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Parkinsonia biocontrol: release and field evaluation of two new agents

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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. 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. 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 the current project that focussed on mass rearing and release of the two most recently approved biological control agents approved for release against parkinsonia in Australia.

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). In this project, we (1) determined an optimal release strategy to ensure widespread establishment of these agents across parkinsonia infestations in the rangelands of northern Australia; (2) mass-reared and released them following this strategy by forming strong partnerships between government and non-governmental agencies and regional landholders; (3) documented the establishment and potential impacts of these agents; (4) undertook a preliminary cost-benefit analysis on the potential benefits of investment in the parkinsonia biological control program, and (5) identified the role for biological control within an integrated management approach for parkinsonia.

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 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) on parkinsonia infestations across northern Australia. Setting up nursery sites was deemed to be the most effective way to get these agents established across the landscape, as these would be regions from which the moths could natural disperse and colonise other infestations. Fourteen and nine nursery sites were set-up for UU1 and UU2, respectively. Permanent populations were established at >60% of the release sites, with establishment success greater than >75% at nursery sites. 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. Glasshouse studies done as part of this project suggest that high levels (>50%) of defoliation are possible at densities comparable to what we are currently seeing in the field, and that such larval densities can impact plant health. The full impacts in the field may take up to a decade to become fully apparent.

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 land-scape 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 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. Ongoing monitoring and impact assessment will be needed to assess these projections.

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1 Background

1.1 *Parkinsonia aculeata*: a rangeland Weed of National Significance

Native to the Americas, parkinsonia (*Parkinsonia aculeata*, Leguminosae) was introduced into Australia as an ornamental tree/shrub and for its potential value for hedging and as fodder (Hawkins *et al.* 2007). Inhabiting semi-arid and tropical rangelands across northern Australia (including across the Kimberley and Pilbara regions of Western Australia), its current distribution extends over an area of some 8000 km^2 (Deveze 2004; van Klinken *et al.* 2009a; van Klinken and Heard 2012; Fig 1a), with potential for further range expansion into bioclimatically suitable areas (van Klinken *et al.* 2009b; van Klinken and Heard 2012; Fig 1b).

It has the ability to form dense thickets in floodplains and grasslands, and along watercourses and bore drains thereby having negative impacts on the pastoral industry (e.g. limiting pasture growth, restricting stock access to water and impeding mustering) and the environment (e.g. providing refuges for feral animals like pigs, increasing evapotranspiration, contributing to soil erosion, suppressing the herb layer and reducing wildlife habitat). Parkinsonia is now a declared weed in all states and territories of Australia, and is considered a Weed of National Significance. Parkinsonia has been a target for biological control in Australia since 1983 (Deveze 2004; van Klinken and Heard 2012).

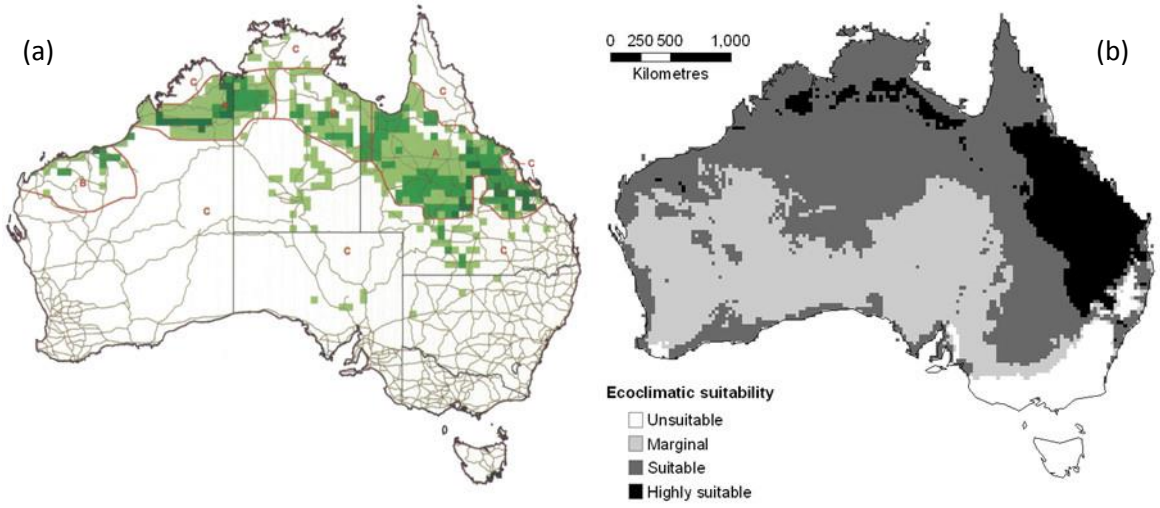

Fig. 1. (a). Current distribution of parkinsonia; the shading indicates relative abundance: dark, mid and pale green corresponds with Abundant, Intermediate and Occasional populations, respectively. National management goals are indicated by red letters; A, B and C are Containment, Active Control and Eradication Zones, respectively (Source: Deveze, 2004 & Queensland Government). (b) Projection of suitable climates for parkinsonia. Shading corresponds to an Ecoclimatic Index predicted using CLIMEX (Source: van Klinken *et al.* 2009b).

1.2 Biological control as a component of integrated management of parkinsonia

1.2.1 Goals of integrated management of parkinsonia

Based on an ecological understanding of the population dynamics of parkinsonia, and consultation with key management stakeholders, the following have been identified as important goals of an integrated weed management program for landscape scale control of parkinsonia (Deveze 2004; Raghu *et al.* 2006; van Klinken 2006; Pichancourt and van Klinken 2012).

- Reduce patch density (< 30% cover) and size (< 1ha)
- Reduce rates of spread and in-fill by reducing seed production/density (< 100 viable seeds/m^2)
- Reduce growth and recruitment (by 50%) and delay time to reproduction (by 1 year; currently 2-3 years)
- Target management in regions at highest risk from parkinsonia impacts

Biological control is a part of the integrated weed management toolbox in meeting these management goals for parkinsonia, and is not intended to work exclusively to control the weed (Deveze 2004; van Klinken 2006). Therefore biological control agents released are intended to be chronic stressors on parkinsonia populations that are particularly hard to cost-effectively control by other means. Impacts of the agents will best be judged by their ability to slow plant vigour and reduce seed production (directly, and indirectly through impacting non-reproductive life stages), and the extent to which they consequently limit the growth and spread of parkinsonia populations (Raghu *et al.* 2006; van Klinken 2006).

1.2.2 Past efforts on parkinsonia biological control

Research by Queensland Government researchers on biological control of parkinsonia has resulted in the introduction of three insect species between 1989 and 1995; a sap-sucking bug (*Rhinacloa callicrates* Herring) and two seed-feeding beetles (*Mimosetes ulkei* (Horn), and *Penthobruchus germaini* Pic). The seed-feeding bruchid, *P. germaini*, is widely established across northern Australia, while *R. callicrates* appears to be common in Queensland (K. Pukallus [QDAF] – pers. comm.). These were inadequate on their own to control parkinsonia populations. CSIRO therefore recommenced native range surveys to identify potential control agents in 2002, with new surveys conducted across central and South America, including in Argentina, Brazil, Costa Rica, Guatemala, Mexico, Nicaragua, Paraguay, Peru, USA and Venezuela (van Klinken 2006; van Klinken and Heard 2012). Several species identified in Mexico and Argentina were imported into CSIRO's quarantine facilities in Brisbane to conduct host-specificity studies to determine the risk associated with releasing these insects into the Australian environment (Heard and van Klinken 2014); these surveys and risk assessments were funded, in significant part, by Meat and Livestock Australia (Projects B.NBP.0366; B.NBP.0620).

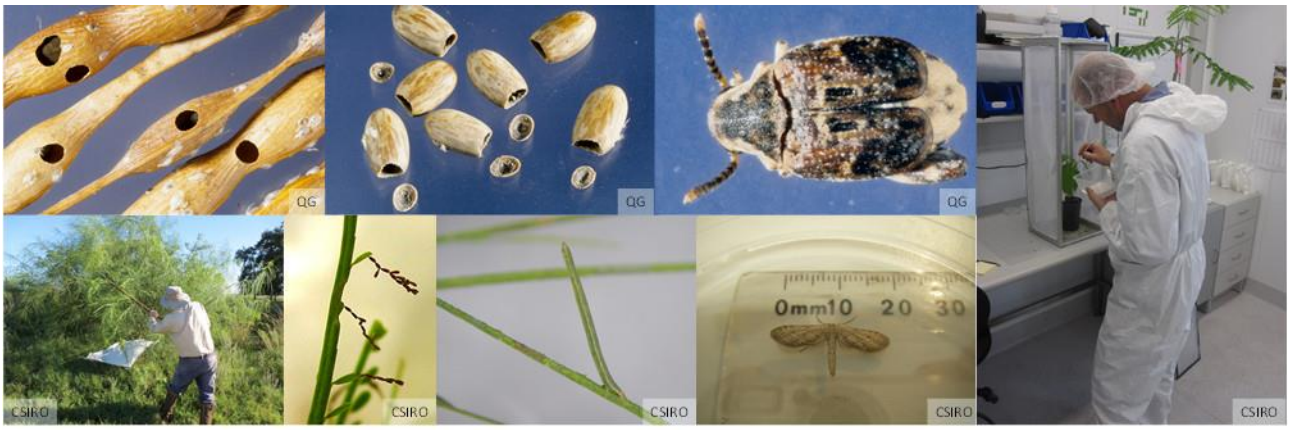

Fig. 2. Established biological control agents on parkinsonia include seed-feeding weevil *Penthobruchus germaini* (top three images) and leaf-feeding moths in the genus *Eueupithecia* approved for release as part of this project. Photo sources: Queensland Government (QG) and CSIRO.

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* (Hausmann *et al.* 2016; see Acknowledgements for permit details). The

overarching aim of this project is to mass-rear, release and assess the performance these two agents, within the context of the broader integrated weed management program on parkinsonia.

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:

- Determine an optimal release strategy for parkinsonia biological control agents UU1 and UU2, and apply this strategy to releases
- Follow the optimal release strategy for UU1 and UU2, have made releases of at least 10,000 individuals of each of UU1 and UU2 in multiple sites in each of at least six locations placed across 3 States in northern Australia
- Determine the impact of the two new biocontrol agents on the health and reproductive output of parkinsonia
- Conduct a benefit cost analysis of the parkinsonia biocontrol agents based on the previous exploration, host specificity projects and the impact from this, the release project
- Provide recommendations for maximising the impact of the two agents into the future including, but not limited to, guidelines for mass-rearing and release, as well as an outline of integrated management actions
- Draft outline of at least 2 journal manuscripts based on the biological control program

3 Methodology

3.1 UU1 and UU2: a general introduction

Eueupithecia cisplatensis and *E. vollonoides* are leaf-feeders and have a similar life-history. The female moth lays her eggs on the leaves of parkinsonia. Development of the moths at a temperature of 25-28^{o}C has the following timelines. Eggs hatch after 5-7 days and newly hatched larvae (caterpillars), less than 2mm long, begin feeding on the leaves. The caterpillars (called loopers because of how they move) continue feeding for around 15 days and grow to approximately 2cm in length before pupating. Adult moths (Fig. 3) emerge from the cocoons after 5-7 days and mate. Female moths then lay their eggs and the cycle begins again. Larvae and adults of UU1 and UU2 are outwardly similar and can only be morphologically distinguished by dissection and examination of features of their genitalia (Table 1; Fig. 4).

