ECOSYTEM SCIENCES
www.csiro.au

Application to release the defoliating caterpillar *Eueupithecia* sp.2 for biological control of the weed *Parkinsonia aculeata*

Dr Tim A. Heard

EcoSciences Precinct, 41 Boggo Rd, Dutton Park, GPO Box 2583, Brisbane, 4001

Phone: 07 3833 5730

Mobile: 0434 416 053

Fax: 07 3833 5503

Email: tim.heard@csiro.au

2013-05-09

For assessment by the Australian Government Department of Agriculture, Fisheries and Forestry and Department of Sustainability, Environment, Water, Population and Communities

Contents

Summary

Parkinsonia aculeata (Leguminosae: Caesalpinioideae) is a shrub or tree from the Americas that can form dense thorn thickets that impact negatively on both environment and the pastoral industry in rangeland Australia. It is recognised as one of twenty worst weeds in Australia (Thorp and Lynch 2000) and has been declared in all states and territories.

The defoliating caterpillar, *Eueupithecia cisplatensis* Prout (Lepidoptera: Geometridae), was released in Australia in 2013 for biocontrol of *P. aculeata*, after testing showed that it was entirely specific to its host. A second, sibling species of *Eueupithecia* has been identified as a potential biocontrol agent (Figure 1). This species has not been formally described and so is referred to as *Eueupithecia* sp.2. *Eueupithecia* sp.2 has a more tropical distribution than its sibling species and so is likely to be more suited to the hotter and drier areas of Australia where its host plant occurs.

Preliminary studies on its host specificity made in the field and laboratory in Argentina, indicated that, like its sibling species, it is specific to *P. aculeata*. *Eueupithecia* sp.2 was then imported into an Australian quarantine where testing was completed on a broad range of plant species, particularly native Australian caesalpinioids, selected on the basis of phylogeny. Excluding *P. aculeata*, a total of 65 plant species were tested, 42 in the laboratory in Australia, 20 in the laboratory in Argentina and five in the field in Argentina. *Eueupithecia* sp.2 has proven, like its sibling species, to be entirely host specific to *P. aculeata*. In laboratory tests, full development to adult occurs consistently on *P. aculeata* with a high rate of success (average of 51%). But no development occurred on any test plant species, with all larvae dying as first instars. No feeding occurred on any test plant species and hence no damage was observed on non-target species.

We conclude that the level of risk associated with releasing *Eueupithecia* sp.2 into the Australian environment is acceptable and that it will potentially be an effective biological control agent for *P. aculeata*. We seek permission for its release in Australia.

Figure 1. Left: a larva of *Eueupithecia* sp.2 resting on a damaged Parkinsonia leaf. The head is in the air, the two pairs of prolegs are grasping the rachis. Most of the pinnules have been eaten and rasping of the surface of the leaf rachis is visible. Right: an adult male of *Eueupithecia* sp. 2.

Acknowledgments

I wish to sincerely thank the following people for their efficient and meticulous lab work in the Brisbane quarantine laboratory: Andrew White, Gio Fichera, Ryan Zonneveld, and Diane Parthenay. Fernando Mc Kay, Alejandro Sosa, Natalia Cuadra and Juan Briano of the Fundación para el Estudio de Especies Invasivas (FuEDEI) Buenos Aires, Argentina, executed native range field and laboratory work with their usual enthusiasm and high level of ability. Axel Hausmann identified specimens and performed taxonomic research on the agent. Meat and Livestock Australia and the Australian Government Department of Agriculture, Fisheries and Forestry through the AWRC program provided funding without which this work, and the preceding studies, would not have been done. Dr Rieks van Klinken provided scientific guidance and discussion. Fernando Mc Kay, Rieks van Klinken and John Scott commented on drafts of the application.

1 Information on target species, *Parkinsonia aculeata*

1.1 Taxonomy

1.1.1 BOTANICAL NAME

Parkinsonia aculeata L.

1.1.2 COMMON NAME

The plant is usually referred to as parkinsonia in Australia and Mexican palo verde and retama in the American literature. However, overseas it has many local names, including Jerusalem thorn, blue palo verde, horse bean tree, sessaban and Barbados flower fence (Hawkins 2001).

1.1.3 RELATIONSHIPS

Parkinsonia aculeata belongs to the family Leguminosae, subfamily Caesalpinoideae, tribe Caesalpinieae. Relationships of the monophyletic Leguminosae to other Angiosperms is still unclear with several families having been proposed as related, but more recent and well supported studies place Surianaceae and Polygalaceae as sister groups (Woyciechowski 2003). Relationships between caesalpinioid genera of the Leguminosae are also unresolved (Herendeen et al. 2003), but the *Peltophorum* group, to which *Parkinsonia* belongs, is strongly supported as monophyletic. The *Peltophorum* group includes *Peltophorum, Parkinsonia*, *Delonix*, *Colvillea* and *Schizolobium* (Haston et al. 2005). The only member of the *Peltophorum* group native to Australia is *Peltophorum pterocarpum.* The genus *Parkinsonia* is considered to be congeneric with the paraphyletic Central American genus *Cercidium* (Hawkins et al. 2007). *Parkinsonia aculeata* is the only *Parkinsonia* species known to have naturalized in Australia. *Parkinsonia aculeata* is easily delimited morphologically from all other *Parkinsonia* species (Hawkins 2001); however, considerable intra-specific genetic variation occurs across its distribution in the native range (Hawkins et al. 2007). More information on the relationships is given in the section “The test plant list”.

1.2 Description

P. aculeata is readily identified in Australia by its smooth, green bark, very distinctive pendulous leaves with minute, easily-shed pinnules, bright yellow, five-petalled flowers, and pods which are straw-coloured when mature and contain 1-11 seeds (Figure 2). Adults typically grow to 5-7 m tall and wide (van Klinken et al. 2009a).

a)

b)

c)

d)

e)

Figure 2. *Parkinsonia aculeata* in Australia: leaves (pinnae and pinnules) and thorns a); flowers b); mature pods c); adult plant in flower d); large infestation in wetlands of the Queensland Gulf Region (e). (Source: Nathan March, Biosecurity Queensland).

1.3 Distribution

1.3.1 NATIVE RANGE

Parkinsonia aculeata is native to the Neotropics. Species level and infra-specific phylogenies have been reconstructed using three chloroplast gene regions, and amplified fragment length polymorphism markers (Hawkins et al. 2007). Several genetically distinct populations of *P. aculeata* have been identified across the Americas: (1) northern and western Mexico, south-western USA and Cuba; (2) eastern and southern Mexico and south-eastern USA; (3) Venezuela; (4) Central America; and (5) Argentina. The Argentine lineage (5) is estimated to have diverged from other lineages (1-4) c. 9.1 million years ago, and the northern Mexico lineage (1) from the Mesoamerican-Venezuelan lineages (2-4) c. 5.2 million years ago (both pre-dating formation of the Isthmus of Panama) (Hawkins et al. 2007). Additional divergent populations may exist in South America, but these have not been analysed genetically.

1.3.2 AUSTRALIAN RANGE

The distribution of *P. aculeata* has been mapped nationally on a 50 x 50 km grid, mainly through existing distributional records held by state departments and through expert knowledge (Figure 3). When considered at that grid scale, *P. aculeata* is now estimated to be present on over 3.3 million ha of Australia, although densities are very low throughout most grid cells (van Klinken et al. 2009a).

Most infestations occur across semi-arid and semi-humid Australia, especially in central and north Queensland, the Barkly Region and the Victoria River District of the Northern Territory, and the Kimberley and Pilbara Regions of Western Australia. Although it is widespread in these regions, dense patches are associated primarily with flood-outs, water infrastructure (such as “turkey nests”), water courses and the edges of seasonally-flooded fresh-water wetlands. Elsewhere in Australia records are mostly of isolated plants, or relatively restricted, scattered infestations (van Klinken et al. 2009a).

The potential distribution in Australia is much greater than the current distribution. Much of northern and eastern Australia is probably climatically suitable for *P. aculeata*, provided adequate soil moisture is available, with conditions being optimal in Central Queensland (van Klinken et al. 2009a). On the broad scale *P. aculeata* has probably naturalized in the majority of suitable catchments. Within catchments *P. aculeata* is generally very sparsely and/or locally distributed, but there is little doubt that *P. aculeata* will continue to spread through the wetter habitats within its current range. Special efforts are currently underway to prevent its spread into Cape York Peninsula, the Lake Eyre and Murray Darling basins in Queensland and the blue-bush (*Maireana* spp.) swamps in the Barkly Tablelands (Deveze 2004).

Climate change is expected to result in a southward extension of highly suitable areas in eastern Australia as a result of reduced cold stress (van Klinken et al. 2009b). Also, in south-west Australia it is expected that there will be improved growing conditions and reduced cold-wet stress. Reduced rainfall is expected to result in the northern (tropical) interior becoming less suitable, while increased rainfall is expected to increase the suitability of much of Australia.

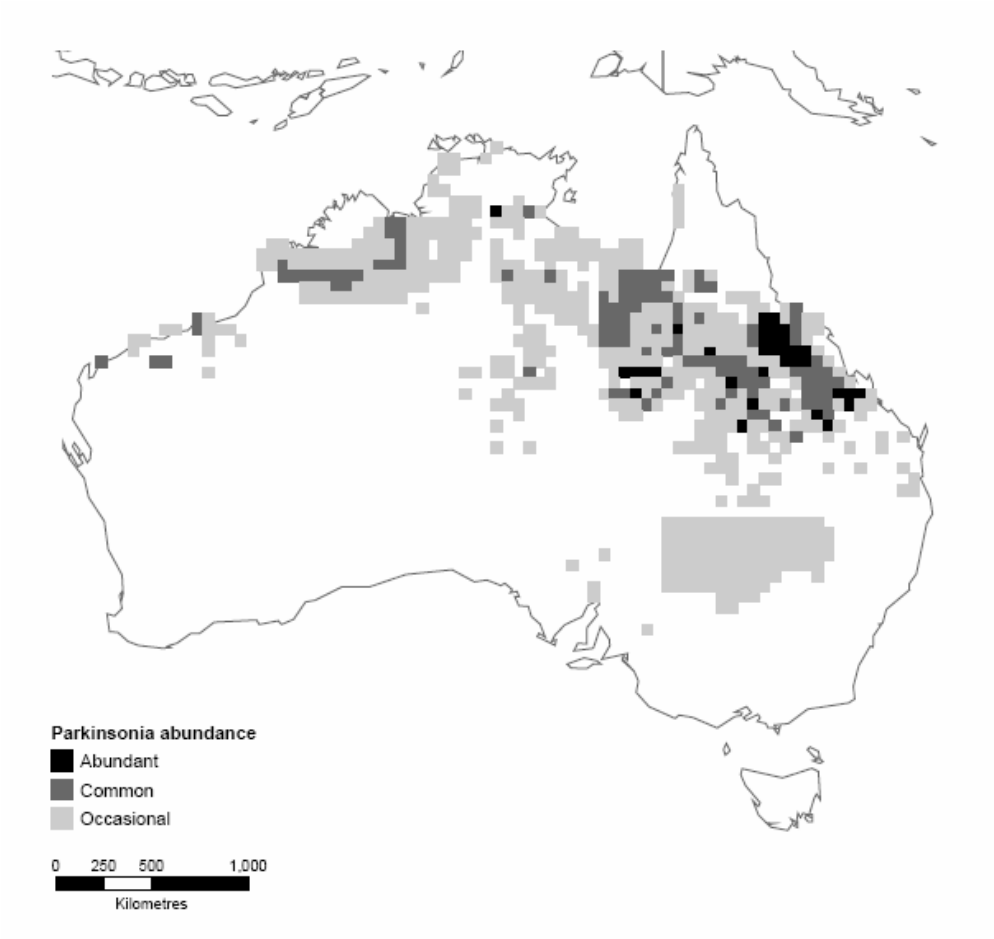

Figure 3. Current distribution and abundance of *P. aculeata* in Australia. Source: Queensland Biosecurity

