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Prepared by: Tim Heard and Rieks van Klinken

CSIRO

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Meat & Livestock Australia Limited

Locked Bag 991

NORTH SYDNEY NSW 2059

Assessment of new biocontrol agents of Parkinsonia

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Abstract

MLA contracted CSIRO (2010-2013) to assess potential new biocontrol agents of parkinsonia, a serious weed of the northern beef industry. The objectives were to select the top three species and conclusively determine whether they are suitable for release and to submit release applications. We exceeded these objectives by determining the suitability of the ten previously-identified highest priority agents. Six were rejected based entirely onnative range work and five (including a new species) were assessed further in an Australian quarantine facility. Of the latter two were shown to be suitable biocontrol agents, both looper caterpillars (family Geometridae). The first of these, *Eueupithecia cisplatensis,* has been approved for release and the first releases have been made in northern Australia; it is too early to know the results of these releases. The second species, *Eueupithecia* sp. 2, is the subject of a pending release application which is expected to be approved. Of the remaining three species, one was not host-specific and two couldn't be cultured. No further biocontrol agents of parkinsonia are known, so it is imperative to maximise the establishment and impact of these two with a thorough release project. An application for funding for this project is pending with MLA.

Executive summary

MLA contracted CSIRO, from 2010 to 2013, to assess biological control agents of parkinsonia (*Parkinsonia aculeata*) to determine if they were likely to be environmentally safe and effective as control agents. Parkinsonia has been identified as one of the most serious weeds of the northern beef industry and also as a high priority candidate for biocontrol. This project was based on a previous MLA funded phase I project which identified the ten most likely candidates following preliminary assessment in the native range. The purpose of this phase II project was to determine if the top three agents were sufficiently specific and safe to release in Australia and to apply for the relase of those that were. Previous evidence suggested that at least one of these agents would be suitable. Hence this project was expected to result in release of at least one agent to assist in the management of this weed. Biocontrol agents can control weeds by damaging their structures and reducing growth rates, survival rates, reproductive output, rates of spread and competitiveness.

We exceeded these objectives by determining the suitability of all ten highest priority agents, and an eleventh species that was discovered during the course of this study. Over the three years of this project, six were excluded from further consideration following work in the native range and five species were imported into Australian quarantine for more intensive assessment. All five species studied only in the native range were eliminated on the grounds of being too rare to work with, not adequately damaging, not being sufficiently specific or being too difficult to breed or test. Two of the five quarantine-tested insects could not be satisfactorily tested as they could not be reared even on the target weed, and a third one failed testing as it was not adequately specific.

The remaining two species tested in Australian quarantine were deemed acceptable for release on the basis that they were both damaging and specific to the target plant. In laboratory tests, full development of egg to adult occurred consistently on parkinsonia with a high rate of success (average of 50%). But no development occurred on any of the 60-plus test plant species. All larvae died in the first stage of their development. No feeding occurred on any test plant species and hence no damage was observed on non-target species. We concluded that the level of risk associated with releasing both agents into the Australian environment was acceptable and that they will potentially be effective biological control agents for parkinsonia. Following this we sought permission for their release in Australia.

Both of these agents are looper caterpillars of the family Geometridae. The first of these loopers, *Eueupithecia cisplatensis*, nicknamed UU, has been approved for release and preliminary test releases have been made in Northern Australia. It is too early to know the results of the releases. The second agent, the related *Eueupithecia* sp.2, nicknamed U2, is the subject of a pending application for release. Given that U2 is as specific as UU, it is expected to be approved. The larvae of both agents feed on leaves of their host plant. Leaf feeding by larvae reduces the total photosynthetic area of the plant causing a 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 parkinsonia are undamaged in Australia, the potential for impact on the plant is great.

No further biocontrol agents of parkinsonia are known, so it is imperative to maximise the establishment and impact of these two with a thorough release project. An application for funding to the WA Cattle Industry Funding Scheme for the release and evaluation of the parkinsonia loopers in Western Australia was successful and that project has commenced with releases being made in the Kimberley and Pilbara regions in May 2013. An initial release has also been made in Queensland in April 2013. A colony has been provided to Queensland and Northern Territory colleagues so that these agencies are ready to contribute to the future national release project for which funding has been sought from MLA. The state and territory collaborators will collaborate in future rearing and release efforts. As field populations of parkinsonia in Australia are patchy and disjunct, a large effort will need to be made to achieve extensive establishment. The proposed national-scale field trial will help ensure that this is achieved efficiently and effectively by optimising release-sizes, stages (adults, larvae or eggs), timing and locations. Once establishment is achieved, populations are expected to be self-sustaining, but ongoing evaluation will be required to quantify the success of biocontrol and to identify any ways that impacts can be further improved.

In addition to achieving all its milestones, this project and its predecessor generated a long list of publications of both an applied and basic nature that contribute to the practise of biological control and will make it more efficient and effective in the future.

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1 Background

MLA has identified the useful role of biological control in the management of weeds that impact on the northern beef industry. MLA has also prioritised parkinsonia (*Parkinsonia aculeata*) as one of the most serious of these weeds and with the best prospect for biocontrol (Grice 2002). Three agents have previously been released on parkinsonia in Australia but with little effect. In 2006, MLA has made a call for projects on weeds of northern Australian with a biocontrol focus. A proposal entitled "Development of new biocontrol agents of bellyache bush and Parkinsonia" was developed by CSIRO and was accepted for funding. This was the phase I project (B.NBP.0366). The second phase is the project reported here (B.NBP.0620).

The phase I project resulted in comprehensive surveying of natural enemies on parkinsonia across its entire native range. Previously, a significant amount of survey work had been done in Central America looking for potential biocontrol agents but the species resulting from those surveys had not been identified or prioritised. The MLA funded project surveyed new areas of South America, identified the entire insect fauna and assessed their potential for release in Australia. It identified the ten insect species that offered the greatest potential as biological control agents in Australia.

Phase II commenced in April 2010 and was focussed on the next stage of biocontrol research, namely the assessment of the highest priority agents for release in Australia. We expect that these species, once released, will become important tools in the management of parkinsonia populations and will help to ameliorate the negative effects of this weed on animal production.

2 Project objectives

By completion of the project on 30 July 2013, CSIRO will have:

1. Collected and assembled all the information necessary to determine whether the three highest priority agents are sufficiently specific to be safe for release in Australia.

2. For each of the three shortlisted agents that prove to be sufficiently specific and effective, develop and submit a proposal for release into the Australian environment.

3 Methodology

Preliminary work was conducted in the native range stations in Argentina and Mexico. Some basic biological studies (especially on lifecycle and culturing methods) and preliminary testing in the native range were continued in order to speed up work in Australian Quarantine. High priority insects were sourced and exported to Australia by collaborators

based at the USDA station in Argentina and the CSIRO station in Mexico, once the necessary export and import permits were obtained. The insects were housed and tested in the CSIRO quarantine facility in Brisbane. The biology of each high priority agent was studied, in particular the duration of life stages and requirements for feeding and egg laying. The methodology for rearing and testing was developed and the host tests for the highest priority agents were completed.

The host specificity of *Eueupithecia* spp. 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 67 plant species were tested for UU and 65 for U2. All test plant species were from the Leguminosae family. The list was compiled according to the modern methods, primarily using degrees of phylogenetic separation, based on published phylogenies. Individuals of plants tested in the laboratory were procured and grown in glasshouses.

1. Surveys of plant use under natural condition in the native range,

Field trips were made to sites with populations of *P. aculeata*. Co-occurring identified legume species were sampled for presence of insects by beating foliage over a one square metre sheet. Resulting insects were held in plastic containers and provided fresh *P. aculeata* leaves until the emergence of adults for identification. A total of five species were tested for both UU and U2.

2. Tests of early larval development on cut plant material in Australia and Argentina

Larval survival was evaluated in laboratory no-choice trials on species of Leguminosae in Argentina (27 species for UU, 20 species for U2) and Australia (0 species for UU, 21 species for U2). 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. 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.

3. Tests of full larval development on living plant species in Australian quarantine

Survival of larvae to adult in whole living plants was evaluated in the laboratory using no-choice trials on species of Leguminosae (42 species for UU, 42 species for U2). 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 a cage in a quarantine glasshosue. Plants were held until all adults had emerged from the *P. aculeata* control plant.