Eggs **Larvae (caterpillars) – 2 weeks old** **Adult moths (female has a larger abdomen)**

Fig. 3. Morphology of life-stages of *Eueupithecia cisplatensis* (UU1). Both UU1 and UU2 (*Eueupithecia vollonoides*) have a similar appearance throughout their life cycle. With experience, the larger size of UU2 relative to UU1 will become apparent to the trained eye. The two species do not interbreed, and can only be told apart by dissection and examination of key anatomical features of their genitalia.

Table 1. Distinguishing morphological/anatomical features of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2)

Morphology	***E. cisplatensis* (UU1)**	***E. vollonoides* (UU2)**
Female genitalia	Length of corpus bursae 1.6 mm, posterior 1/2 sclerotized, slightly folded only	Length of corpus bursae 2 mm, posterior ¾ strongly sclerotized and strongly folded laterally.
Male genitalia	Aedeagus with large basal cornutus (half length of aedeagus) and a smaller, but stout, hook-shaped cornutus at tip. Aedeagus slender, width 0.15 mm.	Aedeagus with two cornuti, neither hook shaped. Aedeagus very broad, width 0.4 mm
Size	On average smaller, wingspan 15-20 mm	On average larger, wingspan 20-25 mm

Fig. 4. Dissection of genitalia of UU1 and UU2. (A) Female: Note the difference in size and sclerotisation (darkening). (B): Male: Note the difference in size and armature

Despite similarities in their biology, the two species appear to have slightly different bioclimatic requirements in their native range (Hausmann *et al.* 2016). Surveys in Argentina have discovered a distinct distribution for each of species, with UU1 occurring in the coastal, slightly cooler and more humid southeast and UU2 occurring in the inland, hotter and drier northwest of northern Argentina (Hausmann *et al.* 2016; Fig. 5). Additional physiological studies are underway on these species with a view to developing bioclimatic models to guide future release efforts across northern Australia.

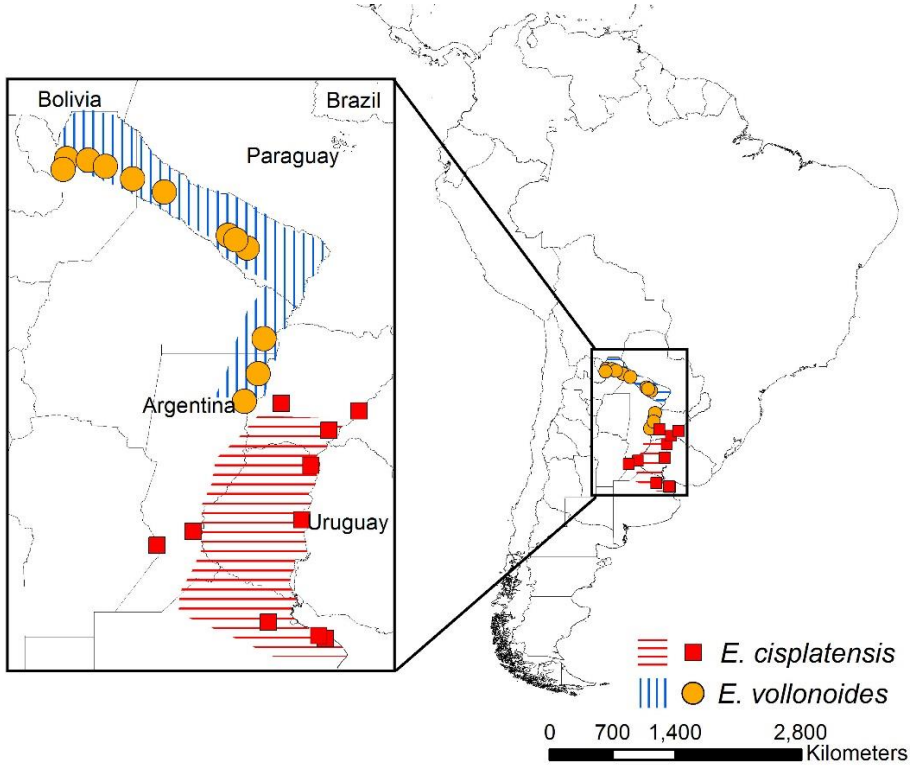

Fig. 5. Distribution of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2) in their native range in S. America.

3.2 Mass-rearing and release of UU1 and UU2

Both UU1 and UU2 were mass-reared at the CSIRO facilities at the Ecosciences Precinct in Brisbane, Queensland, and also at Queensland's Department of Agriculture and Fisheries' Tropical Weeds Research Centre, Charters Towers. In addition UU1 was mass-reared by the Northern Territory Department of Land Resources Management's Weeds Branch in Darwin. Rearing 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. Eggs laid by newly mated females were either returned to colony cages, or were lab-reared in anticipation of field release of larvae/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. Field releases in this project principally focussed on release of larvae, although on occasion pupae were released as well.

A nationally coordinated field release program was developed in discussion with key collaborators in Queensland, Northern Territory and Western Australia (Fig. 6). A consistent protocol was followed for field releases. In each state/territory, we identified several locations to serve as "nursery sites" for each of the two species. The selection of optimal nursery sites were guided by the following features:

- Parkinsonia plants were in healthy condition, as may be the case when they are growing as part of riparian vegetation, or on the bank of a dam/reservoir
- The sites were easily accessible to enable regular releases of the insects, and were not earmarked for other management (e.g. mechanical or chemical control) in the near future
- Plants don't show signs of sooty mould or have scale insects. The latter is usually a good sign that there will be ants tending the scale insects; ants are effective predators of the biological control agents, and can limit their efficacy

The use of nursery sites was important to ensure that the agents became reliably established at least at these locations in the landscape and, over time, populations of these insects would spread and colonize other sites from these nursery sites.

Larvae: Larvae were typically shipped or transported to the release location on sprigs of parkinsonia (Fig. 7). When releasing larvae, several parkinsonia branches were tied together to create a "nest" within which the sprigs containing the larvae can be placed (Fig. 7). This maximized the chances of survival for the larvae by giving them abundant food, and a place to shelter from predators (e.g. ants, wasps, reptiles, birds).

Pupae: Pupae were typically shipped/transported in plastic containers (Fig. 7). When releasing pupae, the container with pupae were housed in a pyramidal shelters (Delta Traps, ISCA Technologies Inc., Riverside, CA, USA), or a clean ice-cream container, and these shelters/container were suspended from a parkinsonia

branch using twine or a cable. A non-toxic glue (Tanglefoot™, The Scotts Company LLC, Marysville, OH, USA) was applied on the twine/cable to prevent ants from predating the pupae (Fig. 7).

Details of releases (including GPS coordinates, photos, dates and number of insects released) were recorded on a standardized data sheet by collaborators doing the field releases, and returned to the project team (See Appendix 1).

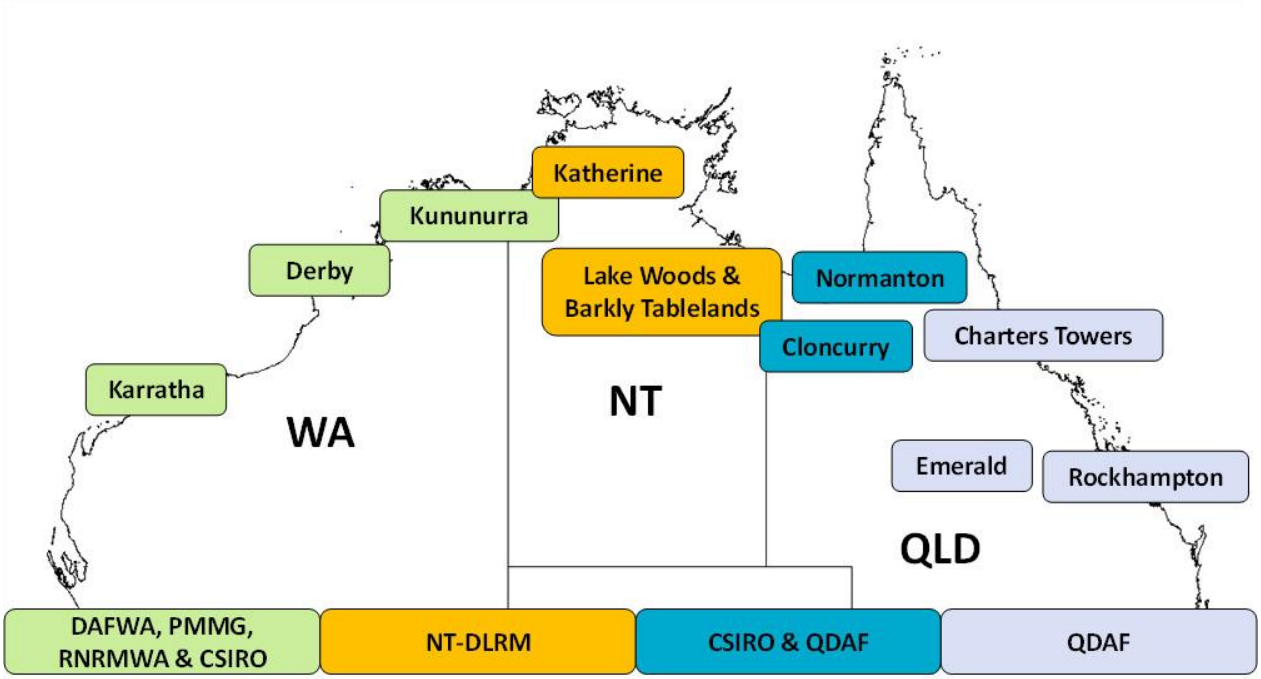

Fig. 6. Coordinated release of *E. cisplatensis* and *E. vollonoides* across parkinsonia infestations in northern Australia was enabled by key collaborations in Queensland (Department of Agriculture and Fisheries (QDAF)), Western Australia (Department of Agriculture and Food WA (DAFWA); Pilbara Mesquite Management Group (PMMG); Rangelands NRM WA (RNRMWA)) and Northern Territory (NT Department of Land Resources Management (NT-DLRM)).

3.3 Assessment of establishment

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 (Fig. 7). 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. 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 (see Appendix 1).