1.4 Ecology

Parkinsonia aculeata has an outstanding ability to survive and grow under a wide range of environmental conditions (Hughes 1989). This includes arid regions to wet-dry tropical regions, with annual rainfall typically ranging between 250 and 1400 mm. Plants probably rarely live more than 20-30 years (van Klinken et al. 2009a). They can produce large numbers of seeds, which are mostly dispersed either by flood waters within floating pods, or become incorporated into the seed bank under or adjacent to parent trees. Seeds are hard-seeded and are released from dormancy by "wet heat" (van Klinken and Flack 2005). Populations are typically very dynamic as a result of often rare major recruitment events and a wide range of mortality factors, including dieback putatively caused by a suite of soil-borne pathogens (Toh et al. 2008; Diplock et al. 2006, 2008; Toh 2009; van Klinken et al. 2009a), severe frosts, fires, and browsing by macropods or sheep (van Klinken et al. 2009a). In fact, most of the 23 initially healthy populations monitored across Australia since 1999-2000 have subsequently declined in adult density, and local extinctions are probably common (van Klinken et al. 2009a). Browsing by sheep, goats and other livestock (generally not cattle) is likely to be an important factor preventing invasions in other countries.

1.5 Importance

Parkinsonia aculeata is an example of a plant that is both weedy and beneficial; however, in Australia its negative aspects far outweigh any actual or potential benefits.

1.5.1 BENEFICIAL

Parkinsonia aculeata is widely used as an ornamental in dry areas throughout the Americas because of its spectacular bright yellow flowers; however, it is not generally considered to produce particularly valuable or high quality products (Hawkins 2001). Uses include hedges, windbreaks, shade, fuel (firewood and charcoal), paper-making and low quality fodder (Hawkins 2001). Although wood can be used for carpentry, it is brittle and of dubious durability (Stewart et al. 1992). *Parkinsonia aculeata* has been used in folk medicine (Barbosa and Prado 1991). Leaves, when made into an infusion, are considered in some areas to have medicinal and antiseptic properties and the infusion has been used to treat fevers, epilepsy and vomiting (Stewart et al. 1992, Hawkins 2001). Raw seeds have been used as a food source by humans in Mexico, children have been reported to eat flowers and seeds in West Africa, and seeds have been investigated as a minor food source in India (Hawkins 2001).

The fodder value of *P. aculeata* pods and foliage varies, and reports range from it being rarely eaten by livestock or wildlife (Everitt 1983) to being a potentially important fodder tree (MacDicken and Brewbacker 1984, Stewart et al. 1992, Hawkins 2001). It appears to be consumed by cattle only in times of shortage (Stewart et al. 1992), such as late in the dry season (Anon 1972, Deveze 2004, p. 35, 45); however, it is browsed by sheep, goats and camels and, in some parts of the world, branches are lopped during dry periods to feed sheep and goats (Hawkins 2001).

Parkinsonia aculeata has been introduced pan-tropically, primarily as an ornamental, hedging and fodder tree (Stewart et al. 1992, Woods 1988, Hawkins 2001). In addition, its tolerance to drought, waterlogging and saline conditions has meant that it has often been promoted for rehabilitation and as a multi-purpose tree, particularly in harsh, degraded or marginal land (Hughes 1986, Hawkins 2001). It has been used for reforestation programs in several countries, including India, Sudan and Cape Verde (Hughes 1989) and continues to attract attention as a candidate for the reforestation of degraded environments. However, its usefulness can be limited by its weedy tendencies (Hughes 1989). In Australia *P. aculeata* appears to have been planted mainly as an ornamental and shade tree.

1.5.2 DETRIMENTAL

Most of the detrimental effects of *P. aculeata* stem from its propensity to form dense, thorny, impenetrable thickets along drainage lines, depressions, ephemeral wetlands and, to a lesser extent, uplands across a large part of Australia. These are of both of environmental and economic significance.

The greatest environmental impact is probably through the exclusion of the herbaceous layer (van Klinken 2006). *Parkinsonia aculeata* trees are relatively shallow-rooted, but they may shorten the duration that ephemeral water bodies hold water. Dense patches are rarely greater than 1 ha so impacts on biodiversity are likely to be localised and limited to the infestation site (van Klinken 2006). At greatest risk are climatically suitable mesic habitats in arid and semi-arid regions, such as wetlands on the Barkly Tablelands (Northern Territory), wetlands and gorges in the Pilbara Region (Western Australia) (van Klinken 2006) and waterbird habitats of national significance across its potential distribution (Humphries et al. 1991).

In production systems *P. aculeata* can also replace pasture, but existing infestations probably do not occur at a sufficient scale to cause significant and widespread reductions in carrying capacities (van Klinken 2006). Thicket formation does, however, interfere with stock management, impedes stock access to water, makes the maintenance of water points difficult and provides refuge for feral pigs (Deveze 2004). Both the formation and control of thickets may also exacerbate erosion problems (Wilson and Miller 1987). Thorns may injure hooves of animals and affect leisure and recreational activities, while its flowers are known to cause hay fever (Wilson and Miller 1987; Deveze 2004).

Although *P. aculeata* is already widespread in Australia, existing infestations are not yet of sufficient scale to cause substantial production losses at the property scale or to cause catchment or regional scale environmental impacts. Most of the direct costs are related to increased property management costs, especially in relation to mustering, accessing water points and maintaining vehicle tyres, and on-ground control work to prevent *P. aculeata* from becoming a more serious problem. Costs to Australia will increase dramatically if *P. aculeata* continues to spread and thicket formation continues. However, actual and potential impacts have not been quantified.

1.6 Information on all other relevant Commonwealth, State and Territory legislative controls of the target species

Parkinsonia aculeata has been declared in all states and territories other than Victoria, Tasmania and the Australian Capital Territory (Deveze 2004). In Queensland it is classified as a Class 2 declared pest (landholders must take reasonable steps to keep land free of the weed; it is also prohibited to introduce, feed, keep, release, take for commercial use, supply or transport). In the Northern Territory the species is classified as Category B (growth and spread to be controlled). In Western Australia it is declared as P1 (prevention of trade, sale or movement), P2 (eradicate) or P4 (contain) according to districts. In New South Wales it is declared in Category W1 (presence must be notified to the local control authority and the weed must be fully and continuously suppressed and destroyed). In South Australia *P. aculeata* is notifiable throughout the state, and plants must be destroyed.

1.7 When the target species was approved for biological control

The Australian Weed Committee approved *P. aculeata* as a target for biological control in Australia in 1983 (Donnelly 2000).

1.8 History of biological control

Three insect species have been released in Australia for biocontrol of *P. aculeata*. *Rhinacloa callicrates* (a sap-sucking mirid) and *Mimosestes ulkei* (a seed-feeding bruchid) were released in Queensland in 1993 (Julien and Griffiths 1998) and the Northern Territory in 1989 (Donnelly 2000) and 1994 (Flanagan et al. 1996), respectively. A third insect from Argentina, the seed-feeding bruchid *Penthobruchus germaini* Pic., was identified from the literature as a potential agent and was released in Australia from 1995 (Briano et al. 2002). *Rhinacloa callicrates* has established in Central Queensland but has never been observed to reach damaging densities there and did not establish in the Kimberley (Donnelly 2000). *Mimosestes ulkei* has established at relatively few sites and, where measured, the seed mortality rates have been low (Donnelly 1998, Lockett et al. 1999). It has not been reported in the past several years. In contrast, *Penthobruchus germaini* established easily, and dispersed readily (van Klinken and Flack 2008). *Penthobruchus germaini* passes through several generations a year, and oviposits primarily on pods on the tree (Briano et al. 2002, van Klinken 2005, van Klinken and Flack 2008). However, seed consumption rates were relatively low during a national

survey conducted between 2000 and 2004 (van Klinken 2005*,* van Klinken and Flack 2008), and the agent is therefore unlikely to be causing any population-level impacts. Studies showed that beetle populations were unable to track sudden seasonal fluctuations in pod supply, resulting in a lag-phase between seed availability and beetle numbers. Also, high egg parasitism (10-70%) by a trichogrammatid wasp (*Uscana* sp.), is likely to be a key regulating factor through its effect on egg survival, and indirectly on adult densities. Existing agents therefore do not appear to be having a significant impact. The defoliating caterpillar, *Eueupithecia cisplatensis* Prout (Lepidoptera: Geometridae), was released in Australia in 2013 for biocontrol of *P. aculeata*, after testing showed that it was entirely specific to its host (van Klinken and Heard 2012).

2 Information on the potential agent *Eueupithecia* sp.2

2.1 Taxonomy

Order: Lepidoptera

Family: Geometridae:

Subfamily Sterrhinae,

Tribe Sterrhini

Genus and species: *Eueupithecia* sp.2

Image: Figure 4

Identification: Dr. Axel Hausmann (Geometridae specialist, Bavarian State Collection of Zoology, Munich, Germany).

Figure 4. *Eueupithecia* sp.2, female left and male right

Voucher specimens (at least two individuals of each sex) and slide mounted genitalia preparations have been prepared and will be deposited with AQIS and the Australian National Insect Collection.