Proposals for the release of agents were written based on the results of this research. A new system is in place for assessing biocontrol agents by the commonwealth department, DAFF. Thus an internal import risk assessment was made on the proposals, rather than the use of the previous system of assessors (co-operators) spread across many federal and state departments. The impact of this is an extension of the assessment process from 6-12 months to 1-2 years. This meant that release of agents couldn't be included as a milestone in this project. However, a preliminary release of the first insect was made following mass-rearing at the CSIRO facility in Brisbane

It is imperative to improving the efficiency and effectiveness of biological control that the results of research be published. This project and its predecessor generated a long list of publications of both an applied and basic nature (see Section 6) that contribute to the practise of biological control.

4 Results and discussion

The ten highest priority species were identified during the phase I MLA project, and this was increased to eleven following the splitting of *Eueupithecia* into two species (Table 1). The top five were introduced into Australian quarantine and the results are discussed in the five sections below. The remaining six species were further assessed in the native range. All were eliminated. The flower feeding wasps Eulophidae spp. and *Tetrastichus* sp. were eliminated on the grounds of being too rare to work with following extensive and intensive field searches, which failed to reveal more than the occasional specimen. The fungal pathogen, *Septoria* sp., was not as rare but was never observed to inflict serious damage to its host. The stem boring beetle *Agrilus parkinsoniae* and the leaf beetle *Glyptoscelis sonorensis* were found in good numbers but proved too difficult to breed and test in the lab and so their suitability cannot be determined. The unidentified Cerambycidae were finally identified. The five species common enough to be considered as viable agents (*Atrypanius irrorellus, Gnaphalodes trachyderoides, Lissonotus flavocinctus, Lophopoeum carinatulum, Sphaenothecus maccartyi*) proved to be not sufficiently specific following literature reviews. The remaining Cerambycidae were too rare (Table 1).

Table 1. List of the top potential biocontrol agents for parkinsonia identified during the MLA phase I project, and conclusions reached during the phase II project. Species tested in quarantine are indicated with an asterisk.

Species	Notes	Results
*Eueupithecia cisplatensis**	Defoliating looper caterpillar	Approved to release
Eueupithecia sp.2*	Defoliating looper caterpillar	Application pending
*Ofatulena luminosa**	Stem borer	Highly damaging insect. Likely to be host-specific but cultures required for testing failed to establish
Neolasioptera sp.*	A gall fly that attacks growing tips	Potentially damaging insect. Likely to be host-specific but cultures required for testing failed to establish
nr *Rudenia leguminana**	A defoliating / flower bud feeding caterpillar	Not specific
Agrilus parkinsoniae	Stem borer	Cannot be reared or tested
Glyptoscelis sonorensis	Defoliating leaf beetle	Cannot be reared or tested
Cerambycidae spp.	Stem borers	Not specific or too rare
Eulophidae spp.	Flower feeding wasp	Could not be found in sufficient numbers
Tetrastichus sp.	Flower feeding wasp	Could not be found in sufficient numbers
Septoria sp.	Leaf and stem fungal cankers	Rare, not damaging

4.1 Assessment of the highest priority agent, the looper Eueupithecia cisplatensis

Eueupithecia cisplatensis (Lepidoptera: Geometridae), nicknamed UU, is a looper caterpillar that is abundant and widespread in Argentina. Two species of *Conura* (Hymenoptera: Chalcidoidea) parasitise the larvae. Free of its natural enemies in Australia, it may cause heavy damage to leaves.

Both field and laboratory studies in Argentina provided convincing evidence of the host specificity of this species. On three field trips to eight sites in northern Argentina, with populations of *P. aculeata* and four co-occurring legume species, plants were sampled for presence of insects. A total of 391 larvae of UU were collected on *P. aculeata* but none on any of the other surveyed *Acacia, Prosopis* or *Parkinsonia* species. It is particularly instructive that UU was not found even on the conspecific *Parkinsonia praecox*. At the same sites, this species was consistently collected on *P. aculeata*.

Host testing commenced in quarantine followed the first shipment of this agent into Australia in March 2010. The fifth generation of the lab colony from the first shipment was heavily affected by a Nosema-like Microsporidian pathogen in August 2010. This invalidated the tests of this generation. A few females survived the epidemic and were used to successfully initiate a new colony. We introduced a regimen in which we established iso-female lines which consisted of eggs laid by one female only. The female was then checked microscopically for disease. Only eggs laid by clean females were used to start the next generation. In this way, we minimized the vertical transmission of the pathogen from mother to offspring and produced a healthy colony. However, the genetic diversity of the colony was greatly reduced and to address this we imported a fresh collection of pupae from Argentina in April 2011 and used these individuals to start a new colony.

We successfully moved the colony to the new quarantine facility situated on the roof of the Queensland EcoSciences precinct at Dutton Park, in February 2011 where host testing was completed. Laboratory no-choice larval survival was evaluated on 40 species of Leguminosae. The Australian no-choice tests showed a consistent failure of larvae of UU to develop on any plant species other than *P. aculeata*. No feeding or damage was observed on any non-target test plant species.

A taxonomic complication required urgent attention towards the end of the assessment process. This complication proved to be an opportunity, in addition to a challenge. In the process of collaborating with an international expert in the taxonomy of Geometridae, in order to gain information relevant to the Application for Release, a second cryptic species was found. This species is cryptic in that no external features separate it from the *Eueupithecia cisplatensis.* However the genitalia of both males and females are very different as is the CO1 gene sequence. We successfully checked representative voucher species of the individuals tested to ensure that we confined our reporting in the Application for Release to only the results of tests on *Eueupithecia cisplatensis.*

The application for release of this first agent was made in October 2011 (Appendix 1) and was successful. We received a release permit from the two government agencies, DAFF and SEWPaC by November 2012.

A fresh colony of *Eueupithecia cisplatensis* was imported into Australian quarantine in January 2013 and reared for one generation prior to release. This fresh importation ensured that there was no risk of inbreeding or laboratory adaptation.

An application for funding to the WA Cattle Industry Funding Scheme for the release and evaluation of this species in Western Australia was successful. This has boosted the funding available for this release activity.

The first releases of *E. cisplatensis* have been made in Queensland in April 2013 and Western Australia. Colonies of *E. cisplatensis* has been provided to Queensland and Northern Territory colleagues who will collaborate in future rearing and release efforts.

4.2 Assessment of the second highest priority agent, the tip borer *Ofatulena luminosa*

Ofatulena luminosa (Lepidoptera: Tortricidae) is a consistently common species in the Tampico delta area of the Mexican Gulf and is also known from the USA (California, Arizona and Texas) (Brown *et al*. 2010). Larvae bore in growing tips, mature green stems and green seeds. Up to five larvae have been dissected from the distal 25 cm of a *P. aculeata* stem. A single larva may also develop in a green seed, eating out the seed and killing it. Larvae of *O. luminosa* are heavily parasitized by Hymenoptera.

This species has been the subject of a survey of natural host plant use in Mexico and has only been recovered from two species of parkinsonia. In these surveys, twenty-three legume species growing in the same habitat as *P. aculeata* were recognized (five species of Caesalpinioideae, eleven species of Mimosoideae, and seven species of Faboideae). Stems with evidence of damage were bagged for adult emergence. All insects that emerged were pinned, labelled and identified. *Ofatulena luminosa* emerged only from *Parkinsonia aculeata* and *Parkinsonia texana*, providing strong support that it would be safe to release in Australia.

A shipment of *Ofatulena luminosa* was imported into the CSIRO quarantine insectary from Mexico in September 2010. They arrived in very good condition. Approximately 1575 stems were sent, most containing one or more larvae of a variety of sizes. The stems were set up in oasis foam in quarantine to maintain their condition and allow larvae development (Figure 1a). Many of the larvae were parasitized as demonstrated by the emergence of adult parasitoids, but approximately 100 *O. luminosa* adults emerged. The adults were sexed, paired and placed in cages with living plants (Figure 1b), or in plastic containers. Eggs were laid in the plastic containers, confirming the adults were fecund. We expected that the adults placed on living plants in cages would lay on those plants which would then become infested with larvae. However, no next generation adults emerged. We are still uncertain as to the reason behind this disappointing result. A possibility is that the glasshouse grown plant stems were too slender for the larvae.

We are confident that this insect is specific based on the survey of native plants in Mexico. We also know this species is capable of damaging the plant and potentially could inflict higher levels of damage in Australia where it will be free of specialized parasites. For that reason we persevered and made another shipment of more than 1600 larvae in 2011 with a similar result, despite improvements in the biophysical environment of the new quarantine facility and improvements in plant quality, in particular, the successful growing of thicker stemmed plants which more closely resemble infested plants in the field.

