGGTCAGTTAATAATATT GTAATTGCTCCTGCTAATACTGGTAAAGATAATAATAATAAGAAAGCTGTAATTCCTACGGCTCAAACAAATAATGGAAGTTGATCAAATGATAT ATTATTAAGTCGTATATTAATAATTGTTGTGATAAAATTAATTGCACCTAAAATAGATGAAATACCTGCTAAATGAAGTGAAAAAATAGCTAAGTC AACTGAACTACCCCCGTGAGCAATATTAGAGGATAGTGGGGGATAAACTGTTCATCCAGTACCAGCTCCGTTTTCAACGATTCTTCTAGAAATTAA TAAAGTAATTGAAGGGGGTAAAAGTCAAAATCTTATATTATTTATTCGGGGGAAAGCTATATCAGGAGCTCCTAATATTAAAGGAACTAATCAAT TACCAAATCCTCCGATTATAATAGGTATAACTATAAAAAAAATTATAATAAATGCATGGGCAGTAACAATAGTATTATAGATTTGATCATCTCCAA TTAATGATCCTGGATTACCTAATTCAGCTCGAATTATTAATCTTAAA
	28S	CCCCTATACCCAGTTCCGACGATCGATTTGCACGTCAGAATCGCTACGGTCCTCCATCAGGGTTTCCCCTGACTTCGACCTGACCAGGCATAGTTC ACCATCTTTCGGGTCCCAGCATCTGTGCTCAGAGCGCGCCTGCATTCGCAGATTGGAAACGAGACGCCTCGGGAGTGCGAGAACGTCGATCGAG ATCGACGCTCCATCCTCCCTGAGAGGGCGACGAGCGTCCTCCTTCACTTTCGTTACGCCTTTAAGTTTCGTTCGAAATAATCTCAATGACTCGCACA CATGCTAGACTCCTTGGTCCGTGTTTCAAGACGGGTCCTGCGAGTGCCCGAAACTGAATCATCGCAGACGGACACGTGCACGGTCCGAGACCAC GGCTGCGACGTACAGGCGCGTCGCCCCCTCCCTCGCGCTCGGGCAGAGGGCGGTGACGAAGCTGACCTTGCGTCGGGCCGGACGCGCTGCTGA ATAGCACGCGTGCACGTTTCGTTGTTTTACCGTCCGACGGCCGGTCGGCTGCCGCAAAGCGGTCAACCCGGCCGAGACCCGCGAGGGCCGACCG AGAGACTCCCGCGCGGAGCGTAGACCGACATCGAACGGGTCGCGATGTATTACTGAGGGAGAAGTGCACGCCGTCCCGGGACGTTTGCGTACG CAGCGCGTGGCGAACCGTCCGAAGACGCAACACCATCGACGACGACGTCCGCACGCCCGGAAAAGACGATGAATCT
E. vollonoides	CO1	CAAAAAATCAGAATAAATGTTGGTAAAGAATTGGGTCTCCTCCACCAGCTGGGTCAAAAAATGATGTATTTAAATTTCGATCAGTTAATAGTATA GTAATTGCTCCTGCTAATACTGGTAAAGATAACAATAATAAAAAAGCTGTAATTCCTACGGCTCAAACAAATAATGGAAGTTGATCAAATGACAT ATTATTAAGTCGTATATTAATAATTGTTGTAATAAAATTAATTGCACCTAAAATAGATGAAATACCCGCTAAATGAAGGGAGAAAATAGCTAAGTC AACTGAACTACCTCCATGAGCAATATTAGAGGATAGCGGAGGGTAAACTGTTCATCCAGTACCAGCTCCATTTTCAACGATTCTTCTAGAAATTAA TAAAGTAATTGAAGGGGGTAAAAGTCAAAATCTTATATTATTTATTCGGGGGAAAGCCATATCAGGAGCTCCTAATATTAAAGGAACCAATCAAT TACCAAATCCTCCAATTATAATAGGTATAACTATAAAGAAAATTATAATAAAGGCATGGGCAGTAACAATAGTATTATAGATTTGGTCATCTCCAA TTAATGATCCTGGATTACCTAGTTCAGCTCGAATTATTAATCTTAAA
	28S	CCCCTATACCCAGTTCCGACGATCGATTTGCACGTCAGAATCGCTACGGTCCTCCATCAGGGTTTCCCCTGACTTCGACCTGACCAGGCATAGTTC ACCATCTTTCGGGTCCCAGCATCTGTGCTCAGAGCGCGCCTGCATTCGCAGATTGGAAACGAGACGCCTCGGGAGTGCGAGAACGCCGATCGAG ATCGACGCTCCATCCTCCCTGAGAGGGCGACGAGCGTCCTCCTTCACTTTCGTTACGCCTTTAAGTTTCGTTCGAAATAATCTCAATGACTCGCACA CATGCTAGACTCCTTGGTCCGTGTTTCAAGACGGGTCCTGCGAGTGCCCGAAACTGAATCATCGCAGACGGACACGTGCACGGTCCGAGACCAC GGCTGCGACGTACAGGCGCGTCGCCCCCTCCCTCGCGCTCGGGCAGAGGGCGGTGACGAAGCTGACCTTGCGTCGGGCCGGACGCGCTGCTGA ATAGCACGCGTGCACGTTTCGTTGTTTTACCGTCCGACGGCCGGTCGGCTGCCGCAAAGCGGTCAACCCGGCCGAGACCCGCGAGGGCCGACGA CCGAGAGACTCCCGCGCGGAGCGTAGACCGACATCGAACGGGTCGCGATGTATTACTGAGGGAGAAGTGCACGCCGTCCCGGGACGTTTGCGT ACGCAGCGCGTGACGAACCGTCCGAAGACGCAACACCATCGACGACTACGTCCGCACGCCCGGAAAAGACGATGAATCT

