Project summary of biocontrol agent *Eueupithecia cisplatensis* for *Parkinsonia aculeata* in Queensland by Queensland DAF

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Summary The leaf-feeding looper, *Eueupithecia cisplatensis* Prout (Lepidoptera: Geometridae) commonly referred to as UU, is a biological control agent for the invasive Weed of National Significance, *Parkinsonia aculeata* L. (parkinsonia). With funding support from Meat and Livestock Australia (MLA), UU was imported from Argentina to Brisbane by Commonwealth Scientific and Industrial Research Organisation (CSIRO) in 2010. Mass-rearing commenced in 2013 at the Queensland Government's Department of Agriculture and Fisheries (Qld DAF) Tropical Weeds Research Centre (TWRC) in Charters Towers. Field releases of UU have occurred at 105 sites throughout Queensland, with establishment observed at 50% of monitored sites in central Queensland and 75.5% in North Queensland.

Keywords Queensland Department of Agriculture and Fisheries, UU, parkinsonia.

INTRODUCTION

Parkinsonia aculeata L. (parkinsonia) is a small, thorned tree originating from the Americas, and found mainly in riparian areas and flood plains. Parkinsonia impedes grazing due to forming dense thickets, reduces grass production and creates feral animal reserves (Qld DAF 2018a).

Three biocontrol agents have been previously released to help control parkinsonia; a sap-sucking bug (*Rhinacloa callicrates* Herring) and two seed feeders (*Mimosestes ulkei* Horn and *Penthobruchus germaini* Pic). All are established and causing various degrees of impact throughout Queensland (van Klinken and Heard 2012). Native range surveys commenced in central and South America in 2002 by CSIRO for new biological control options. *Eueupithecia cisplatensis* Prout (UU) was imported from Argentina in 2010, with approval granted from Australian Department of Agriculture for release in 2013. Mass-rearing of UU commenced at the Queensland DAF's Tropical Weeds Research Centre (TWRC) in March 2013, providing in-kind services to CSIRO. Funding from Meat & Livestock Australia Ltd through Australian Government's Rural Research and Development (R&D) for Profit Program, was received in March 2016 via an arrangement with CSIRO supporting an increase in the mass-rearing and release of the UU project already underway at the TWRC.

The larval stage of *E. cisplatensis* consumes leaflets, damages the leaf and causes defoliation of parkinsonia. This reduction in photosynthetic capabilities impacts the health of the plant, making it more susceptible to fungal pathogens and reduces the overall vigour and reproductive capability of the plant.

METHODOLOGY AND RESULTS

UU biology The life cycle of UU is completed in 23–27 days on average (25°C, 65% RH, 14:10 day length) under glasshouse conditions, with life stages completed in: egg to larva 5–7 days; larva to pupa 13–15 days; and pupa to adult 5 days. Adults live for up to one week, mating within 24 hours of emergence and females start laying eggs shortly after mating.

Eggs are ovoid, a rusty-brown or green colour and laid individually, or in chandelier formation on the leaflets (Figure 1a). Females lay an average of 60–80 eggs. Larvae are cryptic and camouflaged when in situ on a leaf, laying along the midrib (rachis) or at a 45 degree angle (Figure 1b). Larval colour ranges from lime green, rusty red/brown through to light green/yellow.

Pupae are brown, 10 mm in length, and occur encased in a silken cocoon, which has hydrophobic properties to assist UU to survive in flooded conditions (Figure 2a). They are attached to the stem/branch or along the rachis in the field. Adults are a mottled grey/black colour, have a 15 mm wingspan, and at rest, position their wings along the leaf (Figure 2b).

Rearing methodology Adults were collected and sexed from gauzed rearing cages (150 cm × 120 cm × 140 cm) and placed (15 adults maximum) into décor plastic containers lined with paper towel. The adult boxes were kept at 25°C and opportunistically moistened at normal day/night conditions. Once the eggs hatched, the paper towel was removed and placed, eggs facing down, on a parkinsonia plant in a rearing cage, and removed after four days to allow for total eclosion. Plants were replaced when leaflets were near completely consumed, with most cages going through