These attempts to establish cultures in quarantine followed on from several failed attempts to establish cultures at the Mexican Field Station (MLA phase I project). The challenge to test the specificity of this insect against Australian plant species proved to be so great that this insect was dropped from further assessment.

Figure 1a. Rearing of adults of *Ofatulena luminosa* from infested stems collected in the field in Mexico and carried to Australian quarantine; b. Cage into which adults of *Ofatulena luminosa* were placed for establishing a laboratory culture

4.3 Assessment of the third agent, the parkinsonia stem galler, *Neolasioptera aculeatae*

Neolasioptera aculeatae (Diptera: Cecidomyidae) is a newly described gall midge collected from stem swellings of *Parkinsonia aculeata* (Gagne et al. 2011). It is responsible for a common and conspicuous stem gall that stunts the branches and often curbs further axillary growth.

This agent was the subject of several field-based trials of native plant use in Argentina. At the nine sites visited, a total of 919 galls were consistently collected on 416 *P. aculeata* plants. Adults of *N. aculeatae* emerged only from *P. aculeata* stem galls. A total of 244 coexisting plants of *Acacia aroma, A. caven, Prosopis alba, P. ruscifolia, Senna sp., Sesbania sp., Neptunia sp.,* and *Parkinsonia praecox* were visually inspected for the presence of stem galls. Collected stem galls (n = 356) were kept in plastic containers and brought to the lab for subsequent adult emergence. The results of these trials suggested that this species is entirely specific to *Parkinsonia aculeata*. Even the closely related *Parkinsonia praecox* is not a host of this insect.

The necessary follow-up host-testing in quarantine required the establishment of cultures. The first shipment into Australian quarantine of this agent was made in April 2011. Approximately 800 galls were collected in the field (Figure 2), packed and sent to Australia. Over the next months, 402 adults emerged from the field-collected galls. The adults were sexed and counted into cages with healthy plants. A total of 12 cages containing usually around 4 plants each were set up with varying numbers of adults to provide a diversity of conditions for mating and oviposition. Disappointingly, no laboratory colony was founded as no galls formed on any of the plants. This result mirrors that of *Ofatulena* above.

We resolved to continue to study *N. aculeatae* in the native range to try to solve the lab rearing problem. At the SABCL, colleagues also attempted rearing this species. During a field trip, 1372 galls were collected from 300 *P. aculeata* plants at eight sites. Twenty to forty newly emerged adults were confined inside insect rearing sleeves on *P. aculeata* plants. A total of 21 rearing sleeves were set. No stem galls developed.

Laboratory host specificity testing cannot commence without solving the rearing problem. We were therefore forced to drop *N. aculeatae* from the priority list and replace it with a more promising species.

Figure 2. Collecting the Parkinsonia stem galler in the field in Argentina

4.4 Assessment of the fourth agent, the second parkinsonia tip borer, *Rudenia leguminana* complex sp. B

Rudenia leguminana complex sp. B (Lepidoptera: Tortricidae) had been relegated in the priority list because of the taxonomic uncertainty. Other species in the complex feed on various legume host plants. However we reconsidered this species following the failure to progress two other high priority species. Also, we gathered further evidence that the taxon is a species complex and that the host range of some taxa within the complex may be narrower than the whole complex.

This agent is widely distributed from the USA to Venezuela and has been reared in large numbers from *P. aculeata* in Guatemala, Mexico, Nicaragua and Venezuela (Brown *et al*. 2010). First instar larvae feed inside the rachis before making a tunnel in the axil in which

they hide during the day. Larvae leave the tunnel at night to feed on the pinnules and rachises of leaves. Larvae can also develop in flowers and occasionally in pods. We believe it to be a species complex, as it has an unusually broad geographic range for Neotropical tortricids, and analyses of two molecular markers strongly suggested that the individuals examined belonged to more than one species (Brown *et al*. 2010). Host specificity studies at the Mexican Field Station also lent evidence to the hypothesis that a host specific cryptic species may be included in the currently defined species because in open-field trials it successfully developed only on *P. aculeata*. Records in the literature of use of various legumes by *R. leguminana* may therefore refer to other species in this complex.

We imported *R. leguminana* complex sp. B (Lepidoptera: Tortricidae) from the west coast of Mexico in March 2012 into Australian quarantine. Australian-based project officer Gio Fichera travelled to Mexico and teamed with ex-CSIRO, Mexican-based field assistant, Moises Martinez. They collected approximately 400 larvae of the agent and successfully imported them into Australia. A thriving colony was established, proving that this species is amenable to rearing in laboratory conditions. In addition, a methodology for host testing was developed and testing commenced against 17 representative legume species.

This host-testing showed unacceptable development on 8 of the 17 non-target species, including the Australian native *Acacia fimbriata.* Adults from the test plants were smaller, and the development time generally longer, that on *P. aculeata*. Nonetheless, we believe the risks associated with introducing this agent are too great and that the regulatory agencies would not approve its release. Testing was therefore terminated.

4.5 Assessment of the fifth agent, the looper *Eueupithecia* sp.2

A second, sibling species of *Eueupithecia* (Lepidoptera: Geometridae) has been identified as a potential biocontrol agent (Figure 3). This species has not been formally described and so is referred to as *Eueupithecia* sp.2 and nicknamed U2. *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 (Figure 4).

Figure 3. 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.

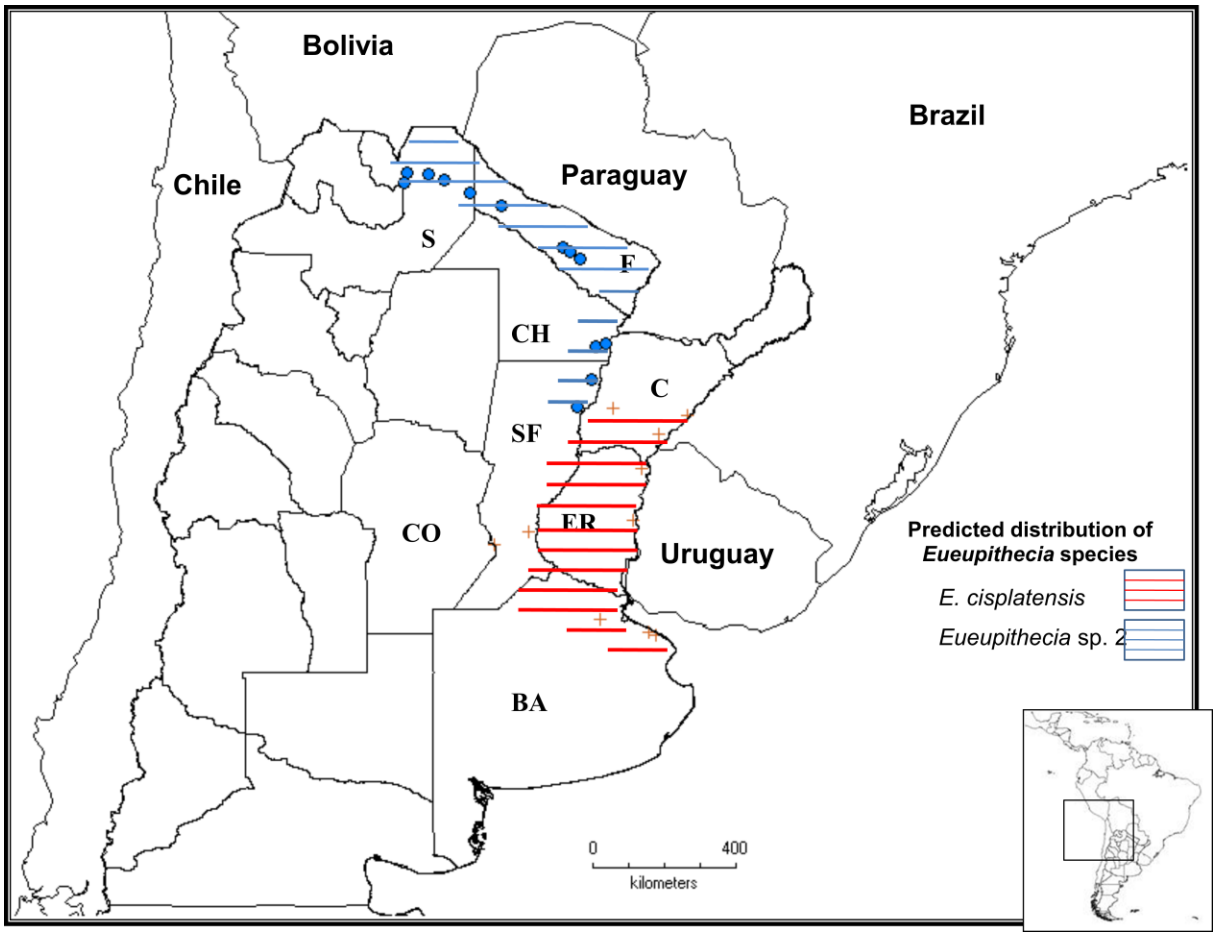

Figure 4. Distribution of Eueupithecia species in Argentina confirmed by genitalia dissections. Red crosses: *E. cisplatensis* localities. Blue dots: *Eueupithecia* sp.2 localities.