Eueupithecia is placed into subfamily Sterrhinae, tribe Sterrhini (see Differential diagnosis below). The Geometridae and all recognized subfamilies are monophyletic (Sihvonen et al. 2011). Also the phylogeny of the Sterrhinae subfamily revealed good support for the subfamily Sterrhinae and the tribe Sterrhini (Sihvonen and Kaila 2004). The tribe Sterrhini consists of approximately 825 species distributed in the following genera: *Anthometra, Arcobara, Brachyglossina*, *Cleta*, *Emmiltis*, *Epicleta, Euacidalia, Eueupithecia, Eumacrodes, Eupithecidia, Idaea, Limeria, Lobocleta, Lophophleps, Odontoptila, Protoproutia, Ptychamalia* and *Tineigidia* (Sihvonen and Kaila 2004).

Parsons et al. (1999) included only one species (*E. cisplatensis*) in the genus *Eueupithecia*. However, Dr Axel Hausmann recently identified the second cryptic species. This species shows striking differences in female and male genitalia (Table 1, Figure 5, Figure 6). In addition the CO1 barcode gene sequence differs by 4%, an amount that normally indicates another species. However, no significant and constant differential features in colour or pattern of adults or larvae have been found. The second species has a more north-westerly distribution to *E. cisplatensis*. No overlap of the

distribution range of the two species has been found, although their ranges come close near the city of Reconquista close to latitude 29°S (Figure 7).

All testing in Australia was conducted on a pure colony of *Eueupithecia* sp.2, as confirmed by genitalia dissections.

Table 1. Differential features between the two *Eueupithecia* species collected on *Parkinsonia aculeata*

	E. cisplatensis	***Eueupithecia* species 2**
Female genitalia (Figure 5)	Length of corpus bursae 1.6 mm, posterior 1/2 sclerotized, slightly folded only	Length of 2 mm, posterior ¾ strongly sclerotized and strongly folded laterally.
Male genitalia (Figure 6)	Aedeagus with large basal cornutus (half length of aedeagus) and a smaller, but stout, hook-shaped cornutus at tip. Aedeagus slender, width 0.2 mm.	Aedeagus with two cornuti, neither hook-shaped. Aedeagus very broad, width 0.4 mm.
Distribution (Figure 7)	NW Argentina, provinces of Salta, Formosa, Chaco and Santa Fe	NE Argentina, provinces of Cordoba, Santa Fe, Corrientes, Entre Rios, and Buenos Aires
Size of adults	On average smaller, wingspan 15-20 mm	On average larger, wingspan 20-25 mm

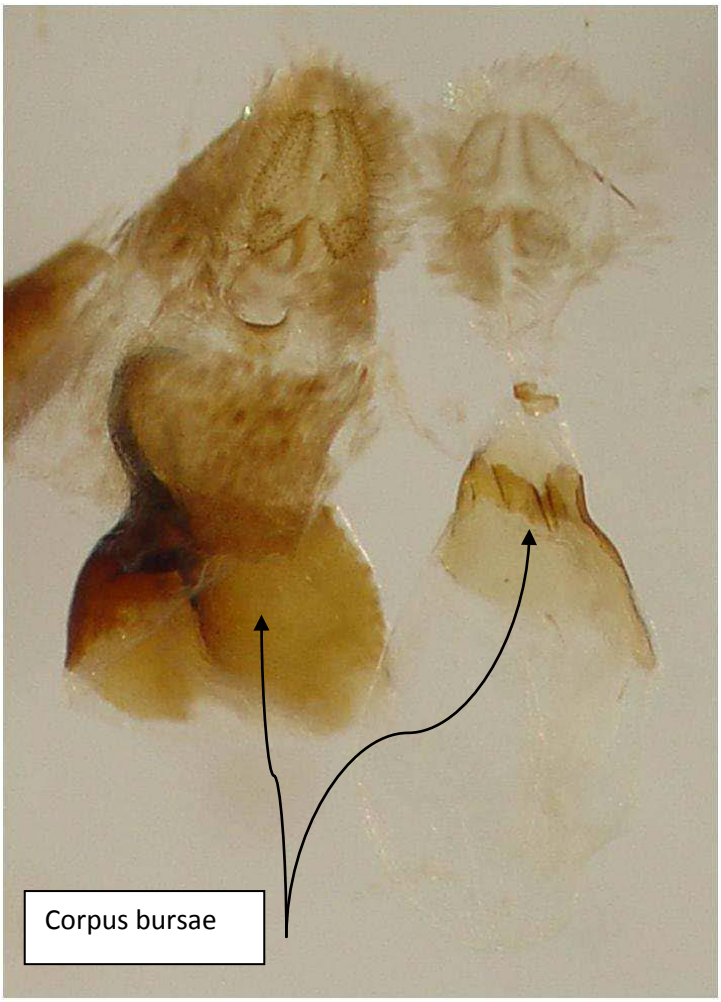

Figure 5. Female internal genitalia of *Eueupithecia* sp.2 (left) and *Eueupithecia cisplatensis* (right). Note the different sclerotisation of the corpus bursa

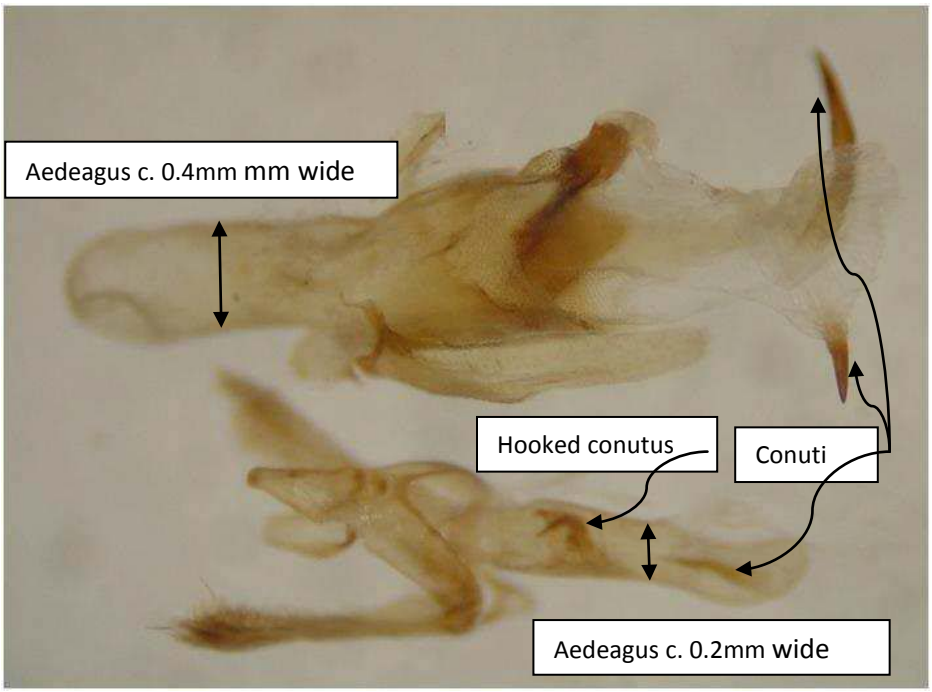

Figure 6. Male internal genitalia of *Eueupithecia* sp.2 (top) and *Eueupithecia cisplatensis* (below). Note the wider aedeagus and lack of a hook shaped cornutus in *Eueupithecia* sp.2.

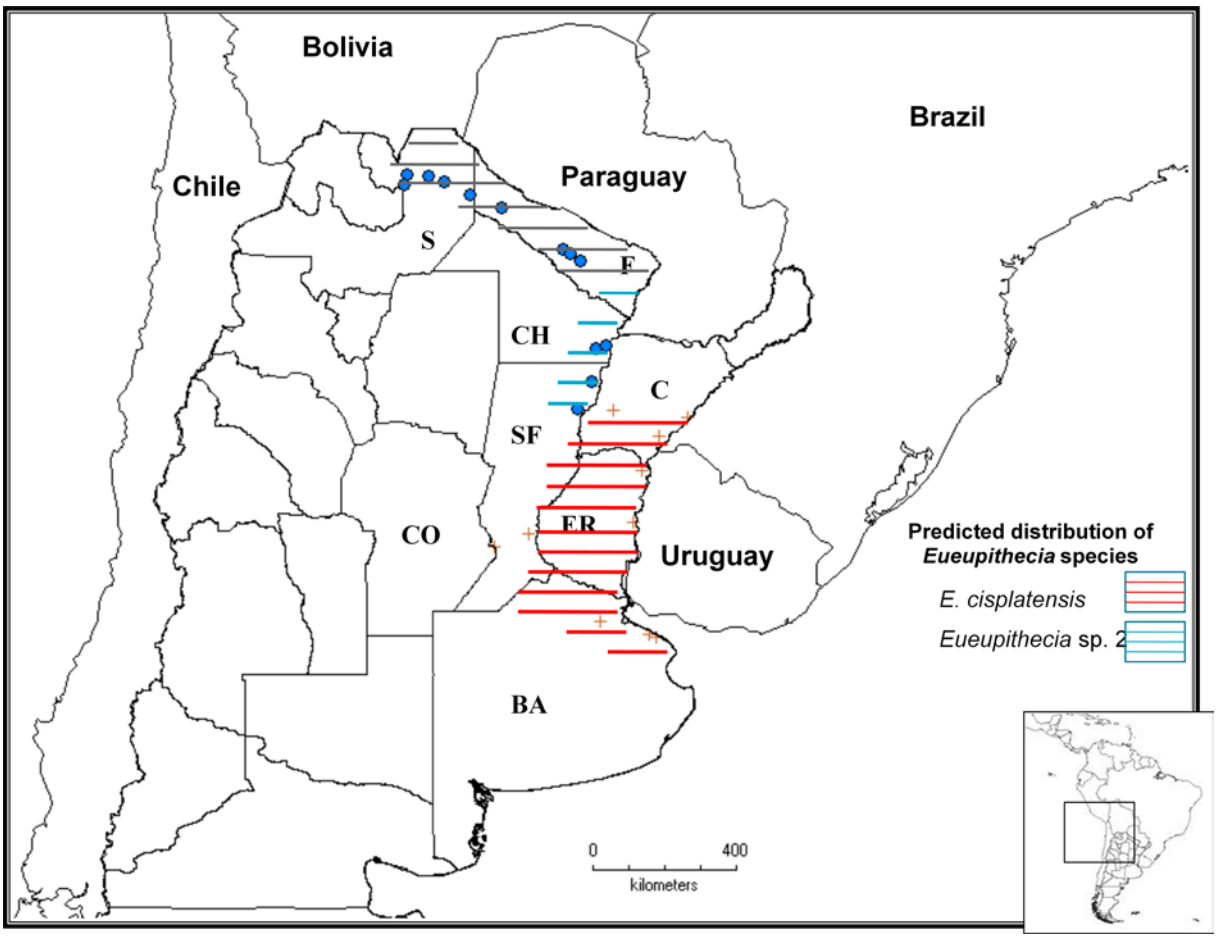

Figure 7. Distribution of *Eueupithecia* species in Argentina confirmed by genitalia dissections. Red crosses: *E. cisplatensis* localities. Blue dots: *Eueupithecia* sp.2 localities. BA, Buenos Aires; CH, Chaco; CO, Córdoba; C, Corrientes; ER, Entre Rios.