Fig. 7. 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), (i) & (j) Beat sheet method for detection of dislodged UU1/UU2. Photo credits: (a,c,d,e,f,g,h,j) – CSIRO; (b,i) – Kelli Pukallus (QDAF).

3.4 Assessment of impact

The full effects of UU1 and UU2 will only be determined in years to come. Establishing self-sustaining populations of these species is the first goal that must be achieved, and we have done this in this project. Given that widespread establishment of populations were only detected in 2014-15, it was deemed to be premature to monitor (beyond qualitative records of larval feeding on plants of different sizes) the impacts of the agents on parkinsonia plants in the field. We therefore chose to undertake studies of impacts of relative abundance of larvae on sapling/juvenile parkinsonia in glasshouse studies. From past demographic studies we know that regulating the growth of the juvenile life-stage of the plant is crucial in bringing parkinsonia populations under control (Raghu *et al.* 2006; Pichancourt and van Klinken 2012). We therefore studied the impacts of larval feeding on juvenile growth rates. Given the similarities in life-history of UU1 and UU2 we only undertook the studies with UU2.

Parkinsonia saplings were exposed under glasshouse conditions to three densities of UU2 larvae representing low (10 larvae/plant), moderate (20 larvae/plant) and high (50 larvae/plant) population densities of UU1/UU2 in the field. These numbers correspond to the range of densities we observed in the field when monitoring establishment of these agents. A control treatment was also imposed where saplings were grown free of any larvae. The experiment was terminated once the larvae pupated in each of the replicates (ca 15 days). Each treatment was replicated eight times and data were collected on the following features of the plant at the start and end of the study: height (cm), basal diameter (mm) and foliage cover (% defoliation). The difference between initial and final condition of the plant was used as an indication of the impact of one generation of UU1/UU2 on parkinsonia saplings. These impact measures correspond to the desired management goals of reduction in parkinsonia cover and growth rate identified for parkinsonia (see Introduction).

Regression analyses were used to understand the influence of larval densities on development rates, and to ascertain the impacts of larval feeding on parkinsonia growth/health.

3.5 Preliminary cost-benefit analysis

A 2006 attempt at an *Ex post* cost-benefit analysis (CBA) of biological control of parkinsonia concluded a meaningful analysis was not possible because no economic benefits were thought to have accrued at that time (Page and Lacey 2006). No additional agents were released between the publication of that study and the releases of UU1 and UU2. As indicated earlier, among the agents released prior to this project, the seed-feeding bruchid *P. germaini* is widespread in distribution and is believed to have a marginal impact of reproductive output of the plant. It accounts for the destruction of between 2-30% of the seeds produced by the plant across sites in northern Australia (van Klinken and Flack 2008). Studies on other legumes suggest that much higher rates of seed predation than that observed for *P. germaini* are needed for population suppression (Kriticos *et al.* 1999; Sheppard *et al.* 2002; Raghu *et al.* 2005), and this is reflected in previous assessments that none of agents released prior to the release of UU1 and UU2 appear to be having a significant impact on parkinsonia populations (van Klinken *et al.* 2009a). Therefore, this CBA was restricted in scope to the potential impacts of UU1 and UU2.

A definitive quantitative CBA of UU1/UU2 impacts requires detailed evaluation data on parkinsonia performance in the field, with and without biological control, once the agents are established at sufficiently high densities across the landscape. Such establishment and impact may take a decade or more to occur (e.g. McEvoy *et al.* 1991; Hoffmann and Moran 1998; Morin *et al.* 2009), and therefore an empirical

evaluation was beyond the scope/resources of this project. Hence we decided to hypothetically examine what the economic benefits of UU1 and UU2 may be under a set of conservative, simplifying assumptions.

We estimated that ca $8 million (2016 dollars) has been invested in the parkinsonia biological control program to date, made up of a combination of investments by research agencies (e.g. CSIRO, Queensland Government; ca 50% of costs) and external funds (e.g. Meat & Livestock Australia, Cattle Industry Funding Scheme-DAFWA; ca 50% of costs, including the investment in this project). We made several simplifying assumptions in undertaking this analysis including that;

- The impacts that we observed in this study from UU1/UU2 (defoliation and its inferred consequent impacts on plant health and reproductive outputs) will be replicated/adopted across 50% of the total area (ca 10000 sq. km = 1,000,000 ha) occupied by parkinsonia in 10 years, and that the adoption of biological control commences 6 years after the research begins (this corresponds with the time it took for UU1 and UU2 to approved for release into Australia) and its takes 10 years from commencement of adoption to reach maximum adoption.
- The annual cost of weed management for parkinsonia is ca $150/ha and is similar to another pricklebush, prickly acacia (*Vachellia nilotica*), where annual costs range from $2/ha to $300/ha depending on density of infestations (Biosecurity Queensland 2015), and that the reduction in parkinsonia management costs would drop by 10% in the presence of successful biological control, resulting in a benefit of $15/ha/y across 500,000 ha (ca 50% of the total area occupied by parkinsonia).
- The impacts of UU1/UU2 would result in a reduction in parkinsonia canopy cover and basal area, which would result in an increase to pasture productivity (Scanlan and Burrows 1990; Carter 1994), and this would be of the order of $1-2/ha/y across 500,000 ha, which are ca 10% of the productivity gains that have been calculated for prickly acacia management (McLean 2015).

Using these assumptions, and a discount rate of 8%, we undertook a CBA using a spreadsheet-based tool developed for evaluating agricultural research and extension projects (Appleyard 1996).

We undertook this CBA to propose a preliminary framework for consideration. All of the above assumptions need to be carefully examined and empirically validated. Therefore, any interpretation or use or communication of our results therefore needs to heed this caution.

4 Results

4.1 Mass-rearing and release of UU1 and UU2

Collectively, over 850,000 UU1 (112 sites; 324 releases) and over 210,000 UU2 (19 sites; 56 releases) were released on parkinsonia infestations across northern Australia (Fig. 8; Table 2). Mass-rearing of these species occurred in Brisbane and Charters Towers in QLD and in Darwin in the NT. Fourteen and nine nursery sites were set-up for UU1 and UU2, respectively. For UU1, nine of the nursery sites were in QLD, two in WA and three in the NT. For UU2, six nursery sites were in QLD and three in WA.

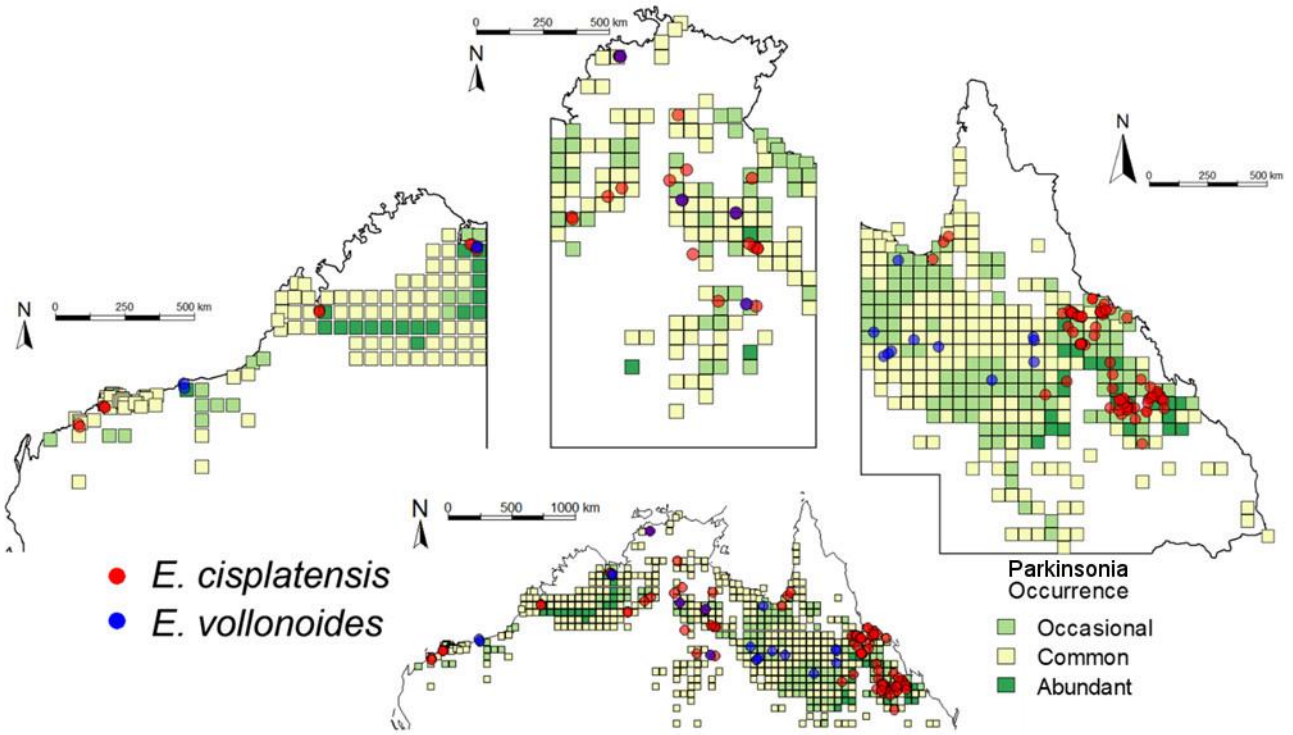

Fig. 8. Releases of *E. cisplatensis* (UU1; red dots) and *E. vollonoides* (UU2; blue dots) on parkinsonia infestations across northern Australia, showing releases relative to parkinsonia occurrence (squares; data source: QDAF – WONS survey 2005).

Table 2. Summary of releases made of the two *Eueupithecia* spp. across different sites in Northern Australia.

Species	State	No. of sites	No. of releases	Total pupae released	Total larvae released
E. cisplatensis	QLD	80	256	193,018	530,180
(UU1)	WA	9	21	2,050	71,520
	NT	23	47	25,171	48,722
	TOTAL	**112**	**324**	**220,239**	**650,422**
E. vollonoides	QLD	11	32	9,253	119,600
(UU2)	WA	4	18	1,310	77,400
	NT	4	6	5,285	950
	TOTAL	**19**	**56**	**15,848**	**197,950**

Releases outside of these nursery sites were made on pastoral properties; these were either directly made by the project team or by pastoralists and regional biosecurity officers receiving these agents from the research agencies managing colonies. The average numbers of insects released at nursery sites of UU1 and UU2 were 41,672 (median = 21,945) and 25,852 (median = 25,635) respectively. For the non-nursery sites, the average release numbers were 4265 and 4370 for UU1 and UU2, respectively.

4.2 Assessment of establishment

Periodic surveys for establishment of UU1/UU2 were made at release sites, with 56 of the 112 release sites surveyed for UU1 and 12 of the 19 release sites surveyed for UU2. As indicated in the Methods, 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. Establishment of UU1 and UU2 was detected at 33 (~60% of surveyed sites) and 8 (~67% of surveyed sites) sites, respectively (Fig. 9). Establishment at nursery sites was higher with establishment of UU1 and UU2 detected at 12 (~86% of the surveyed sites) and 6 (75% of the surveyed sites), respectively.