Preliminary studies of 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 in February 2012, 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 (two species were common to two tests). *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%). In contrast, no development occurred on any test plant species, with all larvae dying as first instars. No feeding, and therefore no damage, occurred on species other than parkinsonia.

We concluded 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 applied for permission for its release in Australia in May 2013 (Appendix 2). This application is pending and a decision is expected in approximately 12 months.

4.6 Development of appropriate rearing and distribution methodologies and networks for agents with submitted applications for release.

Obtaining approval to release an agent wasn't guaranteed during the life of this project, in part because of potential delays in obtaining approvals for release (1-2 years). However, necessary preparatory work for a national release and evaluation programme was to be undertaken as part of the project once applications for release were submitted. This included developing mass-rearing and release methodologies, and establishing networks to facilitate the national release and evaluation of the agents. This was achieved for both insects for which applications have been submitted. Both have similar life histories so methodologies are expected to be similar for each. However, differing climatic associations in the native-range suggest that they will perform best in different parts of Australia. A manual has been drafted for distribution to collaborators which covers the methods for rearing and preliminary release suggestions (Appendix 3).

Mass-rearing methods have been developed for both species as described and illustrated in detail in the manual. However, in short, adults are collected as they emerge, sexed under magnification, and placed in plastic takeaway containers lined with moist paper towelling (2 to 3 pairs in each) to allow them to mate and lay eggs. Most eggs are laid on the paper towelling which makes it easy to track the health of the colony and control the number and age of eggs being used for setting up the next generation. Eggs are inspected daily and emerging adults are removed within 12 hours of hatching and transferred to new parkinsonia plants using a fine, moistened brush (50 larvae per plant). Alternatively a parkinsonia sprig is placed in the egg containers to allow newly emerged larvae to move on to it, and then transferred to a living plant. The sprig can support larvae for several days if it is well set up in a vial of water. In both cases approximately 50 larvae are transferred to each plant to ensure that plants don't become overloaded once they start to grow. Plants with larvae are placed in a gauze-lined cage to contain the larvae and emerging adults. In our case we use an aluminium framed cages measuring 450 x 450 x 900 mm. Larvae feed, grow,

develop, pupate in the cage, and more plants are added as required. The prepupae typically spin cocoons in folds in the gauze roofs of the cage and in concealed positions. It is important maximise genetic diversity and colony vigour by minimising inbreeding. This is achieved by pairing females and males, at each generation, that have emerged from different cages to avoid mating between siblings. In addition, we can avoid potential laboratory adaption by making new importations and incorporating this fresh genetic material into lab colonies. Overall the methodology is relatively efficient however it does require a continuous supply of high quality parkinsonia plants, including through winter months when growth rates slow and defoliation can occur. This is achieved at CSIRO through the use of a range of different glasshouses.

Distribution methodologies have been developed, but will require field testing. The number of insects to release at each site can be varied. Initial releases of *Eueupithecia cisplatensis* with QDAFF in north Queensland (April 2013) compared release sizes of 50, 100 and 200 individuals. This was based on numbers known to be successful in previous biocontrol agent releases, and was also achievable using existing rearing facilities. We also compared the effectiveness of releasing adults (from delta traps, weather-proof, plastic structures that provide good protection and can be hung in a tree) versus young larvae (placed individually on plants) as both methods are practical and have been successfully used for other comparable species. The adult releases required less field time but more laboratory time as it is quicker to set up delta traps in the field compared to larval releases, but time-consuming to find and remove pupae from rearing cages. It is still too early evaluate this trial. Other factors that still need to be trialled in order to develop an effective release strategy include season of release and micro-habitat. Spring is expected to be particularly ideal. Phenological data previously collected from parkinsonia populations across Australia will help in defining this window, which can vary by months between regions. Another factor that needs to be investigated is dispersal ability. Even closely related species can have very different dispersal distances and has a strong bearing on how far apart releases will need to be made to achieve widespread establishment. This data will be derived by establishing transects from release sites when searching for establishment.

The necessary networks have been established as a basis for a national release and evaluation programme. Collaborators in the Northern Territory (Dr Keith Ferdinands, Manager Weed Sciences, Weed Management Branch, Department of Land Resource Management) and Queensland (Dr Shane Campbell, Professional Leader, Invasive Plants and Animals Science Biosecurity Queensland) have both committed to assisting in a phase III national release programme. We have already provided them with start-up cultures. We have also visited both laboratories to explain the methodology, and we continue to be available for troubleshooting. Both agencies have agreed to participate in the proposed national field experiment aimed at developing, testing and implementing optimal release strategies, and evaluating the effectiveness of the agents.

5 Conclusions

The project has resulted in two new biocontrol agents of parkinsonia becoming available for land managers. Additional biocontrol agents for parkinsonia are only possible if there are break-throughs in culturing methodologies. It is therefore imperative to maximise the establishment and impact of these two with a thorough release project. The first releases of *E. cisplatensis* have been made at sites in Queensland in April 2013 and Western Australia (under the WA Cattle Industry Funding Scheme grant) in May 2013, but it is too early to evaluate their success. Colonies of *E. cisplatensis* has been provided to Queensland and Northern Territory colleagues who will collaborate in future rearing and release efforts. As field populations of parkinsonia in Australia are patchy and disjunct, a large effort will need to be made to ensure extensive establishment and maximum impact. A large field trial has been planned to develop and test release methodologies. Once establishment is achieved, populations are expected to be self-sustaining, but ongoing evaluation will be required to quantify the success of biocontrol and to identify any ways that impacts can be further improved. An application for funding for this phase III project is pending with MLA.

6 Acknowledgements

We 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 and Juan Briano (Fundación para el Estudio de Especies Invasivas, Argentina, previously USDA SABCL) and Ricardo Segura, Moises Martinez, Carlos Pascacio, Quiyari Santiago (CSIRO, Mexico) executed native range field and laboratory work with enthusiasm and ability. Jeff Makinson commented on a draft.

7 Publications

The following list is a compilation of publications resulting from or which contributed to this project (B.NBP.0620) or its predecessor (B.NBP.0366) that are already published or are at an advanced stage of preparation. This project and its predecessor generated a long list of publications of both an applied and basic nature that contribute to the practise of biological control and will make it more efficient and effective in the future.

Journals and Book Chapters

Bell, K.L. Heard, T.A. Manion, G., Ferrier, S. and van Klinken, R.D. (2013) Comprehensively characterizing the phytophagous arthropod fauna of a single host plant species: survey completeness through time and space. Ecography (submitted)

Bell, K.L. Heard, T.A., Manion, G., Ferrier, S. and van Klinken, R.D. (2013) The role of geography and environment in species turnover: phytophagous arthropods on a neotropical legume. Journal of Biogeography (in press).

Brown, J.W. Ricardo Segura, Santiago, Q., Jadranka Rota and Heard, T.A. (2010) Leaf-roller moths (Tortricidae) reared from the invasive weed Mexican palo verde (*Parkinsonia aculeata*), with comments on their host specificity, biology, and geographic distribution. Journal of Insect Science Vol. 11 Article 7.

Gagné, R.J., Mc Kay, F. and Heard T.A. (2011) A new species of *Neolasioptera* (Diptera: Cecidomyiidae) from *Parkinsonia aculeata* (Leguminosae) in Argentina for possible use in biological control in Australia, with a key to Neotropical species of Neolasioptera. ZooTaxa 2866: 61-68.

Heard, T.A. Bell, K., Santiago Q. and Segura, R. (2012). The phytophagous insect fauna of *Parkinsonia aculeata* and their potential for biocontrol (In preparation).

Heard, T.A., Segura, R. and al. (2011) Natural host plant use of herbivores of Parkinsonia in Mexico. *Biological Control* (In preparation).