2.2 Description

This insect was first discovered in the unpublished surveys of Cordo and Briano (Heard 2005). The following is a description of the genus *Eueupithecia* obtained by Dr Axel Hausmann (pers. comm. 2011), contained in a manuscript in preparation for publication.

Tongue very short. Palpi very small, tapering, last two segments narrow, length 0.6 times diameter of eye in male, 0.8-1.0 times diameter of eye in female. Frons black, flat, smoothly scaled. Antennae filiform, in female with scarce and very short ciliation, in male ciliate-fasciculate, cilia strongly curved, length 2.5 times width of flagellum. Male hindtibia shortened, without spurs, with weak pencil. Female frenulum developed as a long, single stout bristle, appressed without retinaculum in the fold of the anal vein of the forewing (unknown in any other Geometridae, all other female geometrids have a brush of setae, if they have a frenulum). Hindwing Sc+R1 and Rs+M1 with long anastomosis, ca 2/3 length of cell. M2 much closer to M1 than to M3. Forewing with one single areole. Fore- and hindwing elongate and very narrow, discal spots conspicuous, postmedial line dotted. Hindwings of both sexes with setose lobes at the inner termen. Tympanum with ansa narrow at base, dilated at centre, rounded at tip.

Male genitalia: Small. Uncus single, digitiform. Valvae simple, long spatulate. Saccus very small. Aedeagus with cornuti. Sternum A8 simple, without latero-posterior appendages (cerata).

Female genitalia: Ovipositor with additional ventrolateral ovipositor-lobes. Apophyses fine, comparatively short. Ductus bursae very short. Corpus bursae with posterior part strongly sclerotized. Signum absent.

Synapomorphies: Female frenulum; hindwing anastomosis (Sc, Rs+M1).

Differential diagnosis: Genitalic features (male: uncus, valvae, saccus, cornuti, absence of appendages from sternum A8; female: ovipositor-lobes, sclerotisation of corpus bursae, absence of signum) clearly indicating a position in the tribe Sterrhini. The structure of female frenulum is unique in Geometridae and allows separation from *Idaea*. An isolated lineage of genus *Eueupithecia* with position between Cyllopodini and Semaeopus resulting from COI NJ analysis of neotropical Sterrhinae, but when excluding the (variable) third codon position, the genus falls within the clusters of the tribe Sterrhini. Tympanum is typical for Sterrhinae. The long hindwing anastomosis an extremely rare character in Sterrhinae (but characteristic for Larentiinae). The asymmetric position of hindwing median veins also unusual for Sterrhinae (characteristic for Geometrinae). The eremic species *Idaea volloni* in external appearance and in the long anastomosis of hindwing veins Sc and Rs+M1 (very unusual in Sterrhinae) very similar to *Eueupithecia*, but female frenulum developed as a brush of setae and genitalia of both sexes completely different. The great external similarity, therefore, is probably just a convergence.

Remarks: Both the long vein-anastomosis in the hindwing and the modified female frenulum may be an advantage for wing stability and flight in moths with long and narrow wings.

2.3 Brief biology of the agent

Data was collected while rearing the agent in the quarantine facilities in Brisbane Australia. Colonies of the agent were held in controlled environment chambers at temperatures of 27±1°C day and 23±1°C night; 70±5% relative humidity, with a 14:10 L:D photoperiod. The duration of the egg, larval and pupal stage was recorded. Newly hatched larvae were reared on potted plants of *P. aculeata*.

Brown or green cylindrical eggs, approximately 0.3 mm in length, are usually laid individually or in strings (Figure 8). The eggs hatch and larvae begin to feed about 5 days after eggs were laid. Body colour of larvae ranges from green (Figure 9) to brown (Figure 10). The larvae mimic leaf rachises and young shoots. As larvae develop, they eat most of the pinnules and rasp the surfaces of the rachises. The reduced number of prolegs results in the larvae progressing with a looping motion, hence the common name “loopers”.

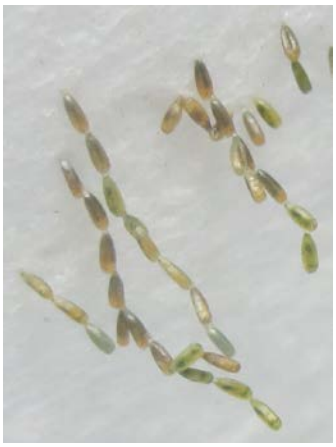

Figure 8. Strings of eggs of *Eueupithecia* sp.2 laid on paper

Figure 9. Green larva of *Eueupithecia* sp.2 on *Parkinsonia aculeata* leaf

Figure 10. Brown larva of Eueupithecia sp.2 on *Parkinsonia aculeata* leaf

Life stage duration. The average duration of egg incubation was 5 days (n=19, range 3-7 days). The number of instars of *Eueupithecia* sp.2 was not determined but is probably four as *E. cisplatensis* undergoes four larval instars. Adults begin to emerge an average of 18 days from egg hatch (n=19, range 16-20 days). The majority of emergence occurs within the first few days and continue for as long as 37 days. A tendency to enter diapause in the pupal stage was noticed when day length decreased. Preoviposition period was two days (n=19, range 1-4 days).

Adult females are bigger than males, with a wider abdomen (Figure 4). The morphology of the antennae also shows sexual dimorphism: pectinate in the male and simple in the female.

Natural enemies. Two species of *Conura* (Hymenoptera: Chalcidoidea) emerged from cocoons of larvae collected in the native range.

2.4 Native range of the agent

Known from field surveys from Argentina only (Figure 7) but probably also occurs in neighbouring Chaco areas in Paraguay, Bolivia and Brazil.

2.5 Related species to the agent and a summary of their host range

The genus *Eueupithecia* has only two known species *E. cisplatensis*, and *Eueupithecia* sp. 2. The later, the subject of this submission has yet to be described but is well diagnosed. A study of the biology and host specificity of *E. cisplatensis* showed that it is a specialist on *P. aculeata*. It is unknown which of the 18 genera in the tribe Sterrhini are closest to *Eueupithecia* (A. Hausmann, pers. comm.), so we are not in a position to summarize the host range of the related species. Preliminary analysis shows that the 825 species distributed in 18 genera in the tribe Sterrhini show a broad spectrum of host specificity, from extreme specialists to generalists.

2.6 The proposed source of the agent

Fernando Mc Kay, Scientist at FUEDEI (Fundación para el Estudio de Especies Invasivas) is the local contact in Argentina. His details follow. Website: www.fuedei.org. Address: Bolívar 1559 (B1686EFA), Hurlingham, Buenos Aires, Argentina. Tel: 54-11-4662-0999 (ext. 107). Email: fmckay@fuedei.org.

The imported material was collected by F. Mc Kay and T. Heard at a mix of locations in the Argentinean provinces of Chaco and Formosa. A shipment of approximately 200 larvae and pupae was hand carried into Australia by T. Heard, on 2012-02-19, under the following permits: AQIS IP11020310, SEWPaC permit WT2011-5601, AQIS order reference no NA12020352. A colony was established which providing individuals for host specificity testing.

Colonies of the genetic material from Argentina that has been tested in Australian quarantine will be maintained and released if permission is granted. The addition of fresh genetic material from Argentina will be incorporated into this colony.

2.7 Possible interactions with existing biological control programs (of same or related targets and other targets)

Three insect species have been released in Australia for biocontrol of *P. aculeata*. But only the seed-feeding bruchid *Penthobruchus germaini* established and dispersed readily. However, seed consumption rates can be high but on average are relatively low and the agent is therefore unlikely to be causing any population-level impacts. Existing agents therefore do not appear to be having a significant impact. The proposed agents feed on vegetation tissue and therefore it is unlikely that they will interact with the existing agent. *Eueupithecia cisplatensis* is being released in 2013. These two species of *Eueupithecia* will potentially interact as they utilise the same resource. However in Argentina, the geographic range of the two species is separate. It is possible that they will occupy different climatic zones in Australia too. In this way, they will complement each other with U2 likely to do best in hotter climates across northern Australia and UU in wetter milder climates, for example in coastal Queensland.

2.8 The agent's potential for control of target

Leaf feeding by larvae reduces the total photosynthetic area of the plant causing reduction in vigour, growth rate and seed production. In the laboratory the larvae are voracious feeders and completely strip all foliage from plants. As the leaves of *P. aculeata* are undamaged in Australia, the potential for impact on the plant is great.

Geometrids have been used successfully in weed biocontrol programs. *Comostolopsis germana* damages shoot tips of bitou bush, *Chrysanthemoides monolifera,* in Australia (Adair and Scott 1989; Adair and Edwards 1996). It is widely established and causes obvious damage to bitou bush. *Aplocera plagiata* established on St John's wort (*Hypericum perforatum*) in Canada and USA but not in Australia (Julien and Griffiths 1998). The Geometridae *Chiasmia inconspicua* and *Chiasmia assimilis* from Kenya, were released in 2000 for biocontrol of *Acacia nilotica* in Queensland. *Chiasmia assimilis* is showing signs of damage to its host in coastal areas of Queensland - particularly the Bowen/Ayr region and is completely defoliating some plants which may lead to reduced flowering and pod production. *Macaria pallidata* and *Leuciris fimbriaria* were released in Australia for control of *Mimosa pigra*. Both have established and *Macaria pallidata* is inflicting heavy damage on the target plant (Heard et al. 2010).

The climatic match between the range of *Eueupithecia* sp.2 and the areas of Australia where *P. aculeata* is most heavily infested is good. The Emerald area of central Queensland is heavily infested

(Figure 3). The climate of Emerald is closely matched to northwest Argentina where this agent was sourced (Figure 11).