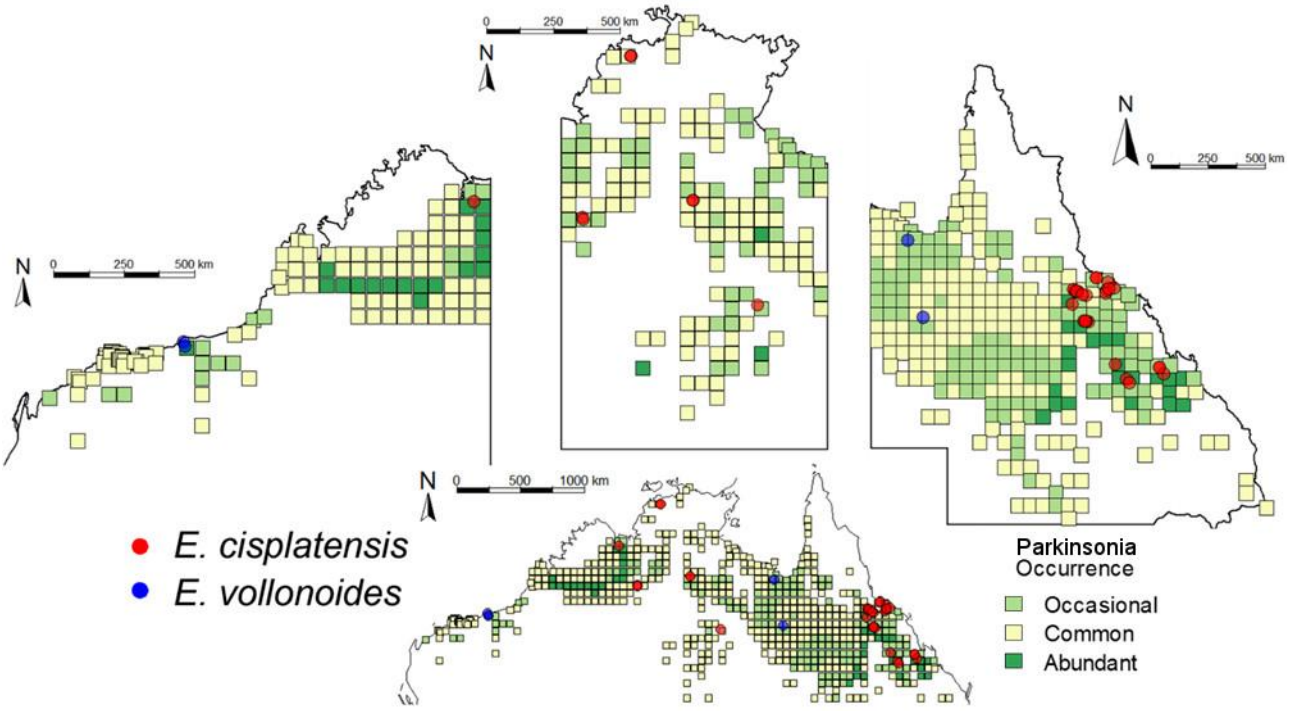

Fig. 9. Establishment of populations of *E. cisplatensis* (UU1; red dots) and *E. vollonoides* (UU2; blue dots) on parkinsonia infestations across northern Australia, showing establishment relative to parkinsonia occurrence (squares: data source: QDAF – WONS survey 2005).

At some of the sites where establishment has been recorded, significant numbers of larvae were detected during surveys. Unsurprisingly, nursery sites are where the populations appear to be the largest, with average numbers of larvae detected in beat sheets to be in excess of 10 larvae/10 beats/plant (Fig. 10), and in excess of 40 larvae/10 beats/plant at some sites at certain times of the year. In addition to local establishment, significant spread from the release sites to parkinsonia infestations where releases were not made was also detected. For example, in central QLD UU1 was detected on parkinsonia plants ca 5km away from the nearest nursery site while, in WA, UU2 was detected in parkinsonia plants ca 3km from the nearest nursery site.

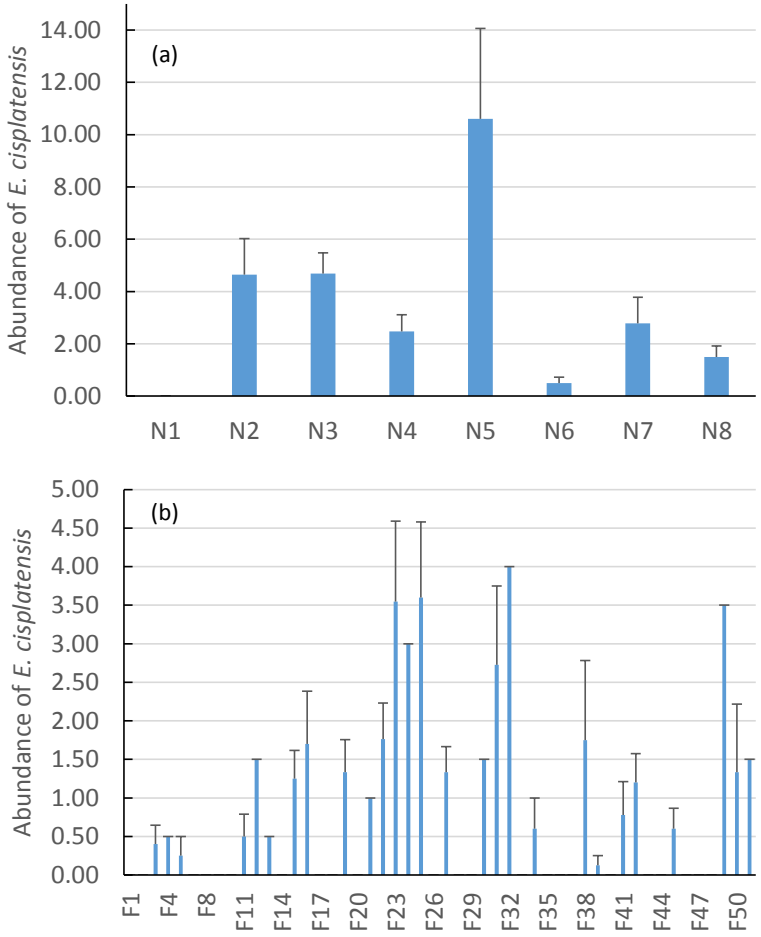

Fig. 10. Abundance (mean ± SE) of *Eueupithecia cisplatensis* (UU1) at (a) Nursery and (b) Non-nursery field sites in Queensland. Sites were surveyed 1-11 times using the beat-sheet method between 2013 and 2016, and up to 12 parkinsonia plants were sampled at each site on each sampling occasion. Note differences in y-axis scales between (a) and (b).

4.3 Assessment of impact

Glasshouse studies of the impact of UU2 larval feeding on parkinsonia plants indicated that individual juvenile plants could adequately support the development of the range of larval densities used in this experiment. However, there was evidence that the initial density of neonate larvae influenced the number of individuals that pupated; on average 25%, 35% and 36% of the neonates failed to reach the pupal stage at the densities of 10, 20 and 50 neonates/plant, respectively (Fig. 11A). Unsurprisingly, higher densities of larvae resulted in higher rates of defoliation with over 50% defoliation occurring at initial densities of 20 larvae/plant and near complete defoliation occurred at neonate densities of 50 larvae/plant (Fig. 11B). The rates of defoliation in turn influenced the rate of change of aspects of the growth of the juvenile plants; this effect was not evident in rate of change of basal stem diameter, while it was strongly apparent in the rate of change of plant height (Fig. 11C).

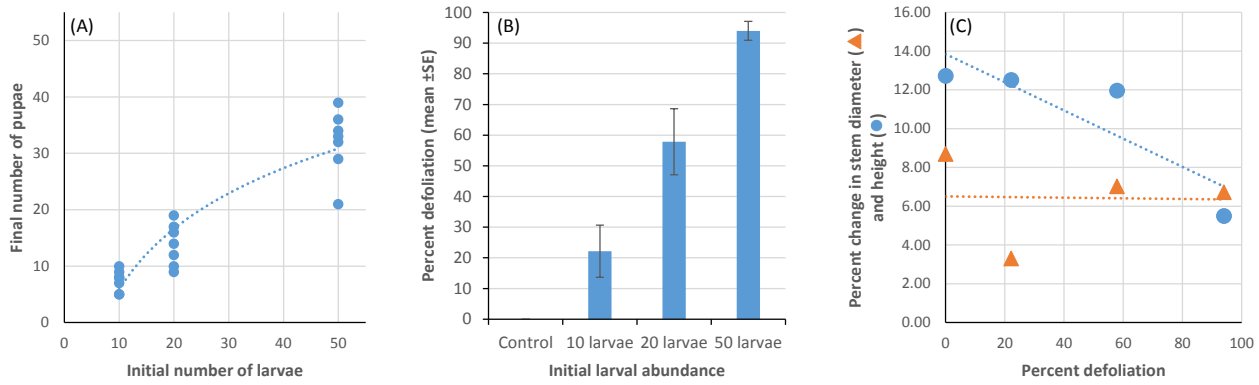

Fig. 11. Development of *Eueupithecia vollonoides* (UU2) on parkinsonia and its impact on the plant. (A) Influence of initial neonate larval densities of UU2 feeding on juvenile parkinsonia plants on the development of neonate larvae through to pupae. Final density of pupae = 15.50*ln(initial density of larvae) - 29.84; R^2 = 0.85. Impact of feeding by different densities of UU2 larvae on parkinsonia (B) foliage cover (% defoliation) and (C) rate of change in stem diameter (mm; orange triangle) and height (cm; blue circle). The duration of feeding was the neonate to pupa development time (ca 15 days), and the response variables in (B) and (C) reflect the change in the juvenile parkinsonia plants over this duration. Regression models in (C): % Stem Diameter Change = -0.073*Percent-Defoliation + 13.84; R^2 = 0.75; % Height Change Stem Diameter Change = -0.002*Percent-Defoliation + 6.51; R^2 < 0.01.

4.4 Preliminary cost-benefit analyses

The development of biological control agents (including UU1 and UU2) for parkinsonia came at an estimated cost of ca $6 million (though joint RDE investment by MLA and CSIRO); these costs also included the screening of other agents that were rejected due to a lack of host-specificity or the inability to rear them for assessment of their safety. Our preliminary CBA indicates that should the establishment and impacts of UU1 and UU2 on parkinsonia result in a savings in control costs and improvements in pasture productivity totalling $17/ha/y across half of the parkinsonia distribution in Australia (ca 500,000 ha), then the biological control program has a Net Present Value (NPV) of $15.6 million and a benefit cost ratio (BCR) of 3.44, at a discount rate of 8.0% (Appendix 3).