Palmer W.A., Heard T.A. and Sheppard A.S. (2010) A review of Australian classical biological control of weeds programs and research activities over the past 12 years. Biological Control, 52: 271-287.

van Klinken, R.D., Campbell S.D., Heard, T.A. McKenzie J. and March N. (2009) The Biology of Australian Weeds *Parkinsonia aculeata* L. *Plant Protection Quarterly* 24: 100-117.

van Klinken, Heard, T.A., *Parkinsonia aculeata* (2011) In *Biological control of weeds in Australia* (Eds. M Julien, R Mcfadyen and J Cullen).), pp. 437-447. CSIRO Publishing, Melbourne.

Conferences

Campbell, S., Heard, T., Galea V. and van Klinken, R.D. (2013) Where do we stand with weeds from a research perspective? Beef Research Update Conference to be held in Cairns between the 13-15 August 2013.

Heard, T.A. (2006) *Parkinsonia aculeata*: surveys for natural enemies, native range ecological studies, and prospects for biological control. Pp. 581-584. Proceedings of the 15th Australian Weeds Conference eds, C. Preston, JH Watts and ND Crossman. Weed management Society of South Australia, Adelaide.

Other

Heard, T.A. (2011) Application to release the defoliating caterpillar *Eueupithecia cisplatensis* (Lepidoptera: Geometridae) for biological control of the weed *Parkinsonia aculeata* (Leguminosae: Caesalpinioideae). Unpublished proposal/report to AQIS, 2011-10-26.

Heard, T.A. (2013) Application to release the defoliating caterpillar *Eueupithecia* sp. 2 for biological control of the weed *Parkinsonia aculeata*. Unpublished proposal/report to AQIS, 2013-05-09.

8 Appendices

8.1 Application to release the defoliating caterpillar *Eueupithecia cisplatensis* (Lepidoptera: Geometridae) for biological control of the weed *Parkinsonia aculeata* (Leguminosae: Caesalpinioideae).

Application to release the defoliating caterpillar *Eueupithecia cisplatensis* (Lepidoptera: Geometridae) for biological control of the weed *Parkinsonia aculeata* (Leguminosae: Caesalpinioideae)

CSIRO Ecosystem Sciences

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

tim.heard@csiro.au

2011-10-26

Figure 1 Eight larvae, seven green one brown, of *Eueupithecia cisplatensis* on a damaged Parkinsonia leaf, most of the pinnules have been removed from the leaves and rasping of the leaf surface is visible on the leaf at the bottom

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

The defoliating caterpillar, *Eueupithecia cisplatensis* Prout, has been identified as a potential biocontrol agent of *P. aculeata*. Preliminary studies on its biology and host specificity made in Argentina, in the field and in laboratory conditions, strongly indicated fidelity to *P. aculeata*. It 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 67 plant species were tested, 40 in Australia and 27 in Argentina.

This species has proven 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 61% in Argentina and 56% in Australia). But no development past the first instar occurred on any test plant species with the exception of was the closely related *Parkinsonia praecox* on which a very low rate of development (3%) was measured. No feeding occurred on any test plant species other than *P. praecox* and hence no damage was observed on non-target species. However, even *P. praecox* was not found to be used by *E. cisplatensis* in the field in the native range.

We conclude that the level of risk associated with releasing *Eueupithecia cisplatensis* 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.

2 Information on target species, *Parkinsonia aculeata*

2.1 Taxonomy

Botanical name

Parkinsonia aculeata L.

2.1.1 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).

2.1.2 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 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. More information on the relationships is given in the section “The test plant list”.

2.2 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).

2.3 Distribution

2.3.1 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.

2.3.2 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. 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.

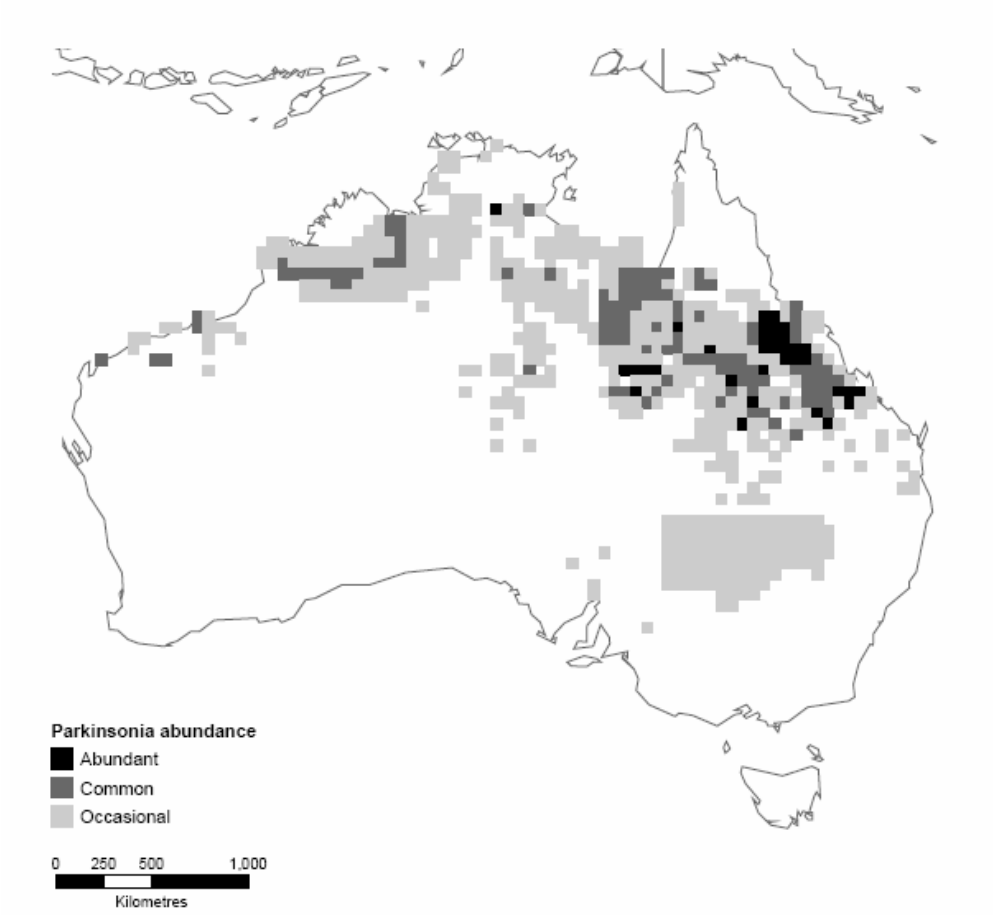

Figure 3. Current distribution and abundance of *P. aculeata* in Australia. Source: Queensland Biosecurity.

2.4 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; van Klinken et al. 2006; 2008). 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.

2.5 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.

2.5.1 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 pantropically, 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.

2.5.2 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.

2.6 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.

2.7 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).

3 Information on the potential agent *Eueupithecia cisplatensis*

3.1 Taxonomy

Eueupithecia cisplatensis Prout 1910 (family Geometridae) (Figure 4), identified by Geometridae specialist Dr. Axel Hausmann (Bavarian State Collection of Zoology, Munich, Germany).

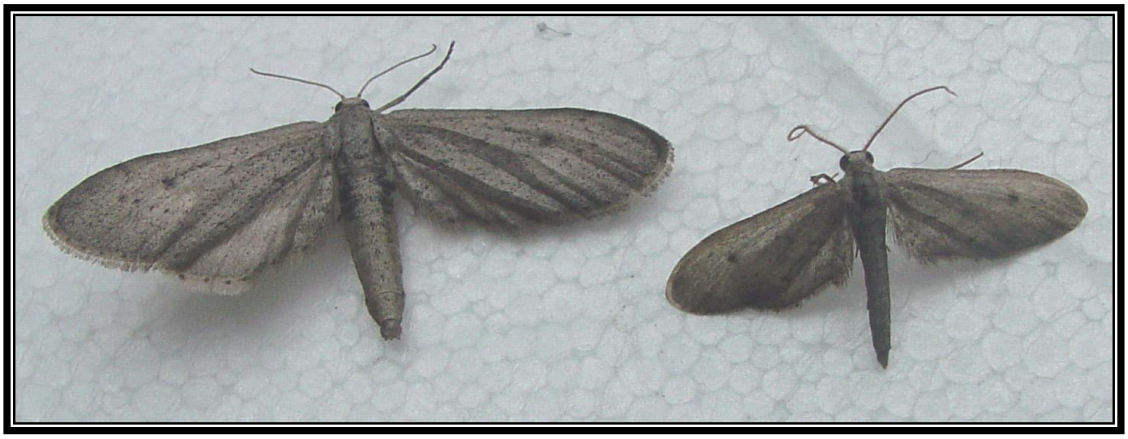

Figure 4. *Eueupithecia cisplatensis*, female left and male right

Eueupithecia cisplatensis 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 a second cryptic species. This species shows striking differences in female and male genitalia and CO1 gene sequence (Table 1). The CO1 barcode gene differs by 4%, an amount that normally indicates another species. But no significant and constant differential features in colour or pattern of adults or larvae have been found. The second species is less common than *E. cisplatensis* and is so far only known from the north western Salta Province of Argentina. Further work is needed to confirm that this second species has not previously been described under the closely related *Euacidalia* genus, the latter including 12 described neotropical and nearctic species.