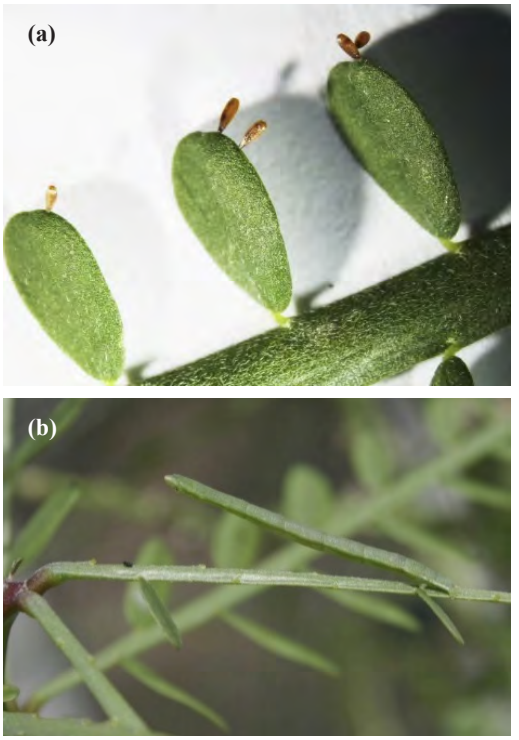

Figure 1. *Eueupithecia cisplatensis* (UU) (a) eggs laid on leaflets and (b) larva at rest (45 degree angle) on a parkinsonia leaf.

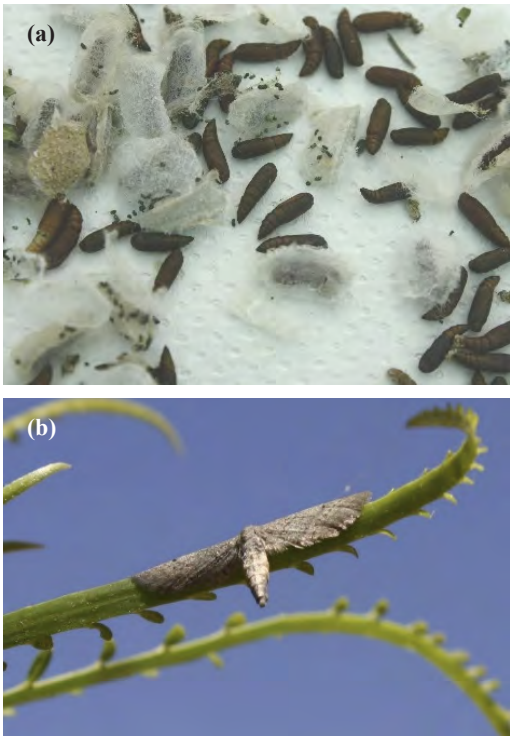

Figure 2. *Eueupithecia cisplatensis* (UU) (a) pupae encased and removed and (b) female adult moth.

7–8 host plants with 250 larvae, over 21 days. Cage floors were cleaned daily to remove fallen plant material and frass and were wiped with methylated spirits, to prevent bacterial and fungal colonies from developing. Larvae wandered to pupate, pupating on the plant, around the pot rim/base or corners/edges of the cage. Pupae were collected with tweezers after browning and hardening, and stored in takeaway containers at 9°C for up to 3 weeks, until released. Adults were collected from uncollected pupa from different cages to ensure genetic diversity within adult boxes.

Release methodology The initial release strategy was using larvae placed in clustered branches, attached with a lock-tie onto tips of parkinsonia branches (Figure 3a). Predation from wasps and ants was immediate, with limited larvae surviving. Eggs and adults from adult boxes were occasionally released, by wrapping and attaching the paper towel with the eggs attached onto branches, similar to larval releases. Pupal releases were the preferred method and are quicker, easier and less bulky for landholder/stakeholders and have reduced transport costs. Shallow plastic takeaway containers, lined with paper towel, containing 600–700 pupae were placed into a delta trap and attached to a parkinsonia branch (Figure 3b). The moths emerge over several weeks, mate and are able to fly to new trees or higher on the plant, compared to the limited mobility offered by larval or egg releases.

Release sites Release sites were on private grazing/agricultural land, mine sites, National parks, roadside verges or council reserves in riparian zones or on flood plains. *Eueupithecia cisplatensis* has been released at 105 sites within five local government areas within Queensland since April 2013. With the assistance of landholders, stakeholders, Queensland government and local council employees.

A total of 297,029 pupae, 504,600 larvae or eggs and 3,270 adult moths have been released (Table 1). Release frequency and quantity have varied at each release site, ranging from one to 20 separate releases over the years, depending on establishment and if they were classified a nursery site.

Figure 3. *Eueupithecia cisplatensis* (UU) (a) larval release method and (b) pupal release method.

Monitoring and establishment Release sites were monitored periodically and opportunistically from six months after release and were conducted using the beat-sheet method. A white sheet is placed under a section of foliage/tree and hit with a hoe handle several times, dispersing the larvae onto the sheet. The number and size range of the larvae were recorded, along with other Lepidopteran larvae or past agents, such as *R. callicrates*. Sites are given a present or non-present rating after monitoring, or unknown if the sites have not been revisited (Figure 4). Establishment (continued present rating) in monitored sites was 75.5% North Queensland and 50% Central Queensland (Table 1).