5 Discussion

5.1 Physiological studies inform mass-rearing and releases

Although similar in morphology and relatively close in distribution in their native range, UU1 and UU2 have significantly different development physiologies in relation to temperature. Specifically, UU1 seems to be more cold-tolerant, while UU2 seems to be able to complete its development once a developmental threshold temperature is reached. The fact that this seems to hinge on the developmental temperatures that eggs are exposed is a significant insight in the importance of microclimatic factors that may influence where mate female moths lay their eggs. This substantiated the approach used in this study to release pupae; this approach ensures that the emerging adult moths can mate and find suitable oviposition sites among the parkinsonia foliage that may be better suited for egg development and larval survival.

The physiological studies undertaken in this study also enabled us to used constant temperature cabinets to slow/temporarily arrest development of insects to ensure they arrived in the release locations in optimal condition. For example, knowing that keeping eggs of UU1 and UU2 at 17 ^{o}C can double and triple the time to egg-hatch (relative to development under ambient [25^{o}C] conditions) enabled us to exchange individuals between the mass-rearing hubs. Such exchange of materials was crucial to minimising the effects of inbreeding and associated loss of genetic diversity of our colonies of these species. The chilling of eggs prior to transport between the rearing hubs enable us to better synchronise mixing of colonies of each species that were maintained in Brisbane and Charters Towers. A similar chilling of pupae at 17^{o}C allowed the extension of the pupa-adult transition from approximately 7 days at 25^{o}C to 14 days at 17^{o}C. This allowed us to better synchronise emergence of adults in lab and glasshouse colonies to improve rearing efficiency. The cooling of pupae also enabled us to hold the insect in this natural resting stage to synchronise it with availability of field personnel and regional stakeholders to undertake the field releases.

5.2 Bioclimatic modelling inform optimal release locations for each species

In biological control, bioclimatic modelling has typically been used to determine the extent of an agent's climatic match with the weed's distribution and in posthoc evaluations of failure of agent establishment. In this project, our prospective use of bioclimatic modelling enabled the careful identification of sites across the parkinsonia infestations in Australia that may be a better fit for UU1 relative to UU2.

Bioclimatically, UU1 seems to be better suited for the relatively cooler and wetter parts of parkinsonia's Australian distribution, while UU2 seems to be better suited to the hotter and drier parts. In addition, the broader bioclimatic range of UU2 appears to be greater than UU1. This knowledge enabled us to concentrate efforts on rearing and releasing UU1 in central QLD (particularly, east of the Great Dividing Range) because this region was a better bioclimatic fit for this species. The siting of the mass-rearing hub for UU1 in Charters Towers (QLD) greatly enhanced these efforts.