All testing in Australia was conducted on a pure colony of *E. cisplatensis*, as confirmed by genitalia dissections. Many provenances were used for Argentinean testing. All insects subsampled for identification were *E. cisplatensis*, although it is possible that a small number of undetected individuals of the new species could have been present among the test material.

Table1. Differential features between the two *Eueupithecia* species collected on *Parkinsonia aculeata*.

	E. cisplatensis	*Eueupithecia* new species
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 one cornutus only. Aedeagus very broad, width 0.4 mm.
Size of adults	On average smaller, wingspan 15-20 mm	On average larger, wingspan 20-25 mm

3.2 Description

The following is a description of the genus *Eueupithecia* obtained by Dr Axel Hausmann (pers. comm. 2011):

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.

3.3 Brief biology of the agent

Experiments were conducted in Argentina in controlled environment chambers at 25±1°C and 60±5% relative humidity, with a 14:10 L:D photoperiod. Cultures of *E. cisplatensis* were established in the laboratory from 50 larvae collected in February 2009 on *P. aculeata* plants growing near La Plata, Buenos Aires Province (60 km south of Buenos Aires city).

Newly hatched larvae were fed bouquets of freshly excised leaves of *P. aculeata* and reared individually in 0.5-liter plastic jars with perforated lids and moist tissue paper. Head capsule width was measured to establish the number and the duration of larval instars. The duration of the pupal stage was also recorded.

Adult longevity and fecundity were estimated from eight pairs of newly emerged *E. cisplatensis*. Each pair was kept in 3-litre plastic jars with moist tissue paper containing bouquets of excised fresh leaves of *P. aculeata*. Every day, bouquets were replaced and eggs removed and counted. A replicate ended when the female died; if the male died first it was replaced. For each replicate, the pre-oviposition period, total number of eggs and longevity of females were recorded.

Brown cylindrical eggs, approximately 0.3 mm in length, are usually laid individually or in strings on the leaflets (Figure 5). The eggs hatch and larvae begin to feed about 5 days after eggs were laid. Body colour of larvae changes progressively from light brown-greenish in the early instars to green-purple in the later instars (Figure 6) mimicking leaf rachises and young shoots. As larvae develop, they eat most of the pinnules and parts of the rachises. The reduced number of prolegs results in the larvae progressing with a looping motion, hence the common name loopers.

Figure 5. Strings of brown eggs of *Eueupithecia cisplatensis* on *Parkinsonia aculeata* leaf.

Figure 6. Two larvae of *Eueupithecia cisplatensis* on *Parkinsonia aculeata* leaf

Life stage duration. *E. cisplatensis* undergoes four larval instars. No overlapping was found in head capsule width ranges, therefore they can be used to distinguish the instars (Table 2). Larval mortality was greater during the first and second instars and the survival to the adult stage was 42 %. The duration of the stages was approximately: 5 days for eggs, 17 days for larvae, and 4 days for pupae.

Table 2. Life stage duration and larval head capsules width of *Eueupithecia cisplatensis* on *Parkinsonia aculeata.*

Stage	n	Life stage duration (days)		Mortality (%)	Cumulative survival (%)	Head capsule width (mm)	
		Mean ± SD	Range			Mean ± SD	Range
Larva 1st instar	43	5 ± 0.24	2-8	35	100	0.26 ± 0.01	0.23-0.26
Larva 2nd instar	28	3 ± 0.46	1-14	21	65	0.42 ± 0.03	0.33-0.42
Larva 3rd instar	22	4 ± 0.21	2-7	5	51	0.68 ± 0.0	0.62-0.72
Larva 4th instar	21	5 ± 0.28	3-9	0	49	1.04 ± 0.06	0.91-1.11
Larva total	21	17 ± 3.1	13-27	61	49	-	-
Prepupa	21	2 ± 0.11	1-2	0	49	-	-
Pupa	21	3 ± 0.11	3-15	14	49	-	-
Adult	18	4 ± 0.11	1-13	-	42	-	-

Female longevity and fecundity. Preoviposion period was 1.8 ± 0.6 days (mean ± SD; n = 6), fecundity was 78.8 ± 62.7 eggs (mean ± SD; n = 8) and the longevity of females was 6.9 ± 3.6 days (mean ± SD; n = 8) (Table 3). The female of pair n° 4, laid a total of 36 green coloured eggs. Previous observations indicate that occasionally, virgin females may lay a few similar green eggs, which never hatch. Based on these observations, we consider these green eggs to be unfertile. The rest of the pairs laid brown fertile eggs.

Table 3. Fecundity and female survival of *Eueupithecia cisplatensis* on *Parkinsonia aculeata*

N° of replicates (pairs)	Female longevity (days)	Preoviposition period (days)	N° of eggs
1	9	3	140
2	13	2	79
3	8	1	168
4	7	2	36[a]
5	2	-	0
6	7	2	117
7	7	1	90
8	2	-	0
Average	6.9	1.8	78.8

[a] green infertile eggs

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

Natural enemies. Two species of *Conura* (Hymenoptera: Chalcidoidea) emerged from cocoons, and probably parasitised the larvae.

3.4 Native range of the agent

Known from field surveys from Argentina and Paraguay only.

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

The genus *Eueupithecia* has only one member other than *E. cisplatensis*, which is yet to be described (see above). A study of the biology and host specificity of the latter is planned but as yet little is known except that we suspect it is also 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.

3.6 The proposed source of the agent

Fernando Mc Kay, Scientist at the United States Department of Agriculture, Agricultural Research Service, South American Biological Control Laboratory (USDA-ARS-SABCL). Address: Bolivar 1559, Hurlingham, Buenos Aires, Argentina. Phone: (54 11) 4662 0999. Email: fmckay@speedy.com.ar.

Cultures of the genetic material from Argentina that has been tested in Australian quarantine will be maintained and released if permission is granted.

3.7 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*. *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 proposed agents feed on vegetation tissue and therefore it is unlikely that they will interact with the existing agents.

3.8 The agent's potential for control of target

Leaf feeding by larvae of Geometridae 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 potted plants of all foliage. 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.

3.9 Information on non-target organisms at risk from an agent

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

3.10 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.

3.11 Report of host specificity testing

3.11.1 Introduction

The host specificity of *E. cisplatensis* was tested using three methods: 1 Surveys of plant use under natural condition in the native range; 2 Tests of larval development on cut plant material in Argentina; and 3 Tests of larval development on living plant species in Australian quarantine. All tests delivered the same result: complete specificity to one plant species, *P. aculeata*. Low rates of pupation were observed on another Parkinsonia species (P. praecox), but that species does not occur in Australia. Each of these tests is considered separately below. But first we discuss the test list which applies to the two latter tests.

3.11.2 The test plant list

The plant list for the Australian plants consists of 40 species from the legume family, in addition to *P. aculeata*. In addition, another 27 legume plant species were tested in Argentina. The list presented here 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 4.

- 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 tested 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 is on the list. *Peltophorum dubium* was also tested in Argentina. Also ornamental member of the group that are exotic to Australia was tested to help define the host range, including *Colvillea racemosa* in Australia and *Schizolobium parahybum* and *Delonix regia* in Argentina.

- The tribe Caesalpinieae is represented in Australia by *Erythropleum chlorostachys*, which 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 by the exotic *Gleditsia triacanthos*, which was tested in Argentina, along with *Gleditsia amorphoides* in Argentina. 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 4 and 5).

- Fourteen species representing eleven of the tribes of the subfamily Faboideae were included.