5 Discussion

The objectives of the project have been mostly met, and are summarised below.

5.1 Success in achieving objectives

- Determine an optimal release strategy for parkinsonia biological control agents UU1 and UU2, and apply this strategy to releases.

Achieved. An optimal release strategy has been developed and adopted in this study, and a protocol/guidelines document outlining has been prepared for the land management agencies/landholders interested in parkinsonia biological control beyond the life of this project. This is included as an Appendix to this report.

- Follow the optimal release strategy for UU1 and UU2, have made releases of at least 10,000 individuals of each of UU1 and UU2 in multiple sites in each of at least six locations placed across 3 States in northern Australia.

Achieved. Releases in excess of numbers anticipated in the objective have been made.

- Determine the impact of the two new biocontrol agents on the health and reproductive output of parkinsonia.

Partially achieved. We have quantified the direct impacts of UU1 and UU2 on growth rate/health of juvenile parkinsonia plants. This information, integrated with the quantitative information on abundance of agents established in the field to date and existing demographic models of parkinsonia, enable us to make some projections/inferences on potential impacts of these agents on parkinsonia populations.

- Conduct a benefit cost analysis of the parkinsonia biocontrol agents based on the previous exploration, host specificity projects and the impact from this, the release project

Achieved. A preliminary cost-benefit analysis has been conducted on the impacts of agents released to date against parkinsonia, and caveats on its use and additional data needs have been highlighted.

- Provide recommendations for maximising the impact of the two agents into the future including, but not limited to, guidelines for mass-rearing and release, as well as an outline of integrated management actions.

Achieved. Recommendations for maximising agent impacts have been provided within the framework of parkinsonia integrated weed management.

- Draft outline of at least 2 journal manuscripts based on the biological control program

Achieved. Journal manuscripts have been prepared from the work based on the biological control program. Details of these are included in the Appendix to this report.

5.2 Inferences and insights from this project

The optimal release strategy for the establishment of UU1 and UU2 is the use of nursery sites to facilitate establishment of local populations of the agents on parkinsonia infestations; the agents can then subsequently naturally spread and find parkinsonia plants across the landscape. Using this approach we released in excess of a million individuals across both species of moths at over a hundred parkinsonia infestations. Establishment occurred at almost all of the nursery sites, validating our release approach, and populations of the agents have begun to spread out across the landscape to be detected at ~60% of the release sites that we surveyed. The mass-rearing, release and monitoring efforts were achieved through close collaborations between CSIRO, state/territory government collaborators in Queensland and the Northern Territory, and multiple regional land management agencies across northern Australia. The success of these collaborations was the result of the following attributes.

- Identification of multiple nursery sites (with attributes outlined in 3.2) across northern Australia where regular releases of agents could be made
- Regular communication between the agencies doing mass-rearing and those doing field releases to identify additional release sites that had parkinsonia plants in the best possible condition to facilitate establishment of agent populations
- Provision of information outlining the release strategy and its goals (e.g. Appendix 1) and the presentation of the approach being taken directly to landholders and local weed management/biosecurity officers at field days and property visits by researchers
- Clarification of the role of biological control within the context of integrated weed management

The mechanism of UU1/UU2 impacts on parkinsonia health and reproductive output is through their ability to defoliate plants. This defoliation results in reduced growth rates, thereby delaying the maturing of the plant and reducing the plants ability to allocate resource to reproductive output relative to regrowth. We quantified the former and inferred the latter based on previous research and our field observations. Our studies on the impacts of feeding by UU2 larvae on parkinsonia show that a larval density of 10 or more larvae per juvenile plant, can result in a reduction in canopy cover by ~60%, and slow the growth rate of the plant. Our surveys for establishment of populations suggest that such larval densities are being achieved in the field by both UU1 and UU2. Where such densities of the agents were seen, anecdotal observations of significant feeding damage was apparent at certain sites. From past demographic studies of parkinsonia we know that the growth rate of juvenile, pre-reproductive plants, needs to be influenced for parkinsonia populations to be controlled (Raghu et al. 2006, Pichancourt and van Klinken 2012). UU1 and UU2 can have 6-10 generations per year under the climate typically experienced by parkinsonia infestations in northern Australia. Hence, if these densities are sustained over time, we can anticipate repeated defoliation and significant impacts at first on juvenile plants and, through that, over time on local parkinsonia populations (Raghu *et al.* 2006; Pichancourt and van Klinken 2012).

It is premature to make definitive conclusions about impact of these agents on parkinsonia at the landscape scale. Our conservative cost-benefit analysis of the parkinsonia biological control program to date suggests that there is the possibility of significant returns on the Research Development and Extension (RDE) investment to date. 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% and improve pasture productivity by $1-2/ha/y, and could result in a benefit cost ratio of ca 3.44. While this is promising, we stress that these economic analyses are preliminary; the assumptions of the analyses will need to be validated against the field performance of the agents in the years ahead to get a more accurate assessment of the accrued benefits of the biological control program.

In addition to the activities of the project focussed on mass-rearing and releases of UU1 and UU2, the research undertaken during the course of this project has also enhanced scientific understanding of the biology/ecology of the two species of moths and of parkinsonia. Two noteworthy advances in this regard are (a) a greater understanding of the relative bioclimatic tolerances of the two species based on an analysis of where they occur in their native range of Argentina and (b) an analyses of parkinsonia demography across different environmental contexts (Appendix 4). The knowledge generated by these studies will continue to inform the integrated management of parkinsonia across northern Australia in the years ahead.

A significant impediment in the current project was the logistics of shipping larvae. It is easy to ship large numbers of larvae, and larvae have the capacity to seek suitable spots in the plant canopy when released in the field. However, larvae are vulnerable to heat stress in packaging and poor handling in transit (e.g. packaging left in the sun by courier companies). This can result in them reaching remote release sites in poor/sub-optimal condition that lowers their odds of survival when released in the field. Furthermore, releases of large numbers of larvae can attract native predators (e.g. ants, wasps, reptiles and birds) to the release site, resulting in potentially significant mortality from predation. Releases in protected parkinsonia "nests" (as we did in this study) are an important way to minimize this risk, but highly mobile predators like ants may still cause significant mortality of agents and impact agent densities in the field. Release of adults is another option but adults of these moths are short-lived and are equally prone to the vulnerabilities of shipment as larvae. These problems may potentially be overcome through the release of pupae, the resting stages of these moths. Adults emerging from pupae can then mate under field conditions and find oviposition sites that may have an optimal microclimate for larval development. To maximise the impacts

of UU1 and UU2, releases of pupae is the intended next phase of this work, beyond this project. This next phase is being supported by funds from Meat and Livestock Australia and the Commonwealth Department of Agricultural and Water Resources' Rural R&D for Profit Scheme. As part of this new project we have established dedicated mass-rearing hubs for the agents in Charters Towers (QLD; QDAF) and in Brisbane (QLD; CSIRO). Our intent is to continue strengthening the current network of nursery sites, and to start new sites where releases of pupae can be made over the course of a 12-24 month period. We anticipate that over time the continued build-up and spread of populations of these moths will assist in reducing the health, reproductive output and spread of parkinsonia.

5.3 Practical implications of research findings

Significant strides have been made in this project towards adding biological control options into the parkinsonia management toolbox for landholders impacted by this weed across northern Australia. The role of biological control in integrated weed management is to provide an additional chronic stressor to the weed throughout the year, and when/where other management may not be possible. It will therefore be important to coordinate integrated management to ensure other methods of management do not interfere with biological control and *vice versa*. Where possible, parkinsonia infestations should continue to be managed using established means such as chemical (i.e. herbicide) and mechanical control, especially to control small infestations that are easily accessible, and to kill reproductively mature plants.

This study has shown how partnerships between public agencies and private landholders can be used to facilitate the landscape-scale releases of biological control agents for a widely distributed weed across northern Australia. There may be an opportunity to adopt the learnings and shortcomings of this project and the established network of collaborators to further the biological control of other similarly widely distributed rangeland weeds.

6 Conclusions/recommendations

6.1 Future R&D on parkinsonia biological control

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 these agents and *P. germaini* 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 test the assumptions and projections of the cost-benefit analyses.

In addition to their chronic effects on plant health, biological control agents, when established across the landscape, can also periodically build up to outbreak densities at certain sites and times and have additional impacts on weed populations (e.g. van Klinken *et al.* 2003). These outbreaks typically coincide with recovery of weed populations from other stressors (e.g. drought). The recovery of areas of northern Australia from drought may therefore present circumstances for opportunistic surveys of parkinsonia infestations to see how UU1 and UU2 perform in such circumstances.

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.

6.2 Development and adoption activities

To achieve full value from the project's findings there is an opportunity make the biological control information from this project more widely and permanently available. Efforts are underway to create web-based content on the biology, mass-rearing, release, field collection for redistribution, and evaluation of UU1 and UU2 on a permanent parkinsonia-focussed website hosted by CSIRO. This information is being prepared in a form (including web-hosted videos, photographs, and documents) that enables its use by a wide range of stakeholders. In addition, details of nursery and release sites where agents have established will be made available through the Atlas of Living Australia's Biological Control portal (http://root.ala.org.au/bdrs-core/wbiocont/home.htm). Collectively, this will facilitate regional bodies or local land management groups interested in maintaining their own agent colonies and nursery sites to do so, and will enable landholders to collect agents from the closest release/nursery site, for release onto parkinsonia infestations on their property.

7 Key messages

The following are the key messages from this project for producers, stakeholders and land managers.

- Management of parkinsonia is best done as part of an integrated management approach (Deveze 2004), and biological control is rarely a "silver bullet" management tactic on its own. Therefore, the following may be a prudent approach to parkinsonia management
    - Manage small parkinsonia infestations early by killing adult/reproductive plants using established chemical or mechanical methods.
    - Use biological control in large infestations to allow them to be a chronic stressor on plant health and reproduction, while resources/time can be garnered to manage parts of parkinsonia infestations where the agents fail to establish due to some reason or are easy to access by other control tactics.
- Biological control represents a good investment despite the high initial development costs of identifying candidate agents and assessing their safety
    - Biological control does not provide instant/ near-term kill, but suppresses weed population growth and spread through impacts on plant health and/or reproduction. The impacts of biological control can take up to a decade to become apparent.
    - Once agents have established permanent populations, biological control is a management tactic that is self-perpetuating requiring little additional ongoing investment other than redistributions of agents to sites that they cannot get to through natural dispersal.