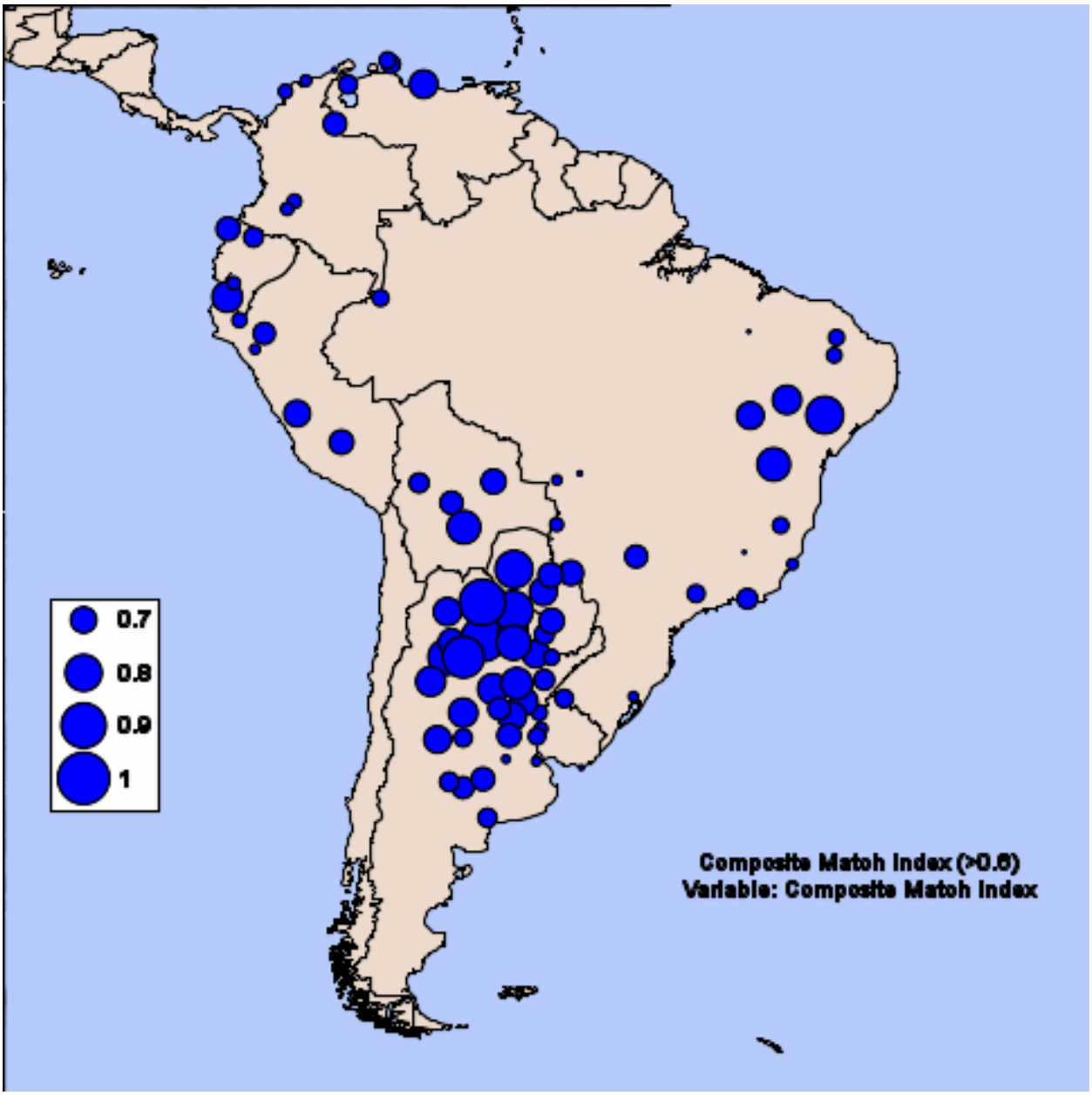

Figure 11. A climate match of South America with Emerald, Queensland, Australia, generated by the computer program Climex

2.9 Details on the quarantine facility and methods of containment

All Australian research was done in the Queensland EcoSciences Precinct QC3 Quarantine Facility for Containment of Arthropod and Pathogen Agents for Weed Biocontrol, situated at the EcoSciences Precinct, 41 Boggo Rd, Dutton Park, Brisbane, 4102. This is an AQIS approved facility, QAP No: Q2275, QC level: 5.3 and QIC level 7.3. Precautions include double glazing of glasshouses, HEPA air filtering, negative air pressure, filtering and heat treatment of liquid waste, air lock entrances, autoclaving or fumigation of solid waste.

All staff are experienced quarantine operators who strictly follow AQIS approved guide-lines. A Standard Operating Procedures document for the facility is available upon request. All staff wear overalls, hairnets and booties when entering the laboratories which they remove before leaving the building. Insects are transported to the facility in sealed containers. Containers are unpacked in a specially designed unpacking room. Insects are held in cages in the laboratories, glasshouses or controlled environment rooms. Changes to new containers are done inside a walk-in cage. Method of disposal and treatment of refuse and packaging is by autoclaving or fumigation.

2.10 Where, when and how initial release will be made

2.10.1 RELEASE FROM QUARANTINE

Eueupithecia sp.2 is currently being cultured within the quarantine facility at the EcoSciences Precinct. Once approval for release is obtained from DAFF and SEWPaC, adults from this culture will be removed from the quarantine after careful inspection to confirm identity and to ensure that no other associated organism such as parasite or pathogen is taken from the quarantine. All requirements imposed by AQIS on the release permit will be followed. Once removed from quarantine, the insects will be placed on *P. aculeata* in non-quarantine glasshouses to initiate a mass-rearing phase.

The following procedure was developed with Tony Robinson, Senior Entomologist, Department of Agriculture, Fisheries and Forestry, for the release of *Eueupithecia cisplatensis*. And a similar one is expected to be used for *Eueupithecia* sp.2.

1. Colonies of *Eueupithecia cisplatensis* segregated from *Eueupithecia* sp.2 in separate glasshouses, laboratories and CT rooms.

2. Maintenance of healthy populations free from parasites (e.g. mites) and pathogens.

3. Confirmation identifications carried out of separate colonies (genitalia dissections) to ensure correct segregation.

4. Prior to work in general laboratory ensure bench, shelf for storing culture containers and surrounding areas are free from unnecessary equipment and stock then swab down with 80% v/v ethanol.

5. Plastic containers that have been disinfected with chlorine are stored in sealed plastic bags within the facility prior to use.

6. New paper towel for use in the culture containers are stored in sealed plastic bags within the facility prior to use.

7. Adult progeny of original *E. cisplatensis* import transferred to clean and disinfected (chlorine solution) plastic takeaway containers with clean paper towel and held in General Laboratory 3 (UU) pending laying of eggs.

8. Container lids have hole cut in middle but are snapped shut over paper towel to ensure integrity of container.

9. When eggs have been laid on the paper towel the adults are removed from the culture containers into vials and placed in the lab freezer for subsequent pinning or placed in ethanol (bench and surrounds are again wiped down with ethanol prior to work).

10. During removal of adults the culture containers are inspected visually and under magnification to ensure no evidence of mites, fungal pathogens or any other contamination is present.

11. The lidded culture containers with only paper towel and eggs present are then placed into a sealed plastic bag. The exterior of the plastic bag is swabbed with 80% v/v ethanol and immediately removed from the quarantine facility.

12. The bag with culture containers is then taken directly to equipment room 4 in the level 3 laboratory (room 3.C.402). The culture containers are removed from the sealed bag and placed in a separate labelled tub on the bench pending hatching of the eggs.

13. When the larvae have emerged the containers are carried directly to CSIRO Tropical Weed Greenhouse.

14. The larvae are hand transferred to Parkinsonia plants in primary cages with the glasshouse.

15. All paper towel is then placed in an autoclave bag for sterilisation in the external autoclave (There was an out of date sticker on this unit, this autoclave is not part of the quarantine facility however it is recommended that it be serviced and calibrated annually).

16. All culture containers are then disinfected with a chlorine solution.

Future releases of this colony can be carried out using this process without DAFF supervision but as discussed we do require a quick notification of each separate release via email.

Should the culture be lost before approvals are granted or any detrimental signs appear as a result of genetic bottlenecks, the insect will be recollected in Argentina and reared through at least one generation in quarantine before being released.

Voucher specimens will be submitted to AQIS and ANIC.

2.10.2 DISTRIBUTING IN THE FIELD

Eueupithecia sp.2 will be distributed to selected sites throughout the weed's range in Australia. Release sites will be recorded with their GPS coordinates. It is expected that state and territory government departments, community groups such as Landcare, Bushcare and schools may contribute to this distribution. Senior representatives of the Queensland government and the Northern Territory government have already expressed interest in participating in release activities. CSIRO will provide "How to" manuals and starter colonies to interested parties.

2.11 Establishment and evaluation

Release sites will be monitored for some years after releases to ascertain whether the insect has established. Should the insect be found to have established, assessments will be made on its effects on the weed.

2.12 Information and results of any other assessments undertaken on the species

None known. This is the first time that this insect has been assessed for biocontrol or any other purpose.

2.13 Non-target organisms at risk

Our thorough host specificity testing (see below), predicts that no non-target plant species are at risk because the host range of *Eueupithecia* sp.2 is confined to *P. aculeata*.

2.14 Report on host specificity testing

2.14.1 INTRODUCTION

The host specificity of *Eueupithecia* sp.2 was tested using three methods: 1 Surveys of plant use under natural condition in the native range; 2 Tests of early larval development on cut plant material in Australia and Argentina; and 3 Tests of full larval development on living plant species in Australian quarantine.

Excluding *P. aculeata*, a total of 65 plant species were tested, 42 in the laboratory in Australia, 20 in the laboratory in Argentina and five in the field in Argentina. Two *Acacia* species, were common to the laboratory and field tests in Argentina explaining why the sum of species tested is 65 and not 67.

All tests delivered the same result: complete specificity to one plant species, *P. aculeata*. Each of these tests is considered separately below. But first we discuss the list of test plants.

2.14.2 THE TEST PLANT LIST

The test plant list consists of 65 species from the legume family, in addition to *P. aculeata*. The list was compiled according to the modern methods, primarily using degrees of phylogenetic separation, based on published phylogenies (Bruneau et al. 2008, and references therein). This is discussed further below and presented in Table 2. This list is very similar to that for the *E. cisplatensis*, except that there are two species fewer, and several substitutions without substantial change to the representation.