- Nineteen species representing the three tribes of the subfamily Mimosoideae were tested. This subfamily contains the large and important tribe and genus *Acacia*. All of the sections of this important genus were represented (Tables 3 and 4) 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 4. Numbers of test plant species in the taxonomic groups of the Leguminosae whether native or exotic to Australia and the number tested in Australian and Argentina

				Number of species:			
Subfamily	**Tribe**	**Group**	**Section**	**Native**	**Exotic**	**Tested Aust.**	**Tested Arg.**
Caesalpinioideae	Caesalpinieae	Peltophorum (*Parkinsonia*)		0	1	1	2
Caesalpinioideae	Caesalpinieae	Peltophorum (not *Parkinsonia*)		1	4	4	3
Caesalpinioideae	Caesalpinieae	Caesalpinia		10	2	2	3
Caesalpinioideae	Caesalpinieae	Dimorphandra		1	0	1	0
Caesalpinioideae	Caesalpinieae	Umtiza		0	1	0	2
Caesalpinioideae	Cassieae			81	15	9	2
Caesalpinioideae	Cercideae			7	1	2	1
Caesalpinioideae	Detarieae			4	2	4	0
Mimosoideae	Acaciae		Botrycephalae			1	1
Mimosoideae	Acaciae		Juliflorae			2	1
Mimosoideae	Acaciae		Phyllodineae			1	0
Mimosoideae	Acaciae		Plurinerves			1	0
Mimosoideae	Acaciae		Acacia			1	3
Mimosoideae	Ingeae					2	1
Mimosoideae	Mimoseae					2	3
Faboideae	Aeschynomeneae					1	0
Faboideae	Bossiaeeae					1	0
Faboideae	Desmodieae					1	0
Faboideae	Mirbelieae					1	0
Faboideae	Phaseoleae					1	2
Faboideae	Robinieae					1	0
Faboideae	Tephrosieae					1	0
Faboideae	Vicieae					1	0

Faboideae	Dalbergiae	0	2
Faboideae	Galegeae	0	1
Faboideae	Milletieae	0	1

After considering phylogeny, the test plant species were selected with regards to the biogeographic overlap with the target or the likely final distribution of the agent within the framework of phylogenetic separation. The concept of testing safeguard species of distant phylogenetic relatedness (Wapshere, 1974) has become redundant in most contexts, as such species do not contribute to the determination of host range (Briese and Walker, 2002; Briese, 2003; 2005). While preferential selection of economic or rare and threatened test plant species can be a useful criterion, providing they pass other selection criteria, systematically testing them is not relevant for risk analysis (Sheppard et al., 2005). As there is no plant on which congeners of the agent have been previously found to feed and reproduce, then this aspect did not result in inclusion of any further species. Taking all these factors into account, we arrived at the test list (Table 5). Such a relatively long list was considered necessary due to the size, diversity and importance of the legume plant family.

Table 5. The complete list of plant species subject to non-choice larval development host specificity tests in Australia and Argentina.

Subfamily	Tribe	group	Section	Genus/species	Tested
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia aculeata*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia aculeata*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Parkinsonia praecox*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Peltophorum dubium*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Peltophorum pterocarpum*	Australia
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia ferrea*	Australia
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia gilliesii*	Argentina
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia paraguariensis*	Argentina
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Caesalpinia pulcherrima*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Colvillea racemosa*	Australia
Caesalpinioideae	Caesalpinieae	Peltophorum		*Delonix regia*	Argentina
Caesalpinioideae	Caesalpinieae	Dimorphandra		*Erythrophleum chlorostachys*	Australia
Caesalpinioideae	Caesalpinieae	Umtiza		*Gleditsia amorphoides*	Argentina
Caesalpinioideae	Caesalpinieae	Umtiza		*Gleditsia triacanthos*	Argentina
Caesalpinioideae	Caesalpinieae	Caesalpinia		*Pterogine nitens*	Argentina
Caesalpinioideae	Caesalpinieae	Peltophorum		*Schizolobium parahybum*	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 corymbosa*	Argentina
Caesalpinioideae	Cassieae			*Senna glutinosa*	Australia
Caesalpinioideae	Cassieae			*Senna notabilis*	Australia

Caesalpinioideae	Cassieae		*Senna spectabilis*	Argentina
Caesalpinioideae	Cercideae		*Barklya syringifolia*	Australia
Caesalpinioideae	Cercideae		*Bauhinia forficata*	Argentina
Caesalpinioideae	Cercideae		*Bauhinia hookeri*	Australia
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
Faboideae	Aeschynomeneae		*Aeschynomene americana*	Australia
Faboideae	Bossiaeeae		*Hovea acutifolia*	Australia
Faboideae	Dalbergiae		*Geoffroea decorticans*	Argentina
Faboideae	Dalbergiae		*Tipuana tipu*	Argentina
Faboideae	Desmodieae		*Desmodium tortuosum*	Australia
Faboideae	Galegeae		*Sesbania virgata*	Argentina
Faboideae	Millettieae		*Lonchocarpus nitidus*	Argentina
Faboideae	Mirbelieae		*Pultenaea villosa*	Australia
Faboideae	Phaseoleae		*Cajanus cajan*	Australia
Faboideae	Phaseoleae		*Erythrina crista-galli*	Argentina
Faboideae	Phaseoleae		*Wisteria sinensis*	Argentina
Faboideae	Robinieae		*Sesbania cannabina*	Australia
Faboideae	Tephrosieae		*Millettia (=Pongamia) sp. McIlwraith*	Australia
Faboideae	Vicieae		*Vicia faba*	Australia
Mimosoideae	Acaciae	Acacia	*Acacia aroma*	Argentina
Mimosoideae	Acaciae	Acacia	*Acacia bidwillii*	Australia
Mimosoideae	Acaciae	Acacia	*Acacia caven*	Argentina
Mimosoideae	Acaciae	Botrycephalae	*Acacia dealbata*	Argentina
Mimosoideae	Acaciae	Botrycephalae	*Acacia decurrens*	Australia

Mimosoideae	Acaciae	Juliflorae	*Acacia disparrima*	Australia
Mimosoideae	Acaciae	Juliflorae	*Acacia julifera*	Australia
Mimosoideae	Acaciae	Juliflorae	*Acacia longifolia*	Argentina
Mimosoideae	Acaciae	Plurinerves	*Acacia melanoxylon*	Australia
Mimosoideae	Acaciae	Botrycephalae	*Acacia oshanesii*	Australia
Mimosoideae	Acaciae	Phyllodineae	*Acacia salicina*	Australia
Mimosoideae	Acaciae	Acacia	*Acacia visco*	Argentina
Mimosoideae	Ingeae		*Archidendron lucyi*	Australia
Mimosoideae	Ingeae		*Enterolobium contortisiliquum*	Argentina
Mimosoideae	Ingeae		*Pararchidendron pruinosum*	Australia
Mimosoideae	Mimoseae		*Anadenanthera colubrina var. cebil*	Argentina
Mimosoideae	Mimoseae		*Dichrostachys cinerea*	Australia
Mimosoideae	Mimoseae		*Leucaena leucocephala*	Australia
Mimosoideae	Mimoseae		*Prosopis alba*	Argentina
Mimosoideae	Mimoseae		*Prosopis chilensis*	Argentina

3.11.3 Surveys of plant use under natural condition in the native range

On three field trips to northern Argentina, over the summer of 2009/10 and 2010/11, eight sites in the provinces of Corrientes, Entre Ríos, Formosa, Salta and Chaco with populations of *P. aculeata* and four co-occurring legume species were sampled for presence of insects by beating foliage over a one square metre sheet (Figure 7). Immature insects were held in plastic containers and provided fresh leaves until the emergence of adults. Voucher specimens of plants and insects collected are maintained at the USDA-ARS-SABCL.

Along the eight sites visited, a total of 391 larvae of *E. cisplatensis* were collected on *P. aculeata* and reared to adult. No *E. cisplatensis* larvae were collected on any of the other surveyed *Acacia*, *Prosopis* or *Parkinsonia* species (Table 6). It is particularly instructive that *E. cisplatensis* was not found even on the conspecific *Parkinsonia praecox*. At the same sites, this species was consistently collected on *P. aculeata*. It is possible that some of the adults reared in this experiment belong to the recently newly identified cryptic species. This only has the effect of reducing the replication obtained for *E. cisplatensis* but not of changing the conclusion. In addition, larvae of *Melipotis acontioides* (Guenee) (Lepidoptera: Noctuidae) and *Macaria* sp. (Lepidoptera: Geometridae) were collected (Table 6).