The relative robustness of UU2 relative to UU1 in terms of a more rapid development time and an increased tolerance/preference to hotter and drier climates enabled us to have UU2 be the focus of

releases across more remote and regional locations in QLD, WA and the NT. The establishment of strong spread of UU2 from release sites despite the tyrannies of distance with shipping to remote locations (e.g. infestations along the Gulf of Carpentaria in QLD, and in the Kimberley and Pilbara in WA) suggest that there is value having bioclimatic modelling guiding the selection of release sites. An important scientific aspect here is that we took advantage of this prospective use of bioclimatic modelling to release agents in sites that may be suboptimal. Future evaluations of persistence of UU1 and UU2 at these bioclimatically suboptimal locations relative to more optimal locations will help to test the value of our modelling approach, and it utility of similar approaches as part of future mass-rearing and release programs in weed biological control.

5.3 Use of advanced tools for detection of establishment in the field

The chemical ecology and genetics aspects of this work were done specifically to develop tools that can help to detect establishment in the field even at low levels.

The chemical ecology work was predicated by initial work that showed that traps baited with a single, live *E. vollonoides* virgin female have caught 17 male moths while traps baited with live *E. cisplatensis* females have only caught a single male moth. The virgin female trap captures indicate that the attractiveness of pheromone blends in these moths is species-specific. This means that, although chemicals in the pheromone glands of females are similar (as identified by the GC-MS study presented in this report, the long range mate recognition of these two species is independent. This suggests the possibility of development of species-specific lures to detect presence and abundance of these species. In addition, such approaches may also enable the detection of aggregation chemicals that help create population aggregations in the field. This could enable conspecifics males and females to find each other even at low densities, overcoming Allee effects, indicating that smaller releases combines with aggregation chemicals could be used to facilitate establishment (Bartelt et al.); this would enable releases to be more spatially extensive than in the current project.

The genetics work will enable us to detect the species that has established, given UU1 and UU2 were released at some sites.

5.4 Practical implications for industry

The key benefit to the pastoral industry is the presence of biological control as a persistent landscape scale weed management tool in the integrated weed management toolbox for parkinsonia. This will enable land managers to prioritise where in the landscape they can deploy other management tactics (e.g. in areas where the agents have failed to establish for some reason or are easy to access by other control tactics), while biological control is a chronic stressor in areas where it has established. A related benefit is that the network of collaborators forged during the life of this project can be used to further the biological control and integrated management of other similarly widely distributed rangeland weeds.

In terms of economic benefits to the industry, if the defoliation capable by UU1 and UU2 are replicated across 50% of the total parkinsonia infestation over the next decade, it could help to reduce current recurring annual weed management costs by 10% (ca $15/ha/y) and improve pasture productivity by $1-2/ha/y. As indicated in a related earlier study, this would translate into a Net Present Value (NPV) of $15.6 million for the investments in the parkinsonia biological control

program to date, and a benefit cost ratio (BCR) of 3.44. Ongoing monitoring and impact assessment will be needed to assess these projections.

5.5 Lessons learnt and Key messages

This project reinforced the importance of developing and sustaining a large network of key stakeholders, especially for the management of widespread weeds.

Another key lesson was the need for contingency resourcing to cover off on unanticipated withdrawal of potentially valuable partners. For example, the NT Government was a key contributor to earlier parkinsonia biological control projects. Unfortunately, dues to budget cuts, they could not formally commit to participate in this project. However, they had informally indicated their ability to contribute to the mass-rearing and release outside of the formal relationships of this project. Over the first year of this project further staffing cuts in the NTG meant that they could not assist in any way at all. Though CSIRO and QDAF were able to stretch resources to cover off on making some releases in the NT, securing additional financial resources from this project may have been a way to sustain NTG's capacity in the context of this project and weed biological control in general.

5.6 Recommendations

Multiple avenues exist for future investigation to add value to the work to date on parkinsonia biological control.

Once the UU1 and UU2 have reached sufficient densities across the landscape, it would be of value to undertake a comprehensive quantitative evaluation of the combined impacts of all agents released on parkinsonia populations. This would enable a better characterisation of the impacts of the agents (including the inferred link between defoliation and demographic consequences for parkinsonia) and help to robustly undertake cost-benefit analyses.

Should there be a need to introduce additional agents for parkinsonia biological control, the stem-galling fly from Argentina (*Neolasioptera aculeatae*) or the stem-boring moth from Mexico (*Ofatulena luminosa*) may warrant further investigation (Heard and van Klinken 2014). Both species have the capacity to reduce the growth and reproduction of parkinsonia, but their host-specificity is yet to be comprehensively evaluated and they need to undergo an appropriate risk assessment prior to being permitted for release into Australia.