8 Acknowledgments

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9 Bibliography

Appleyard, L (1996) 'Appraisal : a spreadsheet for evaluating the returns to agricultural research ' (Performance Evaluation Division, Dept. of Natural Resources and Environment: East Melbourne)

Biosecurity Queensland (2015) 'Flinders Shire Good Neighbour Program Case Study.' (Queensland Department of Agriculture and Fisheries: Brisbane)

Carter, JO (1994) *Acacia nilotica*: A tree legume out of control. In 'Forage Tree Legumes in Tropical Agriculture.' (Eds RC Gutteridge, HM Shelton.) pp. 338–351. (CAB International: Wallingfor, UK)

Deveze, M (2004) 'National Case Studies Manual: Parkinsonia – Approaches to the management of parkinsonia (Parkinsonia aculeata) in Australia.' (Queensland Department of Natural Resources, Mines and Energy: Brisbane, Queensland)

Hausmann, A, Chainey, J, Heard, TA, McKay, F, Raghu, S (2016) Revision of the genus *Eueupithecia* Prout, 1910 from Argentina (Lepidoptera, Geometridae, Sterrhinae). *Zootaxa* **4138**, 392–400.

Hawkins, JA, Boutaoui, N, Cheung, KY, van Klinken, RD, Hughes, CE (2007) Intercontinental dispersal prior to human translocation revealed in a cryptogenic invasive tree. *New Phytologist* **175**, 575–587.

Heard, TA, van Klinken, RD (2014) 'Assessment of new biocontrol agents of Parkinsonia.' (Meat & Livestorck Australia: North Sydney)

Hoffmann, JH, Moran, VC (1998) The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia* **114**, 343-348.

Kriticos, D, Brown, J, Radford, I, Nicholas, M (1999) Plant population ecology and biological control: Acacia nilotica as a case study. *Biological Control* **16**, 230-239.

McEvoy, P, Cox, C, Coombs, E (1991) Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications* **1**, 430-442.

McLean, I (2015) The economics of prickly acacia control. In 'Proceedings of the 13th Queensland Weed Symposium 2015 ' (Queensland Weeds Society: Longreach, Queensland.)

Morin, L, Reid, AM, Sims-Chilton, NM, Buckley, YM, Dhileepan, K, Hastwell, GT, Nordblom, TL, Raghu, S (2009) Review of approaches to evaluate the effectiveness of weed biological control agents. *Biological Control* **51**, 1–15.

Page, AR, Lacey, KL (2006) 'Economic impact assessment of Australian weed biological control.' (CRC for Australia Weed Management, Technical Series: Adelaide)

Pichancourt, JB, van Klinken, RD (2012) Phenotypic plasticity influences the size, shape and dynamics of the geographic distribution of an invasive plant. *Plos One* **7**,

Raghu, S, Wilson, JR, Dhileepan, K (2006) Refining the process of agent selection through understanding plant demography and plant response to herbivory. *Australian Journal of Entomology* **45**, 308–316.

Raghu, S, Wiltshire, C, Dhileepan, K (2005) Intensity of pre-dispersal seed predation in the invasive legume *Leucaena leucocephala* is limited by the duration of pod retention. *Austral Ecology* **30**, 310-318.

Scanlan, JC, Burrows, WH (1990) Woody overstorey impact on herbaceous understorey in *Eucalyptus* spp. communities in central Queensland. *Australian Journal of Ecology* **15**, 191–197.

Sheppard, AW, Hodge, P, Paynter, Q, Rees, M (2002) Factors affecting invasion and persistence of broom Cytisus scoparius in Australia. *Journal of Applied Ecology* **39**, 721-734.

van Klinken, RD (2006) Biological control of *Parkinsonia aculeata*: what are we trying to achieve? *Australian Journal of Entomology* **45**, 268–271.

van Klinken, RD, Campbell, SD, Heard, TA, McKenzie, J, March, N (2009a) The biology of Australian weeds: 54. *Parkinsonia aculeata* L. *Plant Protection Quarterly* **24**, 100–117.

van Klinken, RD, Fichera, G, Cordo, H (2003) Targeting biological control across diverse landscapes: the release, establishment, and early success of two insects on mesquite (*Prosopis* spp.) insects in Australian rangelands. *Biological Control* **26**, 8-20.

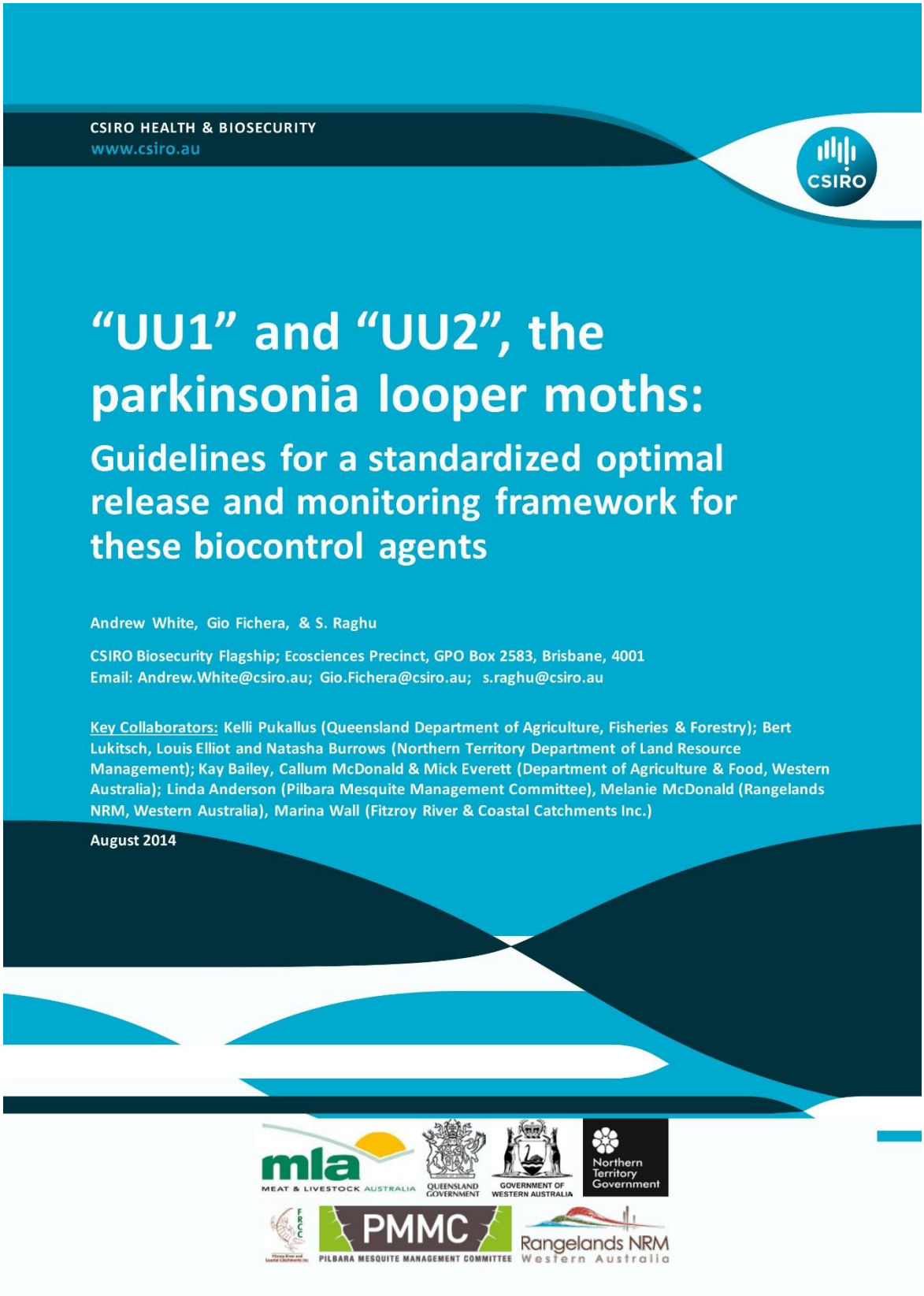
van Klinken, RD, Flack, LK (2008) What limits predation rates by the specialist seed-feeder *Penthobruchus germaini* on an invasive shrub? *Journal of Applied Ecology* **45**, 1600-1611.

van Klinken, RD, Heard, TA (2012) *Pakinsonia aculeata* L. – parkinsonia. In 'Biological Control of Weeds in Australia.' (Eds MH Julien, REC McFadyen, JM Cullen.) pp. 437–447. (CSIRO Publishing: Melbourne, Australia)

van Klinken, RD, Lawson, BE, Zalucki, MP (2009b) Predicting invasions in Australia by a Neotropical shrub under climate change: the challenge of novel climates and parameter estimation. *Global Ecology and Biogeography* **18**, 688-700.

10 Appendices

10.1 Optimal release and monitoring protocols



Why release these insects into Australia?

Parkinsonia aculeata is native to the Americas and is recognised as one of the twenty worst weeds in Australia. It has been declared in all states and territories. The Australian Weed Committee approved parkinsonia as a target for biological control in Australia in 1983.

Three insect species have previously been released in Australia but only one, the seed-feeding bruchid, *Penthobruchus germaini*, from Argentina, established and dispersed readily. Although the bruchid is widespread, it has so far failed to have a significant impact.

The leaf-feeding looper caterpillar, *Eueupithecia cisplatensis* (nicknamed "UU1") and *Eueupithecia species 2* (nicknamed "UU2"), were identified in Argentina, and have the potential to be an effective biological control agent for parkinsonia.

Is it safe? What else does it eat?

After preliminary testing in the field and in laboratory conditions in Argentina, CSIRO imported UU1 and UU2 into a quarantine facility in Brisbane where testing was completed on a broad range of plant species. Testing concluded that both moths are highly specific to parkinsonia with newly hatched larvae surviving no more than a couple of days on non-target test plants before starving to death.

Based on these investigations, Australian regulatory authorities (currently, Department of Agriculture and Department of Environment) have given permission for their field release in Australia.

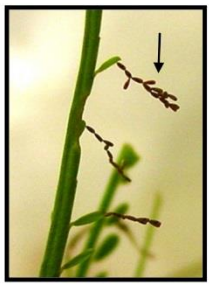
Life cycle of UU1 & UU2

Both UU1 and UU2 have somewhat similar life cycles. The female moth lays her eggs on the leaves of parkinsonia. Eggs hatch after 5-7 days. Newly hatched larvae (caterpillars), less than 2mm long, begin feeding on the leaflets. They continue feeding for around 15 days, growing to approximately 2cm in length before pupating (form a cocoon). Adult moths emerge from the cocoons after 5-7 days, mate, females lay their eggs, and the cycle begins again.

Expectations

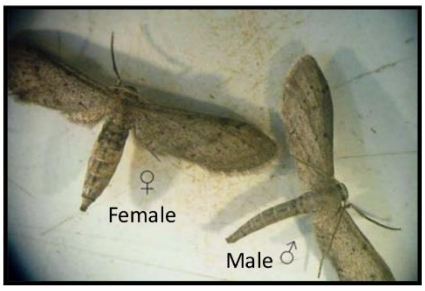
The damage to the plant is done by the larvae feeding on the leaves, defoliating the plant. This reduces the plants' ability to photosynthesise impacting on the health of the plant, making it more susceptible to disease, and reducing growth and seed production.