- The genus *Parkinsonia: Parkinsonia aculeata* is the only *Parkinsonia* species known to have naturalized in Australia and so no other species could be tested. Note, however, that *Parkinsonia praecox* was available in Argentina and was assessed there.
- The group *Peltophorum* is a strongly supported monophyletic group that includes *Peltophorum, Parkinsonia*, *Delonix*, *Colvillea* and *Schizolobium* (Haston et al. 2005). The only member of the *Peltophorum* group native to Australia is *Peltophorum pterocarpum* which was tested. Also ornamental members of the group that are exotic to Australia were tested to help define the host range, including *Colvillea racemosa, Schizolobium parahybum* and *Delonix regia*.
- The tribe Caesalpinieae is represented in Australia by *Gleditsia, Caesalpinia, Haematoxylum* and *Erythropleum* and the genera in the Peltophorum group mentioned in the previous dot point. *Erythropleum chlorostachys* was tested. There are several native *Caesalpinia* species which could not be obtained and so were replaced by *Caesalpinia pulcherrima* and *Caesalpinia ferrea*. The genus *Gleditsia* is represented in Australia only by the exotic *Gleditsia triacanthos* which was tested. The genus *Haematoxylum* is represented in Australia by the exotic *Haematoxylum campechianum*, which could not be obtained.
- The subfamily Caesalpinioideae. In addition to the tribe Caesalpinieae (above), members of the tribes Cassieae, Cercideae and Detarieae occur in Australia. Representatives of all these groups were included on the test list (Table 2).
- Fourteen species representing eleven of the tribes of the subfamily Papilionoideae were included.
- Nineteen species representing the three tribes of the subfamily Mimosoideae were tested. This subfamily contains the large genus *Acacia*. All of the sections of this important genus were represented (Table 2) except Lycopodiifoliae which are very difficult to obtain and grow in cultivation.
- The legume family belongs to the Order Fabales. Traditionally this order contained only the Leguminosae, considered an isolated family. However a novel hypothesis in which the order

Fabales contains also the families Quillajaceae, Surianaceae and Polygalaceae is emerging from recent molecular phylogenies (Stevens 2001 onwards). There is scant morphological support for these relationships (Bello et al. 2009). The Quillajaceae are a small family known only from temperate South America. Surianaceae is mostly Australian with two species of *Cadellia,* one species of *Guilfoylia,* one species of *Suriana* and three *Stylobasium* species. Polygalaceae contains several species of *Comesperma*, *Polygala* and *Salomonia*. Due to the high specificity of the insect being tested, the doubts over the relationships and the lack of morphological similarity, we did not include any non-legume species on the list.

Table 2. The complete list of plant species tested for host specificity in the field in Argentina and in Australian quarantine

Subfamily	Tribe	Group	Section	Genus/species	Tested
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia aculeata*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia aculeata*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia praecox*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Colvillea racemosa*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Delonix regia*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Peltophorum pterocarpum*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Peltophorum dubium*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Schizolobium parahybum*	Australia
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia ferrea*	Australia
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia pulcherrima*	Australia
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia gilliesi*	Argentina
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia paraguayiensis*	Argentina
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Pterogyne nitens*	Argentina
Caesalpinioideae	Caesalpinieae	Dimorphandra		*Erythrophleum chlorostachys*	Australia
Caesalpinioideae	Caesalpinieae	Umtiza		*Gleditsia triacanthos*	Argentina
Caesalpinioideae	Caesalpinieae	Umtiza		*Gleditsia amorphoides*	Argentina
Caesalpinioideae	Cassieae			*Cassia brewsteri*	Australia
Caesalpinioideae	Cassieae			*Ceratonia siliqua*	Australia
Caesalpinioideae	Cassieae			*Chaemacrista mimosoides*	Australia
Caesalpinioideae	Cassieae			*Chaemacrista nomane*	Australia
Caesalpinioideae	Cassieae			*Labichea lanceolata*	Australia
Caesalpinioideae	Cassieae			*Petalostylis labicheoides*	Australia
Caesalpinioideae	Cassieae			*Senna artemisioides*	Australia
Caesalpinioideae	Cassieae			*Senna glutinosa*	Australia
Caesalpinioideae	Cassieae			*Senna corymbosa*	Argentina
Caesalpinioideae	Cassieae			*Senna spectabilis*	Argentina
Caesalpinioideae	Cassieae			*Senna notabilis*	Australia
Caesalpinioideae	Cercideae			*Barklya syringifolia*	Australia
Caesalpinioideae	Cercideae			*Bauhinia hookeri*	Australia

Caesalpinioideae	Cercideae		*Bauhinia forficata*	Argentina
Caesalpinioideae	Detarieae		*Cynometra ramiflora*	Australia
Caesalpinioideae	Detarieae		*Intsia bijuga*	Australia
Caesalpinioideae	Detarieae		*Maniltoa lenticillata*	Australia
Caesalpinioideae	Detarieae		*Schotia brachypetala*	Australia
Caesalpinioideae	Detarieae		*Tamarindus indica*	Australia
Papilionoideae	Aeschynomeneae		*Aeschynomene americana*	Australia
Papilionoideae	Bossiaeeae		*Hovea acutifolia*	Australia
Papilionoideae	Dalbergiae		*Geoffroea decorticans*	Argentina
Papilionoideae	Dalbergiae		*Tipuana tipu*	Argentina
Papilionoideae	Desmodieae		*Desmodium tortuosum*	Australia
Papilionoideae	Mirbelieae		*Pultenaea villosa*	Australia
Papilionoideae	Phaseoleae		*Cajanus cajan*	Australia
Papilionoideae	Phaseoleae		*Erythrina crista-galli*	Argentina
Papilionoideae	Phaseoleae		*Wisteria sinensis*	Argentina
Papilionoideae	Robinieae		*Sesbania cannabina*	Australia
Papilionoideae	Robinieae		*Sesbania virgata*	Argentina
Papilionoideae	Tephrosieae		*Millettia (=Pongamia) sp. McIlwraith*	Australia
Papilionoideae	Tephrosieae		*Lonchocarpus nitidus*	Argentina
Papilionoideae	Vicieae		*Vicia faba*	Australia
Mimosoideae	Acaciae	Acacia	*Acacia aroma*	Argentina
Mimosoideae	Acaciae	Acacia	*Acacia caven*	Argentina
Mimosoideae	Acaciae	Acacia	*Acacia visco*	Argentina
Mimosoideae	Acaciae	Acacia	*Acacia bidwillii*	Australia
Mimosoideae	Acaciae	Botrycephalae	*Acacia decurrens*	Australia
Mimosoideae	Acaciae	Botrycephalae	*Acacia oshanesii*	Australia
Mimosoideae	Acaciae	Juliflorae	*Acacia disparrima*	Australia
Mimosoideae	Acaciae	Plurinerves	*Acacia melanoxylon*	Australia
Mimosoideae	Acaciae	Botrycephalae	*Acacia oshanesii*	Australia
Mimosoideae	Acaciae	Phyllodineae	*Acacia salicina*	Australia
Mimosoideae	Ingeae		*Archidendron lucyi*	Australia
Mimosoideae	Ingeae		*Pararchidendron pruinosum*	Australia
Mimosoideae	Ingeae		*Enterolobium contortisiliquum*	Argentina
Mimosoideae	Mimoseae		*Dichrostachys cinerea*	Australia
Mimosoideae	Mimoseae		*Leucaena leucocephala*	Australia
Mimosoideae	Mimoseae		*Prosopis ruscifolia*	Argentina
Mimosoideae	Mimoseae		*Prosopis alba*	Argentina
Mimosoideae	Mimoseae		*Anadenanthera colubrina*	Argentina

2.14.3 SURVEYS OF PLANT USE UNDER NATURAL CONDITION IN THE NATIVE RANGE

On three field trips to northern Argentina, over the summers of 2009/10, 2010/11 and 2012/13, four sites in the provinces of Formosa, Salta and Chaco with populations of *P. aculeata* and co-occurring legume species were sampled for presence of insects by beating foliage over a one square metre sheet (Figure 12). Each beats was done on a separate sheet to the previous one. Immature insects were held in plastic containers and provided fresh *P. aculeata* leaves until the emergence of adults for identification. Voucher specimens of plants and insects collected are maintained at the FuEDEI laboratory.

Along the four sites visited, a total of 123 larvae of *Eueupithecia* sp.2 were collected on *P. aculeata* and reared to adult. No *Eueupithecia* sp.2 larvae were collected on any of the other five surveyed legume species (Table 3). It is particularly instructive that *Eueupithecia* sp.2 was not found even on the conspecific *Parkinsonia praecox*. At the same sites, *Eueupithecia* sp.2 was consistently collected on *P. aculeata*. In addition, larvae of *Melipotis acontioides* (Guenee) (Lepidoptera: Noctuidae) and *Macaria* sp. (Lepidoptera: Geometridae) were collected but could be readily distinguished from *Eueupithecia* sp.2.

Figure 12. FuEDEI researcher Fernando Mc Kay beating *P. aculeata* plants in northern Argentina

Table 3. Number of *Eueupithecia* sp.2 and other Lepidoptera on various legume plants species, arranged in order of species, from surveys of plant use under natural condition in the native range in Argentina

Date	Locality	Province	Surveyed plant species	Plants sampled	*Eueupithecia sp.2*
2010-03-20	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia aculeata*	10	24
2010-09-26	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia aculeata*	15	2
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Parkinsonia aculeata*	10	35
2013-03-08	RN° 81, 8 km W Cmte Fontana	Formosa	*Parkinsonia aculeata*	25	62
2010-03-19	RN° 81, 8 km S Pozo d Mortero	Formosa	*Parkinsonia praecox*	10	0
2010-03-20	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia praecox*	3	0

2010-09-26	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia praecox*	10	0
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Prosopis ruscifolia*	10	0
2013-03-08	RN° 81, 8 km W Cmte Fontana	Formosa	*Prosopis ruscifolia*	10	0
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Acacia caven*	10	0
2013-03-08	RN° 81, 8 km W Cmte Fontana	Formosa	*Acacia aroma*	10	0
2013-03-08	RN° 81, 8 km W Cmte Fontana	Formosa	*Geoffroea decorticans*	10	0

2.14.4 TESTS OF LARVAL DEVELOPMENT ON CUT PLANT MATERIAL

Larval survival was evaluated in laboratory no-choice trials on species of Leguminosae in the subfamilies Caesalpinioideae, Papilionoideae and Mimosoideae both in Australia (Table 4) and Argentina (Table 5). Initial studies showed that leaves of *P. aculeata* of all ages are suitable for larval development and so no special plant requirements were required concerning leaf age. A total of 42 species were tested in Australia and 20 in Argentina.

To obtain larvae for testing, eggs were collected from the colony and held in a Petri dish until emergence of the neonate larvae. Twelve newly emerged larvae were placed in 15cm petri dishes with moist tissue paper (Figure 13). The larvae were fed freshly excised leaves of the test plant species. Feeding damage and larval stage reached and mortality were recorded at day 5. Four replicates were performed for each plant species. In Argentina the methodology differed slightly. In each replicate, 10 newly emerged larvae were placed in 0.7-liter plastic containers with perforated lids and moist tissue paper. The larvae were fed bouquets of freshly excised leaves with their petioles inserted in small recipients filled with water. The bouquets were replaced every 48-72 hours according to need. Feeding damage and larval mortality counts were recorded daily, until adult emergence.