6 Project Achievements

6.1 Sub-Project level achievements

Achievement against the Key Performance Indicators of the parkinsonia sub-project are outlined below.

	Achievement criteria	**Status against KPIs**	**Progress achieved against KPI**	**Outputs**
1	KPI 1.10 — Progress identifying release sites and establishing mass rearing hubs (as per Output 6(a))	Achieved	At least 18 sites were identified for *Eueupithecia cisplatensis* (UU1) and *Eueupithecia vollonoides* (UU2). Mass-rearing hubs have been established for UU1 at Charters Towers, and for UU2 in Brisbane.	A spatial database of release sites
2	KPI 2.7 — Report on physiological requirements of insect biological control agents (Output 6(b))	Achieved	A detailed understanding of the physiological differences in UU1 and UU2 has revelaed that UU1 may be more cold tolerant, but UU2 may be more vigorous once its minimal developmental threshold temperature has been reached.	Data on thermal physiology
3	KPI 3.10 — results of physiological studies to a scientific journal (Output 6(b))	Partially achieved	The data have been analysed and graphs have been prepared for inclusion in this report. These need to be adapted for publication in a scientific journal.	Draft manuscript on thermal physiology
4	KPI 3.11 — release of agents at field sites (Output 6(c))	Achieved	Releases in excess of the initially anticipated releases have been achieved across northern Australia. Over 200,000 UU1 (76 sites; 116 releases) and 75,000 UU2 (24 sites; 37 releases) on parkinsonia infestations across northern Australia. This is in addition to the 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) released as part of an earlier MLA-funded project.	180K pupae and >100K larvae released at over 100 release sites across northern Australia (spanning QLD, WA and NT)

	Achievement criteria	Status against KPIs	Progress achieved against KPI	Outputs
5	KPI 4.9 — progress with mass-rearing process on the basis of diapause studies (Output 6(c))	Achieved	The physiological studies had strongly informed the mass-rearing and release processes, principally in relation being able to hold insects in suitable temperatures to exchange materials between mass rearing hubs to ensure genetic mixing of populations. The studies also help guide the holding of pupae at cooler temperatures as part of shipments to remote release locations.	Increased efficiency of mass-rearing and improved maintenance of colony genetics between mass-rearing hubs
6	KPI 5.8- Report on release and monitoring of at least 10,000 pupae of each agent at 18 sites across northern Australia (Output 6(c))	Achieved	Mass rearing and releases are continuing despite having already achieved the project milestones. The rearing hubs in Brisbane (CSIRO) and Charters Towers (QDAF) are continuing to produce substantial number of insects for release on parkinsonia infestations across northern Australia. In addition, a S. America was undertaken to import fresh material of *E. vollonoides* to refresh the genetics of colonies.	>180K pupae and >100K larvae released at over 100 release sites across northern Australia (spanning QLD, WA and NT)
7	KPI 6.9 — Report on level of establishment in at least 18 pupal release sites across northern Australia (Output 6(c))	Achieved	Establishment of UU1 and UU2 has occurred in some 69% and 39% respectively of the monitored release sites. We anticipate that these number will be revised upwards with more this year, given much of parkinsonia's distribution was under drought conditions. The breaking of the drought is likely to improve conditions for the plant and there for the insects as well.	Spatial database on establishment relative to release sites
8	KPI 6.10 — Submission of an article on results of pupal diapauses studies to a peer-reviewed journal (Output 6(b)).	Partially achieved	As indicated in an earlier milestone report, diapause in pupae seems to have been completely eliminated from the colonies. So in the place of a manuscript on diapause, a manuscript on the bioclimatic model is being prepared. The graphs and maps of that modelling effort is included in this report, and will be adapted for publication in a scientific journal.	Draft manuscript on bioclimatic modelling

Sub Performance Indicators and Measures in the Sub Project Log Frame - (Parkinsonia), through to current (M6) milestone period