There are no guarantees as to how successful UU1 and UU2 will be. Like all insects, they will be preyed upon, particularly by spiders, ants, and wasps. Our aim is to establish populations throughout northern Australia. If UU1 and UU2 populations can overcome predation, they could have a significant effect on parkinsonia infestations. Biological control is rarely a "quick fix" solution and only time will tell how much of an impact UU1 and UU2 will have on controlling parkinsonia.



Eggs

Larvae (caterpillars) – 2 weeks old



Adult moths (female has a larger abdomen)

Both UU1 and UU2 have a similar appearance throughout their life cycle. With experience, the larger size of UU2 relative to UU1 will become apparent to the trained eye. The two loopers do not interbreed, and can only be told apart by dissection of genitalia.

Andrew White, Gio Fichera, & S. Raghu

Guidelines: field release and monitoring

Given that field releases of UU1 and UU2 are being made across QLD, NT and WA, it would be helpful if release and monitoring methods were standardized. The following are some guidelines in this regard.

Selection of nursery sites

In each of the release regions, we recommend that 3-6 sites be selected to serve as nursery sites for each of the two species. At present, it will be helpful if UU1 and UU2 are not released at the same sites. The following criteria may assist in the selection of good nursery sites:

- Parkinsonia plants are in healthy condition, as may be the case when they are growing as part of riparian vegetation, or on the bank of a dam/reservoir
- The sites are easily accessible to enable regular releases of the insects
- The sites are not earmarked for other management (e.g. mechanical or chemical control) in the near future
- Plants do not show signs of sooty mould or have scale insects. The latter is usually a good sign that there will be plenty of ants tending the scale insects. Ants are very efficient predators of the biocontrol agents, and will undo the good work you put into the releases.

These nursery sites will be important for ensuring that the agents get established reliably at least at these locations in the landscape. Over time, these populations of insects will spread and colonize other sites from these nursery sites.

Any additional releases that you may be able to do, will also be very helpful but, at the very least, please plan for establishing nursery sites. Our goal for 2014-2017 is to have a minimum of 3-6 such sites/agent each, in QLD, NT and WA.

Suggested methods of release

Larvae: Larvae are typically shipped or transported to the release location on sprigs of parkinsonia. When releasing larvae, try and tie together several parkinsonia branches using zip ties to create a "nest" (see picture below) within which the sprigs containing the larvae can be placed. This maximizes the chances of survival for the larvae giving them an abundant food, and a place to shelter from predators (e.g. wasps, birds).

Pupae: Pupae are the inactive stage of the life cycle, are typically shipped on transported in take-away food containers. When releasing pupae, house the container with pupae in a delta/pyramid trap, or a clean ice-cream container, and suspend the trap/container from a parkinsonia branch using twine or a cable (see picture). Use adequate amounts of Stick-em, a non-toxic glue, on the twine/cable to prevent ants from raiding the pupae.

Maintain detailed records of releases (including GPS coordinates, photos, dates and number of insects released), as this will be useful in understanding why establishment of the insects succeeded or failed. A sample data sheet for each release event is shown in Appendix 1.1.

Suggested methods of monitoring

Try and monitor the nursery sites at least 3 times in a year; twice in the dry season, and once in the transition from the dry to the wet season. Since the larvae are very good at mimicking parkinsonia foliage or thorns, detecting their presence by searching plants is very difficult and laborious. The beat sheet method is a useful monitoring tool for these insects.

Beat sheets (see pictures below) can either be hand-held or laid on the ground. Select 6-10 of the healthiest parkinsonia plants close to the release area at a site. Beating the healthy foliage of each of these trees above the beat sheet using a stick will dislodge any UU1 and UU2 present; it will also dislodge any other insects. Examining the sheets and recording the numbers of UU1/UU2, and the presence of other insects (notably predatory insects) will help record the evidence of successful establishment of populations of the biocontrol agents. It can take a little time for the larvae to become active. So be patient and search the sheet for several minutes. After an initial scan, a gentle shake of the beat sheet to remove large foliage and plant materials, may help to better detect small larvae. It is useful to standardize the number of beats per tree, and to also record the size of beat sheet being used to collect the data, as this will allow comparisons of establishment success across locations (see Appendix 1.2).

(A) Shipment of larvae; (B) Shipment of pupae; (C) & (D) Releases of larvae into a parkinsonia "nest"; (E) Setting up a delta/pyramid trap for release of pupae; (F) Coating the trap handle with Stick-em; (G) Take-away container with pupae placed in delta trap (with adult UU1 emerging); (H) Modified ice-cream container for release of pupae

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(I) Placement of pupal release containers amid the parkinsonia canopy; (J),(K),(L) & (M) Beat sheet method for detection of dislodged UU1/UU2 (note differences in size of beat sheets used, and their relative advantages).

Photo Sources: (B), (G), (H), (J) & (L): Kelli Pukallus, Queensland Department of Agriculture, Fisheries and Forestry; (A), (C), (D), (E), (F), (I), (K) & (M): CSIRO

Monitoring the spread of insects

Once populations establish, over time, insects will spread out and colonize the entire patch of parkinsonia where releases were made. To detect this spread of insects, use the beat sheet method to survey 6-10 healthy parkinsonia trees close to the original release area at a site. Then, radiate outwards from the release area and sample a similar number of parkinsonia trees, at a sequence of fixed distances (e.g. every 25m from the release area) in different directions. If you note the distance from the release area at which you no longer detect UU1 or UU2, this will give you a measure of the extent of dispersal by these insects in the time since the original release. This information will be very useful to understand how well the insects are likely to colonize nearby parkinsonia patches, including patches where releases have not been made.

A schematic representation of a parkinsonia patch where biocontrol agents have been released, showing sampling locations at set distances from the release area where one could sample to monitor spread of these insects over time.

Appendix 1.1: Sample data sheet for release and site data

MLA Parkinsonia Biological Control Project

Release and establishment of *Eueupithecia spp.* (UU1 and UU2)

Date:

Site:

UU1 or UU2
(circle)

GPS coordinates:

State: **Nearest major town centre:**

Landowner: **Address:**

phone contact:

email:

Contact/collaborator:

Release details: **Released by:**

Initial release or follow up release:

Life stage:

eggs

larvae (+ approx age)

pupae

adults

Other information: **Is site prone to flood: y / n**

Photos taken: **Is there access in wet season: y / n**

Description of infestation & other notes:

Important: Please send this information to one of the contacts listed on the cover page of this document.

Appendix 1.2: Sample data sheet for beat-sheet data on insect establishment and spread

MLA Parkinsonia Biological Control Project
Release and establishment of *Eueupithecia spp.* (UU1 and UU2)

Site name:
GPS Coordinates:
Dates of releases of UU1/UU2:
Size of beat sheet (length x breadth in m):
Number of beats/tree:

Distance (in m) from release area	Tree #	No. of UU1/UU2	Predatory Insects	Notes
0 (i.e. at release area)	1			
0 (i.e. at release area)	2			
0 (i.e. at release area)	3			
0 (i.e. at release area)	4			
0 (i.e. at release area)	5			
0 (i.e. at release area)	6			
25	1			
25	2			
25	3			
25	4			
25	5			
25	6			
50	1			
50	2			
50	3			
50	4			
50	5			
50	6			
75	1			
75	2			
75	3			
75	4			
75	5			
75	6			
100	1			
100	2			
100	3			
100	4			
100	5			
100	6			
125	1			
125	2			
125	3			
125	4			
125	5			
125	6			

These distances from the release area are provided as examples. Please select distances appropriate to the parkinsonia patch that you are monitoring. The distances need not necessarily be at regular intervals.

Sample a minimum of 6 trees at each distance from the release area.

Important: Please send this information to one of the contacts listed on the cover page of this document.

10.2 Summary of releases (to August 2016) of *E. cisplatensis* (UU1) and *E. vollonoides* (UU2) across northern Australia, and status of establishment of permanent populations

Eueupithecia cisplatensis (UU1)

State	Region	Site name	No. releases	Total Pupae	Total Larvae	Establishment status
Qld	**Central Qld**	Airlie	3	1,851		Not surveyed
		Anabranch	4	1,590	19,620	No evidence
		Banyula	1	466		Not surveyed
		Big Bend site 1	1	200		No evidence
		Big Bend sites 2/3/4	21	15,107	49,620	Established
		Blackwater Council paddock	1	566		Not surveyed
		Blackwater Creek	1	1,132		Not surveyed
		Borilla Park	1	1,401		Not surveyed
		Bridge Flats	1	1,249		Not surveyed
		Burdekin River bank, Dalrymple NP	61	48,747	191,880	Established
		Caerphilly	6	6,863	7,320	Established
		Carse O'Gowrie	1		1,500	Not surveyed
		Cassidy Paddock	4	2,883	2,940	Established
		Clermont Coal	2	3,024		Established
		Cleveland Bay Purification Plant	3	2,465	3,420	Established
		Codenwarra	6	3,740		No evidence
		Coreen	2	1,315		Not surveyed
		Cranbourne	1	500		Not surveyed
		Donohue Rd	1	679		Not surveyed
		Doohan Rd	1	700		Not surveyed
		Eastmere	1	1,423	5,760	Not surveyed
		Elimnet	1	1,050	2,940	Established

State	Region	Site name	No. releases	Total Pupae	Total Larvae	Establishment status
		Ensham Mine	1		3,000	Not surveyed
		Eumara Springs	3	3,795	1,620	Established
		Fernlea	4	4,751	7,620	Established
		Fletchervale site 1	7	4,250	15,660	Established
		Fletchervale site 2	1	50		No evidence
		Glenample	1	366	12,840	Not surveyed
		Gordon	1	470		Not surveyed
		Jumba	2	1,300	1,500	Established
		Kaiuroo	3	3,460		Not surveyed
		Karamarra	3	3,590		Not surveyed
		Kirknie Road	2	445	4,140	Established
		Lake Mary	2	1,826		Not surveyed
		Lascelles	3		9,420	No evidence
		Macrossan Bridge	6	1,654	17,520	Established
		Mayview	1	1,178		Not surveyed
		McMullen Rd	4	2,671		No evidence
		Merinda	1	832		Not surveyed
		Myola Rd	1	690		Established
		Nogoa River	1	928		Not surveyed
		Pandanusvale	1	255		Established
		Prairie	1	1,511		Not surveyed
		Retro Magenta	1	511		Not surveyed
		Rifle Range Rd	1	840		No evidence
		Rookwood	6	9,999		Not surveyed
		Royles	1	697		Not surveyed
		Ruan	3	4,790	14,940	Not surveyed
		Solferino	2	628		No evidence
		Star Rain	1	1,060		Not surveyed