Monthly monitoring at a nursery site along the Burdekin River, north of Charters Towers (−19.80489° S, 146.07713° E), shows the larval population increases during summer and autumn. Averages are 4.3 and 5.42 larvae per tree for these seasons, coinciding with major rain events and new leaf growth, compared with spring (2.49) and winter (1.59). Three monthly monitoring was conducted at additional sites along the east-coast in the Burdekin region and in Central Queensland region since September 2017.

Establishment at non-release sites has been observed at several locations within Queensland, and has occurred more than 20 km from known release sites. At a non-release site near Brandon in North Queensland (−19.50228° S, 147.30742° E) in April 2016, over 40 larvae were counted from monitoring one section of a small tree (<2 m tall). Significant damage was noted to the tree, with limited leaflets, browned midribs and no flowers.

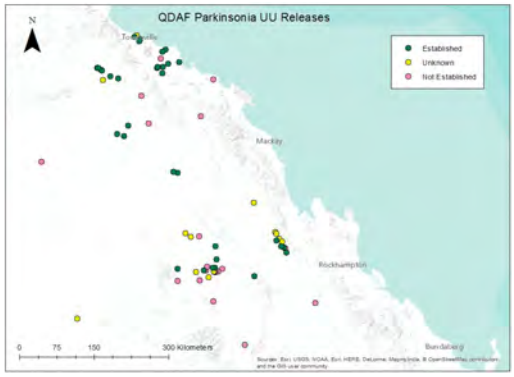

Figure 4. *Eueupithecia cisplatensis* (UU) release sites on parkinsonia throughout Queensland (April 2013–2018).

Table 1. *Eueupithecia cisplatensis* (UU) release numbers on parkinsonia in Queensland (April 2013–2018).

Region	# sites	Pupa	Larva/eggs	Adults	No. sites establishment noted	% established of monitored sites
Central Queensland (−24.8 to −22.2° S)	56	115,692	23,100	0	19	50
North Queensland (−21.6 to −19.2° S)	49	181,337	481,500	3,270	37	75.5
Totals	105	297,029	504,600	3,270	56	65.1

Impact The impact of biological control agents is often difficult to quantify in the field. Laboratory experiments on larval consumption of parkinsonia's bipinnate leaf structure have shown that a single UU larva can consume up to 118 leaflets (average 53.4) over a 15 day larval stage at 25°C (12:12h day/night). Consumption peaks when larval length reaches 20 mm, at around day 13. In the field the larvae are dispersed on the plant, rather than clumped, causing dispersed impacts across the plant. Larvae consume the leaflets, either whole or partly, and damage the rachis causing the leaf to eventually abscise.

Time lapse photographs taken every hour in rearing cages have shown daily larval movement throughout the plant architecture, and nocturnal feeding habitats. A UU larva can move up to 21 nodes on a single stemmed parkinsonia seedling (average height of 359.1 mm) over 13 days, travelling approximately 240 mm in stem length. Daily larval movement and leaflet consumption is sporadic and they rarely stay in the one location on a leaf.

DISCUSSION

With good field establishment, equating to seasonal persistence and agent dispersal, what does it mean in terms of impact and control of parkinsonia?

We know one larva consumes on average 53.4 leaflets within its development, and the average number of UU larvae sampled on parkinsonia in the field ranges from 1.59–5.42 per part tree, compared to the real impact that has been observed in the field from a population density of 40 UU on sampling a part tree. Accordingly, the current field density needs to increase significantly (by over 700%) to have an impact on the number of leaflets on a mature tree, conceivably to slow the reproductive potential and therefore the spread of parkinsonia, based on those numbers. With the completion of upcoming seedling exclusion and further cage trials, the impact of UU and the potential to reduce the population of parkinsonia seedlings will be better quantified.

Since 2014, release sites have been in local government drought declared or partly declared areas (Qld DAF 2018b), providing limited food resources at times for UU. Yet throughout these years, UU has persisted and spread to new locations. Seasonal peaks in population densities of UU are created, with a lag effect once rain events occur, as shown at the monitoring site at Burdekin River. The long term environmental events are unknown, but if UU can survive through the current conditions it will persist in the long term. This project has mass-reared and released the largest number of agents from Qld DAF's TWRC facility and has been a long-running program, due for completion shortly.

We are on the way to deeming the project a success due to the large numbers of UU released, establishment success, but whether a reduction in seedling recruitment and associated reduction of control activities for parkinsonia is observed remains to be seen in the years ahead.

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