Project Details	Performance Indicators and Measures	M&E Methods	When	Progress through to current milestone report
1 - Determine physiological thermal requirements for *Eueupithecia* species to improve their mass-rearing as Parkinsonia biological control agents, and facilitate field-release processes to improve their potential in bioclimatically suitable areas.	• Physiological studies on *Eueupithecia cisplatensis* and *Eueupithecia vollonoides* completed	• Milestone report 3 and 4 reporting on physiological requirements of *Eueupithecia cisplatensis* and *Eueupithecia vollonoides* • Draft manuscript completed for submission to peer-reviewed journal	• 30-Nov-2016, 30-Apr-2017 • 30-Apr-2017	• Studies on Egg-Neonate, Neonate-Pupa and Pupa-Adult transitions completed for *E. cisplatensis* and *E. vollonoides*, and preliminary analyses have been completed • Fresh importation of *E. vollonoides* from S. America in Nov 2017 will enable reinvigorating the genetics of the colonies in Brisbane and Charters Towers.
2 - Engage a broad coalition of regional NRM bodies, and local land management groups to develop strong local networks for coordinated biological control of Parkinsonia through access to the established mass-rearing hubs for the agents.	• Compilation of key network contacts for releases of biological control agents for parkinsonia • Establishment of mass-rearing hubs for *Eueupithecia* spp. • *Eueupithecia cisplatensis* pupae released at 18 sites across, QLD, WA and NT • *Eueupithecia vollonoides* pupae released at 18 sites	• Contact of regional land protection, weeds and biosecurity officers to build a database on contacts interested in participating in weed biological control • Mass-rearing protocols developed and refined, specifically for the generation of pupae for most effective long-distance despatch of agents	• 30-March-2016 • 30-Nov-2017 • 30-Sep-2018	• Network contacts established for all release sites • Mass rearing hubs established in Brisbane (*E. vollonoides*) and Charters Towers (*E. cisplatensis*) • Releases of *E. cisplatensis* and *E. vollonoides* pupae are ongoing into the sites in QLD, WA and NT • Releases of *E. cisplatensis* and *E. vollonoides* pupae in excess of the proposed releases have been achieved through effective collaboration between project team and engagement of a diversity of on-ground stakeholders.

Project Details	Performance Indicators and Measures	M&E Methods	When	Progress through to current milestone report
	across, QLD, WA and NT			
3 - Provide the best evidence-based on-farm best practice recommendations to integrate biological control into production systems	• Best-practice guidelines for mass-rearing, release and monitoring of Eueupithecia species for parkinsonia biological control.	• Details of protocols developed as part of this project and the previous MLA-funded project (WEE.0134) will be collated to facilitate interested landholders, local authorities and community groups interested in continuing to mass-rear *Eueupithecia* species beyond the life of the project. This will be made available both via MLA and through the CSIRO webpage.	• 30-Sep-2018	• Draft guidelines have been developed and amended to include the methods to release pupae and the optimisation of releases in relation to the bioclimatic and physiological preferences/tolerances of *E. cisplatensis* and *E. vollonoides*.
4 - Producers and their advisers have improved access to information	• Article in Feedback magazine on mass-rearing hubs (Nov 2016) • Information on Parkinsonia biological control through CSIRO website (Nov 2016) • Webinar through MLA website (Nov 2017) • Peer-reviewed publication on physiological requirements of	• Precis provided to MLA, followed by interview of project leader by MLA reporter. Draft of article will be fact-checked by project leader prior to publication • Creation of website through CSIRO webpage, followed by quarterly updates to content (where required) • Sub-project leader will prepare content for inclusion in and assist with the delivery of webinar. • Publication of physiological requirement will be made	• 30-Sept-2018	• Interview completed with Cat Nicholls for MLA • Template for Parkinsonia biological control site on CSIRO website completed; content upload is completed, and website to be live in mid-May. • Brief information videos on the project and rearing and releases have been filmed and in the final stages of production by QDAF. MLA and DAWR are formally acknowledge in their credits. They need to go through the Queensland Government's approval processes as part of being released online.

Project Details	Performance Indicators and Measures	M&E Methods	When	Progress through to current milestone report
	Eueupithecia species and its implications of biological control (Sep 2018)	open-source to ensure free and widespread access		• Information based on the parkinsonia subproject has been compiled and submitted as part of the Biological control app. • Data analyses of physiological studies have been completed and a draft outline for a manuscript has been developed.