All larvae were dead on all test plant species by day 5. In contrast, a mean of 75% of larvae survived on the control plant, *P. aculeata* (Table 4). No feeding occurred on any test plant species and hence no damage was observed on non-target species.

Figure 13.Test of larval development on cut plant material

Table 4. Result of host specificity testing in Australian quarantine including the early larval development test in petri dishes and the entire larval development test on whole living plants. The plants are arranged in alphabetical order. For phylogenetic relationships, see Table 2.

	Cut plant in Petri dish		Living plant in cage		
Plant species	**No. Replicates**	**%Survival to 5 days**	**No. Replicates**	**%Survival to adult**	**Total plant replicates**
Parkinsonia aculeata	**28**	**75 (33-100)**	**12**	**51 (16-76)**	**40**
Acacia bidwillii	4	0			4
Acacia decurrens	4	0			4
Acacia disparrima	4	0	2	0	6
Acacia fimbriata	4	0			4
Acacia melanoxylon	4	0	2	0	6
Acacia oshanesii	4	0	2	0	6
Acacia salicina	4	0			4
Aeschynomene americana	4	0	2	0	6
Archidendron lucyi	4	0			4
Barklya syringifolia	4	0	2	0	6
Bauhinia hookeri	4	0			4
Caesalpinia ferrea	4	0			4
Caesalpinia pulcherima	4	0	2	0	6
Cajanus cajan	4	0	2	0	6
Cassia brewsteri	4	0			4
Ceratonia siliqua	4	0	2	0	6
Chamaecrista mimosoides	4	0			4
Chamaecrista nomane	4	0			4
Colvillea racemosa	4	0	2	0	6
Cynometra ramiflora	4	0			4
Delonix regia	4	0			4
Desmodium tortuosum	4	0			4
Dichrostachys cinerea	4	0	2	0	6
Erythrophleum chlorostachys	4	0	2	0	6
Hovea acutifolia	4	0			4
Intsia bijuga	4	0	2	0	6
Labichea lanceolata	4	0			4
Leucaena leucocephala	5	0	2	0	7
Maniltoa lenticillata	4	0	2	0	6
Millettia sp. *McIlwraith*	4	0	2	0	6
Pararchidendron pruinosum	4	0	2	0	6
Peltophorum pterocarpum	4	0			4
Petalostylis labicheoides	4	0			4
Pultenaea villosa	4	0	2	0	6
Schizolobium parahybum	4	0	2	0	6
Schotia brachypetala	4	0	2	0	6
Senna artemisioides	4	0	2	0	6
Senna glutinosa	4	0			4
Senna notabilis	4	0			4
Sesbania formasa	4	0			4
Tamarindus indica	4	0	2	0	6
Vicia faba	4	0			4

Table 5. Result of host specificity testing in Argentina including the early larval development test. The plants are arranged in alphabetical order. For phylogenetic relationships, see Table 2.

Plant species	No. Replicates	%Survival to pupae	%Survival to adult
Parkinsonia aculeata	**11**	**76**	**62**
Acacia aroma	3	0	0
Acacia caven	2	0	0
Acacia visco	5	0	0
Anadenanthera colubrina var. *cebil*	6	0	0
Bauhinia forficata	4	0	0
Caesalpinia gilliesii	4	0	0
Caesalpinia paraguariensis	10	0	0
Enterolobium contortisiliquum	10	0	0
Erythrina crista-galli	10	0	0
Gleditsia amorphoides	3	0	0
Gleditsia triacanthos	10	0	0
Lonchocarpus nitidus	10	0	0
Peltophorum dubium	4	0	0
Prosopis alba	5	0	0
Pterogine nitens	10	0	0
Senna corymbosa	4	0	0
Senna spectabilis	4	0	0
Sesbania virgata	4	0	0
Tipuana tipu	10	0	0
Wisteria sinensis	4	0	0

2.14.5 TESTS OF LARVAL DEVELOPMENT ON LIVING PLANTS

Survival of larvae to adult in whole living plants was evaluated in the laboratory using no-choice trials on 21 species of Leguminosae (Table 4). This trial complemented the previous trial; living plants give a more realistic result than cut plants but the cut plant trial allowed the observation of individual mortality. Fifty neonate larvae were counted and placed on the foliage of an individual test plant species growing in a pot. The plants were held for larval development in an aluminium frame cage lined with gauze and measuring approximately 250 x 250 x 800 mm. The cages were kept in quarantine glasshouses to allow plants to maintain good condition (Figure 14). When day lengths decreased, trials were conducted in quarantine controlled environment rooms under artificial lighting (Figure 15). Plants were monitored regularly and extra plants of the same species were added if the larval feeding depleted the original plant (this only occurred on the control plant). Plants were held for an average of 47 days (range 28 to 69 days), by which time all adults had emerged from the *P. aculeata* control plant, confirmed by checking that all pupal cases were empty.

One *P. aculeata* control plant and two to six test species, depending on the availability of larvae, were used in each trial. The inclusion of a *P. aculeata* control plant in each trial ensured that the larvae and other

conditions were suitable for development to adult. A total of 18 trials were done to complete the tests. Of these, six trials were invalid due to poor larval development on the control plant as a result of poor plant quality. These trials were repeated. For each plant species, different individual plants were used for each replicate throughout all trials. Only two replicates were done as these species were already tested on cut plants in petri dish trials.

Eueupithecia sp.2 larvae failed to develop on any plant species other than *P. aculeata* (Table 4). No feeding or damage was observed on any non-target test plant species.

Figure 14. Andrew White transferring newly hatched larvae of *Eueupithecia* sp.2 onto a plant during no-choice tests in an Australian quarantine glasshouse

Figure 15. Andrew White transferring newly hatched larvae of *Eueupithecia* sp.2 onto a plant during no-choice tests in an Australian quarantine controlled environment room

2.14.6 DISCUSSION AND CONCLUSION OF HOST SPECIFICITY TESTS

Three methods were applied to evaluate the specificity of *Eueupithecia* sp. 2. All delivered the same result: total specificity to one plant species, *P. aculeata*. The methods used differed, but complemented and supported each other. The field survey in the native range could only be done on a small number of legume species that could be found coexisting with *P. aculeata*. But this method had the advantage of showing the natural host plant use. Even the closely related *Parkinsonia praecox* was not found to be used by *Eueupithecia* sp. 2. in the field in the native range.

The two laboratory tests had the common element that they assessed the larval developmental host range. That is, they evaluated the suitability and acceptability of the test plant species for feeding, growth and progression of larvae to later developmental stages. The test on early larval development on cut plants in Petri dishes had the advantage that it allowed early instar larvae to be observed directly. It showed that all larvae on non-target tests plants died as first instars. A disadvantage is that the work was done on cut plant material which could potentially be different chemically, nutritionally or physically from living tissue. Hence a further test on living plants was done. This test followed the larvae right through to adult emergence. It did not allow the observation of the fate of the larvae, but it did show that even healthy living test plants cannot support the development of larvae.

Larval development tests are conservative in the sense that it is extremely unlikely to under-estimate the host range (Sheppard et al. 2005). If a larva is behaviourally and physiologically able to feed and grow when placed on a food source, then it will do so. For some insect species, these types of tests over-estimate the host range. That is they feed and develop on food sources upon which they would not in nature. The fact that our larvae died rather than feed on all test plant species except *P. aculeata*, proves, to a very high level of confidence, that this insect species will not feed on or damage any other plants species in the field and hence the risks of damage to non-target plants following its release are extremely low.

References

Adair RJ, Edwards PB. 1996. An attack strategy against *Chrysanthemoides monilifera*, a weed of native vegetation in Australia.Moran VC, Hoffmann JH. Proceedings of the 9th international symposium on biological control of weeds, Stellenbosch, South Africa, 19-26 January 1996.Rondebosch, South Africa: University of Cape Town.

Adair, R.J. and Scott, J.K. 1989. The life-history and host specificity of *Comostolopsis germana* Prout (Lepidoptera: Geometridae), a biological control agent of *Chrysanthemoides monilifera* (Compositae). Bulletin of Entomological Research 79: 649-657.

Bello, M.A., Bruneau, A., Forest, F. and Hawkins J.A. 2009 Elusive relationships within order Fabales: Phylogenetic analyses using matK and rbcL sequence data. Systematic Botany. 34: 102-114.

Briano, J.A., Cordo, H.A. and DeLoach, C.J. (2002). Biology and field observations of *Penthobruchus germaini* (Coleoptera: Bruchidae), a biological control agent for *Parkinsonia aculeata* (Leguminosae: Caesalpinioideae). *Biological Control* 24, 292-9.

Bruneau A; Mercure M; Lewis, GP, Herendeen, PS 2008. Phylogenetic patterns and diversification in the caesalpinioid legumes. Botany 86: 697-718.

Deveze, M. (ed). (2004). 'Parkinsonia national case study manual'. (QNRME: Brisbane).

Diplock, ND, Galea, VJ, van Klinken R, and Wearing, AH (2006). A preliminary investigation of dieback on Parkinsonia aculeata. In: C. Preston and J.H. Watts and N.D. Crossman, 15th Australian Weeds Conference. 15th Australian Weeds Conference, Adelaide Convention Centre, Adelaide, South Australia, (585-587). 24-28 September 2006

Diplock, N, Galea, V and van Klinken, RD (2008). Movement of dieback through a stand of parkinsonia - a time series study. In: van Klinken, R.D., Osten, V.A., Panetta, F.D. and Scanlan, J.C., 16th Australian Weeds Conference Proceedings. 16th Australian Weeds Conference, Cairns, (186-186). 18-22 May 2008 www.16awc.com.au

Donnelly, G.P. (1998). Levels of attack and destruction of *Parkinsonia aculeata* seeds by bruchid biological control agents. Proceedings of the Sixth Australasian Applied Entomological Research Conference Volume 2, eds M.P. Zalucki, R.A.I. Drew and G.G. White, p. 310. (University of Queensland Press, Brisbane)

Donnelly, G.P. (2000). Biology and host specificity of *Rhinocloa callicrates* Herring (Hemiptera: Miridae) and its introduction and establishment as a biological control agent of *Parkinsonia aculeata* L. (Caesalpiniaceae) in Australia. *Australian Journal of Entomology* 39, 89-94.