State	Region	Site name	No. releases	Total Pupae	Total Larvae	Establishment status
		Stockham Rd	2	3,297		Not surveyed
		Taemas 3P dam sites 2&3	4	5,809	40,680	Established
		Taemas front paddock	2	3,434		Established
		Taemas site 1	1		7,800	No evidence
		Tartrus	1	1,508		Not surveyed
		Theresa Creek, Bullery Road	2	1,244	1,500	Established
		Theresa Creek, Peak Downs Hwy	4	2,377		Established
		Theresa Downs	1	377		Not surveyed
		Valencia	1	515		Not surveyed
		Valeria	1	1,757		Not surveyed
		Waranilla	1	1,285		Not surveyed
		Weir	5	4,238	11,100	Established
	Central Qld coastal	Apis Creek	3		11,820	Established
		Bingegang	1		400	Not surveyed
		Bundaleer	1		200	Not surveyed
		Clements Creek	8	2,497	18,840	Established
		Dunloe	1		200	Not surveyed
		Forest Home	1	100		Not surveyed
		Groper Creek	7	6,230	18,660	Established
		Heleen Downs	4	5,719	10,980	Established
		Honeycombe	1		400	Not surveyed
		Langley	1		600	Not surveyed
		Leichhardt Downs	5	3,133	5,760	Established
		Leura	1		400	Not surveyed
		Mourindilla	3		15,400	No evidence
		River Lea	1		600	Not surveyed
		Scrubbee	1		200	Not surveyed
	Qld Gulf	Delta Downs site 1 Pelican Swamp	2		6,880	No evidence

State	Region	Site name	No. releases	Total Pupae	Total Larvae	Establishment status
		Delta Downs site 2 Revolver Swamp	1		3,300	No evidence
		Magowra Station, Beazley's Paddock	1		1,200	Not surveyed
		No. sites = 80	**256**	**193,018**	**530,180**	
WA	**E Kimberly**	Burr Camp	1	50	1,500	No evidence
		Buttons Crossing	1		1,500	No evidence
		Dunham R.	1		6,000	No evidence
		Mambi	9	1,900	18,480	Established
		Valentine Falls	1		1,200	No evidence
		Maitland River	5	50	38,580	No evidence
	Pilbara	Robe River Mouth	1		1,260	No evidence
	W Kimberly	Cockatoo Yard	1	50	1,500	No evidence
		Minnie Bridge	1		1,500	No evidence
		No. sites = 9	**21**	**2,050**	**71,520**	
NT	**Alice Springs**	Elkedra Station 1	2	1,150	0	No evidence
		Elkedra Station 2	1	0	5,915	Established
	Barkly	Alroy Downs Station 1	2	716	233	Not surveyed
		Alroy Downs Station 2	3	20	3,391	Not surveyed
		Alroy Downs Station 3	1	693	0	Not surveyed
		Anthony Lagoon Station	4	5,469	200	No evidence
		Anthony Lagoon Station 2	1	1,400	0	Not surveyed
		Brunette Downs Station	1	0	941	Not surveyed
		Buchanan Downs	1	1,261	0	Not surveyed
		Hayfield Station	1	100	2,755	Not surveyed
		Newcastle Waters Station 1	4	0	1,632	Established

State	Region	Site name	No. releases	Total Pupae	Total Larvae	Establishment status
		Newcastle Waters Station 2	3	1,350	0	Established
	Gulf of Carpentaria	Mallapunyah Springs Station	1	3,390	0	Not surveyed
	Katherine	Cave Creek Station	1	0	1,853	Not surveyed
	Tennant	Murray Downs Station	2	1,864	0	Not surveyed
		Warrego Township	1	770	0	Not surveyed
	Top End	Adelaide River Station	1	0	235	Not surveyed
		Snake Creek Station 1	6	2,213	4,991	Established
		Snake Creek Station 2	2	700	16,240	Established
	Victoria River District	Birrindudu Station 1	1	0	3,449	Established
		Birrindudu Station 2	4	1,600	2,203	Established
		Camfield Station	3	1,475	4,684	No evidence
		Wave Hill Station	1	1,000	0	Not surveyed
		No. sites = 23	**47**	**25,171**	**48,722**	

Total:	**Releases**	**Pupae**	**Larvae**
No. sites = 112	**324**	**220,239**	**650,422**

Eueupithecia vollonoides (UU2)

State	Region/Town	Site name	No. of releases	Total pupae	Total larvae	Establishment status
QLD	**Qld Gulf**					
	Burketown	Escott Station	8	1,760	32,000	Established
	Qld inland central					
	Boulia	Boulia Site 1 Ardmore Station	1	994		Not surveyed
		Boulia Site 2 Oban Station	1	849		Not surveyed

State	Region/Town	Site name	No. of releases	Total pupae	Total larvae	Establishment status
		Boulia Site 3 Ardmore Station	1	601		Not surveyed
	Cloncurry	Corella Dam	5	2,789	20,600	Established
		Maronan Station	3		8,200	No evidence
	Mt Isa	Barkly Downs	4	680	18,200	No evidence
	Prairie	Prairie Excavation site	3		17,600	No evidence
		The Plains	4	1,580	19,400	Established
		Waterview Station	1		1,400	Not surveyed
	Winton	Corella - Winton	1		2,200	Not surveyed
		No. sites = 11	**32**	**9,253**	**119,600**	
WA	**Pilbara**					
	Port Hedland	DeGrey Station	5		28,000	Established
		DeGrey Station site 2	4	260	18,400	Established
	Kimberly_East					
	Kununurra	Button's Crossing	7	300	25,200	Established
		Ivanhoe Station	2	750	5,800	No evidence
		No. sites = 4	**18**	**1,310**	**77,400**	
NT	**Alice Springs**	Elkedra Station 2	1	2,010	0	Not surveyed
	Barkly	Anthony Lagoon Station 2	1	577	0	Not surveyed
		Newcastle Waters Station 2	1	1,486	0	Established

State	Region/Town	Site name	No. of releases	Total pupae	Total larvae	Establishment status
	Top End	Adelaide River Station	3	1,212	950	Established
		No. sites = 4	**6**	**5,285**	**950**	

Total:	**Releases**	**Pupae**	**Larvae**
No. sites = 19	**56**	**15,848**	**197,950**

10.3 Cost Benefit Analyses of parkinsonia biological control using UU1 and UU2

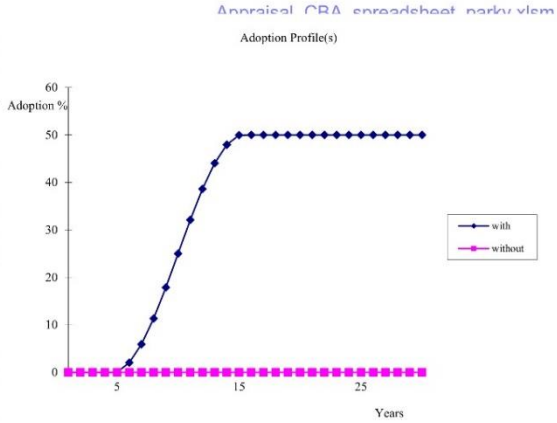
Appraisal_CBA_spreadsheet_parky.xlsm

PROJECT NAME IN FULL (below)

Parkinsonia biological control (UU1 and UU2)

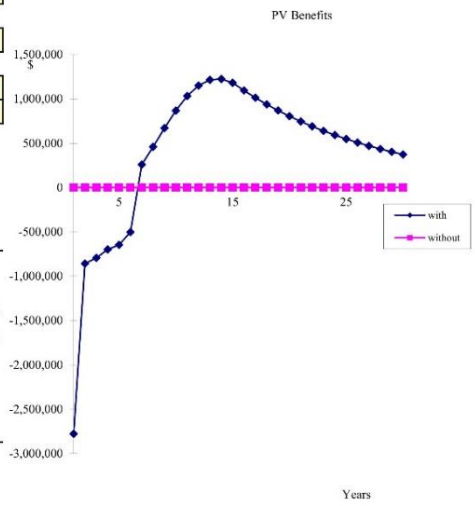
Adoption Profile

With Project	
Year Adoption Begins	6
Maximum Adoption (%)	50
Years to Obtain Maximum Adoption	16
Without Project	
Year Adoption Begins	0
Maximum Adoption (%)	0
Years to Obtain Maximum Adoption	0
Depreciation Decay Rate (%)	0.00
Discount Rate (%)	8
Probability of Research Success (%)	50
Probability of Successful Implementation of Research Results (%)	50



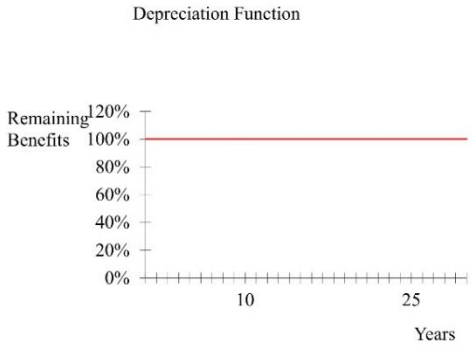
Results - Total Expenditure

PRESENT VALUE BENEFITS	(PVB)	$21,930,415
PRESENT VALUE COSTS	(PVC)	$6,372,442
NET PRESENT VALUE	(NPV)	$15,557,972
BENEFIT COST RATIO	(BCR)	3.44
INTERNAL RATE OF RETURN	(IRR)	17.12%
NPV/$ INVESTED		$2.44



Project Identifiers

Project Title (from cell B18, IN FULL)	Parkinsonia biological control (UU1 and UU2)
File (EXCEL) Name	Appraisal_CBA_spreadsheet_parky.xlsm
Project Number	B.WEE.0134
Project Leader (surname|space|first name)	Sathyamurthy, R
Research Category	Biocontrol
Industry Program Code (G,D,M,W,P,N,C,H)	
Industry Sub Program Code	
Commodity	Rangelands
Appraisal (growth, normal)	normal
Economist (surname in full)	Raghu
Institute	CSIRO
Factor Change (default = 1)	1
Ex Ante or Ex Post	Ex Post
Project Status (f, l, nyf, u)	
Qualitative (ql) or Quantitative (qn)	ql
Policy Initiatives	
MIS number	
Funding body number	
Keyword	
Other	



10.4 Publications resulting from the parkinsonia biological control programme

Copies of publications/drafts are available on request

White, A. Fichera, G. Pukallus, K. Clark, J. 2015. UU and UU2: The latest tools in Parkinsonia biocontrol in Australia. Proceedings of the 13th Queensland Weeds Symposium, Longreach, Queensland.

Hausmann, A., J. Chainey, T. A. Heard, F. McKay, and S. Raghu. 2016. Revision of the genus *Eueupithecia* Prout, 1910 from Argentina (Lepidoptera, Geometridae, Sterrhinae). *Zootaxa* 4138: 392–400.

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

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: *Ecological Applications*)

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*)