6.2 Contribution to project expectations

Provide a description of how the sub-project achievements contribute to the achievement of the expected outcomes for the whole project. Limit this to 1-2 paragraphs for each point.

a) Greatly increase the on-farm populations of 8 weed biocontrol agents

This project has resulted in the release of over 200,000 UU1 (76 sites; 116 releases) and 75,000 UU2 (24 sites; 37 releases) on parkinsonia infestations across northern Australia. This is in addition to the 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) released as part of an earlier MLA-funded project.

b) Reduce weed competition and herbicide use across more than 25 million ha

The two released agents have the capacity defoliate parkinsonia which can slow the growth rate of plant; in particular, the growth rate of juvenile plants can be reduced by up to 50% when defoliation by the agents is in excess of 50% of the leaves of the plant.

c) Reduce the densities of the six target weeds across northern and southern Australia

Over time, the impacts of biological control (by these two agents and others that are already established), as a component of integrated weed management, are anticipated to reduce the densities of parkinsonia across it distribution in Australia (principally northern Australia).

d) Increase long-term annual yield and reduce annual weed control costs

The impacts of agents (assuming 50% defoliation of the plants across 50% of the parkinsonia infestations) could help reduce the annual costs of weed management by about $15/ha/y.

e) Improve agricultural natural resource management nationally

The impact of the agents identified above could results in improved pasture productivity of $1-2/ha/y. This productivity gain is on the basis of what is know for other similar prickle bushes.

f) Inform producers of weed management options and

The parkinsonia subproject has involved over 100 primary producers, land holders and regional stakeholders. In addition this direct engagement the media releases and online engagement is anticipated to have reached at least 10 times as many people.

g) Establish a new collaborative national approach to weed biocontrol

The parkinsonia subproject worked across the entire distribution of parkinsonia in northern Australia and spanned collaborations between researchers, extension officers, biosecurity officers and stakeholders in QLD, NT and WA. This close collaboration is being extended through work on other weeds beyond the life of this project through a related project funded by the DAWR's Rural R&D for Profit Scheme.

6.3 Contribution to Rural Profit R&D programme objectives

The objective of the programme is to realise significant productivity and profitability improvements for primary producers, through:

- generating knowledge, technologies, products or processes that benefit primary producers

The project has generated new knowledge on the physiological tolerances and bioclimatic preferences of biological control agents in the context of parkinsonia. In addition the methods related to these scientific outputs have broad relevance and applicability in the context other mass-rearing and release project in weed biological control.

- strengthening pathways to extend the results of rural R&D, including understanding the barriers to adoption

The vast networks of primary producers, weeds officers, extension officers, biosecurity officers and regional stakeholders reached through this project has enabled the extend the results of the parkinsonia sub-project across the entire distribution of parkinsonia in Australia. In addition to overcoming any barriers in the context of this weed, it has opened up collaborations with these stakeholders for future projects on weeds.

- establishing and fostering industry and research collaborations that form the basis for ongoing innovation and growth of Australian agriculture.

If the impacts of the agents (50% defoliation of plants) are replicated across 50% of the parkinsonia infestation across northern Australia, it could help reduce recurrent annual weed management costs by up to $15/ha/y and improve pasture productivity by $1-2/ha/y.

7 Collaboration

Cash and/or in-kind contributions were formally made to this project by CSIRO, QDAF and PMMG.

Free information sharing occurred across the project partners and the extensive network of stakeholders. Information exchange occurred via both print (e.g. reports, guidelines) and email, and via online media platform (e.g. project website).

8 Extension and adoption activities

- Project website (https://research.csiro.au/parkinsonia)
- Feedbase Focus article
- Feedback Magazine article interview scheduled with Riccarda Burley in May
- Youtube videos on UU1 and UU2 biology, mass-rearing, release and evaluation (available after formal approval by QDAF)
- November 2016: QDAF media release (http://statements.qld.gov.au/Statement/2016/9/1/very-hungry-caterpillars-join-queenslands-bug-army-to-fight-pest-weeds) and regional (e.g. Burdekin Advocate) and national (ABC Rural) media articles.
- March 2017: Northern Beef Producer Expo, 3-4th March 2017. Charters Towers Showgrounds. TWRC & DAF stand. Attended by general public, landholders, stakeholders, Govt agencies. Promoted current biological control projects.
- March 2017: NQ Dry Tropics woody weed control Demo Day, 20 March 2017. Crooked waterhole, Giru. UU & Parkinsonia update & pupae available for release to local landholders.