Doyle, J.J., Chappill, J.A., Bailey, C.D. and Kajita, T. (2000). Towards a comprehensive phylogeny of legumes: analyses of rbcL sequences and a general data set. *In* 'Advances in Legume Systematics', Part 9, eds P.S. Herendeen and A. Bruneau. (Royal Botanic Gardens, Kew).

Everitt, J.H. (1983). Seed germination characteristics of two woody legumes (Retama and Twisted Acacia) from South Texas. *Journal of Range Management* 36, 411-4.

Flanagan, G.J., van Rangelrooy, D.S. and Kerin, S. (1996). Integrated management of Parkinsonia aculeata on the Roper River, Northern Territory, Australia. Proceedings of the IX International Symposium on Biological Control of Weeds, eds V.C. Moran and J.H. Hoffmann, pp. 441-3. (University of Cape Town, Cape Town).

Haston, E.M*.,* Lewis, G.P. and Hawkins J.A. 2005. A phylogenetic reappraisal of the *Peltophorum* group (Caesalpinieae: Leguminosae) based on the chloroplast *trnL-F, rbcL* and *rps16* sequence data. *American Journal of Botany* 2, 1359-71.

Hawkins, J.A. (2001). *Parkinsonia aculeata* (Mexican palo-verde). *In* 'Forestry compendium', (CABI Publishing, Oxon, UK).

Hawkins, J.A., White, L., Hughes, C.E., Contreras-Jiménez, J.L. and Mercado, P. (1999). Investigation and documentation of hybridisation between *Parkinsonia aculeata* and *Cercidium praecox* (Leguminosae: Caesalpinioideae). *Plant Systematics and Evolution* 216, 49-68.

Hawkins, J.A., Boutaoui, N., Cheung, K.Y., van Klinken, R.D. & Hughes, C.E. (2007) Intercontinental dispersal prior to human translocation revealed in a cryptogenic invasive tree. New Phytologist, 175, 575-587.

Heard, T.A. (2006). *Parkinsonia aculeata*: surveys for natural enemies, native range ecological studies and prospects for biological control. Proceedings of the 15th Australian Weeds Conference, eds C. Preston, J.H. Watts and N.D. Crossman, pp. 581-4. (Weed Management Society of South Australia, Adelaide).

Heard, T.A., Elliott, L., Zonneveld, R., Mira, A., Chan, R. and Fichera, G. A. Routley, B., Wirf, L., Burrows, N., Segura, R. (2010) Biology, host specificity, release and establishment of *Macaria pallidata* and *Leuciris fimbriaria* (Lepidoptera: Geometridae) potential biocontrol agents of *Mimosa pigra*. *Biological Control* 55: 248-255.

Herendeen, P.S., Bruneau, A. and Lewis, G.P. (2003). Phylogenetic relationships in the caesalpinioid legumes: a preliminary analysis based on morphological and molecular data. *In* 'Advances in legume systematics, Part 10', eds B.B. Klitgaard and A. Bruneau, pp. 37-62. (Royal Botanic Gardens, Kew).

Hughes, C.E. (1986). Progress in collection and evalution of NFT germplasm from Central America. *Nitrogen Fixing Tree Research Reports* 4, 49-51.

Hughes, C.E. (1989). Intensive study of multipurpose tree genetic resources. Oxford Forestry Institute, UK: Unpublished Report.

Hughes, C.E., and Styles, B.T. (1984). Exploration and seed collection of multiple-purpose dry zone trees in central America. *The International Tree Crops Journal* 3, 1-31.

Humphries, S.E., Groves, R.H. and Mitchell, D.S. (1991). Plant Invasions of Australian Ecosystems: A Status Review and Management Directions. *In* 'Kowari 2' (Australian National Parks and Wildlife Service, Canberra).

Julien, J.H. and Griffiths, M.W. (1998). 'Biological control of weeds: a world catalogue of agents and their target weeds', 4th edition. (CABI Publishing, Wallingford).

Lewis, G.P. Simpson, B.B. and Neff, J.L. (2000). progress in understanding the reproductive biology of the Caesalpinioideae (Leguminosae). *In* 'Advances in legume systematics, part 9', eds P. S. Herendeen and A. Bruneau, pp. 65-78. (Royal Botanic Gardens, Kew).

Lockett, C., Gray, E. and Donnelly, G. (1999). The prevalence of seed damage by the introduced bruchid, *Penthobruchus germaini* (Coleoptera: Bruchidae), a biological control agent for parkinsonia (*Parkinsonia aculeata*), in far north and central Queensland. Proceedings of the 12th Australian Weeds Conference, eds A.C. Bishop, M. Boersma and C.D. Barnes, pp. 431-5. (Tasmanian Weeds Society, Hobart)

MacDicken K.G. and Brewbaker J.L. (1984). Descriptive summaries of economically important nitrogen fixing trees. *Nitrogen Fixing Tree Research Reports* 2, 46-54.

Parsons MS, Scoble MJ, Honey MR, Pitkin LM, Pitkin BR. 1999. The Catalogue. In: Scoble MJ, ed. *Geometrid Moths of the World: a Catalogue (Lepidoptera, Geometridae)*. Collingwood: CSIRO Publishing, 1–1016.

Prout L. B. 1910. On the Geometridae of the Argentine Republic. London Transactions of the Entomological Society Volume: 1910 Pages: 204-345

Polhill, R.M. and Vidal, J.E. (1981). Caesalpineae. *In* 'Advances in legume systematics', Volume 1, ed. R.M. Polhill, P.H. Raven, pp. 81-95. (Royal Botanic Gardens, Kew).

Ross, R.H. (1998). Caesalpiniaceae: *Parkinsonia*. *Flora of Australia* 12, 69-70.

Scoble, M. J. 1999. Geometrid moths of the world : a catalogue (Lepidoptera, Geometridae) CSIRO Publishing, Collingwood, Vic. / edited by Malcolm J. Scoble. **OR** Parsons, M.S.; Scoble, M.J.; Honey, M.R.; Pitkin, L.M.; Pitkin, B.R. 1999. Geometrid moths of the world : a catalogue (Lepidoptera, Geometridae) CSIRO Publishing, Collingwood, Vic.

Sheppard, A.W., van Klinken R.D. and Heard, T. A. (2005) Scientific advances in the analysis of direct risks of weed biological control agents to non-target plants. *Biological Control* 35: 215-226.

Sihvonen, P; Mutanen, M; Kaila, L; Brehm, G; Hausmann, A; Staude, HS 2011. Comprehensive Molecular Sampling Yields a Robust Phylogeny for Geometrid Moths (Lepidoptera: Geometridae). PLOS ONE 6 DOI: 10.1371/journal.pone.0020356.

Sihvonen P; Kaila L (2004) Phylogeny and tribal classification of Sterrhinae with emphasis on delimiting Scopulini (Lepidoptera : Geometridae). Systematic Entomology 29: 324-358.

Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, June 2008 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/.

Stewart, J.L., Dunsdon, A.J., Hellin, J.J. and Hughes, C.E. (1992). Wood biomass estimation of central american dry zone trees. Oxford Forestry Institute Tropical Forestry Papers 26, Oxford.

Thorpe, J.R. and Lynch, R. (2000). 'The Determination of Weeds of National Significance'. (National Weeds Strategy Executive Committee, Launceston).

Toh, R., Galea, V., Diplock, N. and van Klinken, R.D. (2008). Evaluation of fungal isolates for potential use as mycoherbicides for seedbank reduction of Parkinsonia aculeata. Proceedings of the 16th Australian

Weeds Conference, eds R.D. van Klinken, V.A. Osten, F.D. Panetta and J.C. Scanlan, pp. 284-86. (Queensland Weeds Society, Brisbane).

van Klinken, R.D. (2005). Total annual seed loss on a perennial legume through predation by insects: the importance of within-season seed and seed-feeder dynamics. *Austral Ecology* 30, 414-25.

van Klinken, R.D. (2006). Parkinsonia biocontrol: what are we trying to achieve? *Australian Journal of Entomology* 45, 268-71.

van Klinken, R.D. and Flack. L. (2005). The relationship between wet heat and hard-seeded dormancy and germination. *Weed Science* 53, 663-9.

van Klinken, R.D. and Flack, L. (2008). What limits predation rates by a specialist seed feeder? *Journal of Applied Ecology*. In press.

van Klinken R.D., Heard, T.A., (2012) Parkinsonia aculeata. In Biological control of weeds in Australia (Eds. M Julien, R McFadyen and J Cullen), pp. 437-447. CSIRO Publishing, Melbourne.

van Klinken, R.D., Campbell S.D., Heard, T.A. McKenzie J. and March N. (2009a) The Biology of Australian Weeds *Parkinsonia aculeata* L. *Plant Protection Quarterly* (in press).

van Klinken RD, Lawson BE and 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.

Wilson, C.G. and Miller, I.L. (1987). Parkinsonia in the Northern Territory. Northern Territory Department of Primary Industry and Fisheries Technical Bulletin. No. 106.

Woods, W. (1985). Bruchid seed beetles for control of Parkinsonia aculeata in Australia. *In* 'Proceedings of the VI International Symposium on Biological Control of Weeds, ed. E. Delfosse, pp. 855-62. (Agriculture Canada, Ottawa).

Woods, W. (1988). The potential for biological control of Parkinsonia aculeata L.: phytophagous insects collected from the U.S.A., Mexico and Costa Rica and the effect of insect damage on the growth and survival of the plant. Master of Science Thesis. (University of Western Australia, Perth).

Woods, W. (1992). Phytophagous insects collected from Parkinsonia aculeata (Leguminoseae: Caesalpiniaceae) in the Sonoran Desert Region of the south western United States and Mexico. *Entomophaga* 37, 465-74.

Woyciechowski, M.F. (2003) Reconstructing the phylogeny of legumes (Leguminosae): an early 21st centur perspective. *In* 'Advances in legume systematics, Part 10', eds B.B. Klitgaard and A. Bruneau, pp. 5-35. (Royal Botanic Gardens, Kew).

Copies of any references referred to in the application

Copies of the many references cited in this application are available from the author upon request.

CONTACT US

t 1300 363 400
+61 3 9545 2176
e enquiries@csiro.au
w www.csiro.au

YOUR CSIRO

Australia is founding its future on science and innovation. Its national science agency, CSIRO, is a powerhouse of ideas, technologies and skills for building prosperity, growth, health and sustainability. It serves governments, industries, business and communities across the nation.

FOR FURTHER INFORMATION

Dr Tim A. Heard

EcoSciences Precinct, 41 Boggo Rd, Dutton Park,
GPO Box 2583, Brisbane, 4001

Phone: 07 3833 5730

Mobile: 0434 416 053

Fax: 07 3833 5503

Email: tim.heard@csiro.au