- June 2017. Dry Tropics Pest Advisory Forum & TWRC open day. Practical demonstration. TWRC, Charters Towers. Landholders, industry & general public attended. Overview of current biological control projects & supplied UU for release for attendees.
- July 2017. “Biological Control Overview & current projects” UQ St Lucia Northern Tour students. PPT presentation and glasshouse walk-through, TWRC, Charters Towers, 13 July 2016.
- October 2017: 18 October 2017, Certificate II in Conservation and Land Management Program students from Jenagar, TWRC tour of glasshouses & Biological control overview of current projects.
- QDAF Technical Highlights 2016, 2017, 2018 - project overview and update on release date & sites.
- Over 100 on-farm visits have occurred over the life of this project; on several of these visits engagements with multiple farm managers/land holder occurred wherein the role of weed biological control in integrated weed management was discussed. Over 50 landholders/farm managers/regional weeds officers participated in field releases. See network of release sites identified in the Appendix.

9 Financial Statement

Attached.

9.1 Unexpended funds

Unexpended funds identified in the financial statement will be used to support salary and operating costs of project staff to enable collation of all project information through to the final publication of scientific papers from data collected over the life of this project.

9.2 Project partners

In-kind contributions for this sub-project were provided by CSIRO, QDAF and PMMG as indicated in the contract. Regular financial statements on these contributions have been provided by all these agencies in association with milestone reports. The NT Weeds branch was anticipated to be a project partner for local mass-rearing, release and evaluation of agents. However, because of budget cuts in the relevant NTG department they were unable to fully participate; work in the NT was achieved by CSIRO and QDAF providing agents for release in the NT. In addition to the above formalised partners, gratis contributions were received from a wide range of stakeholders including landholders, regional biosecurity officers in QLD and WA and agriculture/primary industry officers in QLD, WA and NT; these contributions were made in the form of access to field sites and assistance with field releases.

9.3 Additional Funds

If additional funding was available for the parkinsonia sub-project additional work on chemical ecology and genetics would be undertaken to improve detection of established populations, and improve the understanding behind establishment success in relation to local environmental conditions.

10 Appendix

10.1 Project, media and communications material and intellectual property

10.1.1 Database of landholder sites on which work was conducted

Landholder_network_B.WBC.0060&B.WEE.0134_2014-18.pdf

10.1.2 Database on release and establishment

UU1_releases_B.WBC.0060_2016-18.pdf

UU2_releases_B.WBC.0060_2016-18.pdf

10.1.3 Release and monitoring guidelines

UU1&UU2_releases&monitoring_guidelines.docx

10.1.4 Likely publications (these overlap the work done on this project and the related MLA-funded B.WEE.0134)

Pichancourt, J.B., van Klinken, R.D. and Raghu, S. (in review). Testing the limits of demographic generalization: environmental context and the population dynamics of the widespread invasive species *Parkinsonia aculeata*. (Target journal: *Ecosphere*)

Heard, T.A., McKay, F., Pariso, M., White, A., Fichera, G., Sosa, A. and Raghu, S. (in prep). Biology and host specificity of two *Eueupithecia* species (Lepidoptera: Geometridae), biological control agents of *Parkinsonia aculeata* (Leguminosae) in Australia. (Target journal: *Austral Entomology*)

Mukherjee, A. and Raghu, S. (in prep.). Bioclimatic projections of species distributions with limited occurrence records: a method and application to guide translocations of weed biocontrol agents. (Target journal: *Journal of Biogeography*)

Raghu, S., White, A., Fichera, G. (in prep.). Temperature-dependent development of *Eueupithecia cisplatensis* and *Eueupithecia vollonoides*, biocontrol agents for *Parkinsonia aculeata* (Target journal: *Physiological Entomology*)

Copies of publications will be available on request from project team upon submission to the journal.

10.2 Equipment and assets

Not applicable.

10.3 Staffing levels

10.3.1 CSIRO

Raghu Sathyamurthy (0.1 FTE; project leader); Andrew White (0.2 FTE; Technical Officer); Gio Fichera (0.2 FTE; Technical Officer); David Comben (casual; Technical Officer)

10.3.2 QDAF

Kelli Pukallus (0.4 FTE; Experimentalist); Judy Clark (1 FTE; Technical Officer (2016-17)); Joshua Nicholls (0.8 FTE; Technical Officer (2017-18)); Centaine Ferris (1 FTE; Technical Officer)

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