Figure 7. USDA-ARS-SABCL researchers Marcelo Parisi and Fernando Mc Kay beating *P. aculeata* plants in northern Argentina

Table 6. Number of *Eueupithecia cisplatensis* and other Lepidoptera on various legume plants species from surveys of plant use under natural condition in the native range in Argentina

Date	Locality	Province	Surveyed plant species	Beats	*Eueupithecia cisplatensis*	*Melipotis acontioides*	*Macaria sp.*	Unidentified Geometridae
2009-12-03	RN° 14, Pucheta	Corrientes	*Parkinsonia aculeata*	50	44	0	0	0
2009-12-03	RN° 14, Cuatro Bocas	Corrientes	*Parkinsonia aculeata*	32	43	0	0	0
2009-12-03	RN° 14, Mocoretá	Corrientes	*Parkinsonia aculeata*	17	13	0	0	0
2009-12-03	RN° 14, Chajarí	Entre Ríos	*Parkinsonia aculeata*	46	195	0	0	0
2009-12-04	RN° Concepción del Uruguay	Entre Ríos	*Parkinsonia aculeata*	30	35	0	0	0
2010-03-20	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia aculeata*	10	24	-	5	0
2010-09-26	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia aculeata*	15	2	20	0	0
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Parkinsonia aculeata*	10	35	-	0	0
2010-03-19	RN° 81, 8 km S Pozo d Mortero	Formosa	*Parkinsonia praecox*	10	0	-	29	0
2010-03-20	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia praecox*	3	0	-	12	0
2010-09-26	RN° 81, 60 km NW Juarez	Salta	*Parkinsonia praecox*	10	0	15	0	0
2009-12-03	RN° 14, Pucheta	Corrientes	*Prosopis affinis*	2	0	0	0	2

2009-12-03	RN° 14, Cuatro Bocas	Corrientes	*Prosopis affinis*	8	0	2	0	3
2009-12-04	RN° Concepción del Uruguay	Entre Ríos	*Prosopis affinis*	4	0	0	0	0
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Prosopis ruscifolia*	10	0	-	0	0
2009-12-03	RN° 14, Pucheta	Corrientes	*Acacia caven*	10	0	9	0	0
2009-12-03	RN° 14, Mocoretá	Corrientes	*Acacia caven*	5	0	1	0	0
2009-12-03	RN° 14, Chajarí	Entre Ríos	*Acacia caven*	10	0	1	0	0
2009-12-04	RN° Concepción del Uruguay	Entre Ríos	*Acacia caven*	5	0	0	0	0
2010-03-23	RN° 95, near Fortín Lavalle	Chaco	*Acacia caven*	10	0	-	0	0

3.11.4 Tests of larval development in Argentina

Laboratory no-choice larval survival was evaluated on 28 species of Leguminosae in the subfamilies Caesalpinioideae and Mimosoideae (Table 7). Plants were selected on the basis of taxonomic relatedness to *P. aculeata* and availability. The plants were a mix of species native to Argentina and introduced from other countries including two species of Australian *Acacia*. Experiments were carried out in controlled environmental chambers (25±2°C: 60-80% RH; 16:8 L:D).

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 of the test plant species, with their petioles inserted in small recipients filled with water. The bouquets were replaced every 48-72 hours as needed. Feeding damage and larval mortality were recorded daily until adult emergence. The various test plant species and the control plant (*P. aculeata*) were tested using insects of five different provenances (Table 7). Usually 10 replicates were performed for each plant species, although fewer were done for some plant species (last column in Table 7).

Table 7. The number of replicates of each plant species tested with the various provenances of *Eueupithecia cisplatensis* in Argentina

	The number of replicates (in table body) tested with the various provenances (in column header)					
Test plants	1*	2*	3*	4*	5*	Total
Order Fabales						
Family Fabaceae						
Sub Family Caesalpinioideae						
Tribe Caesalpinieae						
Group Peltophorum						
Parkinsonia aculeata	5	6	2	5	3	21
Parkinsonia praecox	10					10
Peltophorum dubium		3		6	1	10
Schizolobium parahybum	3				2	5
Delonix regia	4	1			5	10
Group Caesalpinia						

Test plants	The number of replicates (in table body) tested with the various provenances (in column header)					
	1*	2*	3*	4*	5*	Total
Caesalpinia gilliesii		3		6	1	10
Caesalpinia paraguariensis		10				10
Pterogine nitens		2	8			10
Group Umtiza						
Gleditsia amorphoides		2		7	1	10
Gleditsia triacanthos		10				10
Tribe Cassiae						
Senna corymbosa		3		6	1	10
Senna spectabilis		3		6	1	10
Tribe Cercideae						
Bauhinia forficate		3		6	1	10
Sub Family Mimosoideae						
Tribe Acaciae						
Acacia aroma	7	3				10
Acacia caven		1		8	1	10
Acacia visco		4		5	1	10
Acacia dealbata	10					10
Acacia longifolia	10					10
Tribe Ingeae						
Enterolobium contortisiliquum		5			5	10
Tribe Mimoseae						
Anadenanthera colubrina var. *cebil*			6			6
Prosopis alba		4		5	1	10
Prosopis chilensis	10					10

Test plants	The number of replicates (in table body) tested with the various provenances (in column header)					
	1*	2*	3*	4*	5*	Total
Sub Family Papilionoideae						
Tribe Dalbergiae						
Geoffroea decorticans	10					10
Tipuana tipu		10				10
Tribe Galegeae						
Sesbania virgata		4				4
Tribe Phaseoleae						
Erythrina crista-galli		10				10
Wisteria sinensis		4				4
Tribe Millettieae						
Lonchocarpus nitidus		10				10

*Detail on the various provenances used: 1. Plants tested in Jan 2010 with northern populations (Corrientes and Entre Ríos); 2. Plants tested in Oct 09 with northern populations (Formosa and Salta); 3. Plants tested in Nov 09 northern populations (Formosa and Salta); 4. Plants tested in Feb-Apr 09 with southern populations (La Plata, Buenos Aires); 5. Plants tested in Sep 2009 with northern populations (Formosa and Salta)

Voucher specimens at USDA-ARS-SABCL: 1♀+ 2♂(Chajarí, Entre Ríos province); 6♂(RN°14, km 455, Corrientes province); 4♀+ 5♂(La Plata, Buenos Aires province); 1♂+ 1♀(Yuchán, Salta Province).

Eueupithecia cisplatensis was able to complete larval development only on *P. aculeata* and *P. praecox*, with 61% and 3% of adult emergence recorded, respectively (Table 8). Larvae exposed to the other tested species died between 2-4 days of initiation of testing. No feeding occurred on any test plant species other than *P. praecox* and hence no damage was observed on non-target species.

Table 8. Results of no-choice larval survival tests on *Eueupithecia cisplatensis* in Argentina

Test plants	Replicates	Pupation (%)	Adult emergence (%)
Order Fabales			
Family Leguminosae			
Sub Family Caesalpinioideae			
Tribe Caesalpinieae			
Group Peltophorum			
Parkinsonia aculeata	**21**	**70 (20-100)**	**61 (20-100)**
Parkinsonia praecox	**10**	**6 (0-30)**	**3 (0-10)**
Peltophorum dubium	10	0	0
Schizolobium parahybum	5	0	0
Delonix regia	6	0	0
Group Caesalpinia			
Caesalpinia gilliesii	10	0	0
Caesalpinia paraguariensis	10	0	0
Pterogine nitens	10	0	0
Group Umtiza			
Gleditsia amorphoides	10	0	0
Gleditsia triacanthos	10	0	0
Tribe Cassiae			
Senna corymbosa	10	0	0
Senna spectabilis	10	0	0
Tribe Cercidae			
Bauhinia forficata	10	0	0
Sub Family Mimosoideae			
Tribe Acaciae			
Acacia aroma	10	0	0
Acacia caven	10	0	0
Acacia visco	10	0	0
Acacia dealbata	10	0	0
Acacia longifolia	10	0	0
Tribe Ingae			
Enterolobium contortisiliquum	10	0	0
Tribe Mimosae			
Anadenanthera colubrina var. *cebil*	6	0	0
Prosopis alba	10	0	0
Prosopis chilensis	10	0	0
Sub Family Papilionoideae			
Tribe Dalbergiae			
Geoffroea decorticans	10	0	
Tipuana tipu	10	0	0
Tribe Galegeae			
Sesbania virgata	4	0	0
Tribe Phaseoleae			
Erythrina crista-galli	10	0	0
Wisteria sinensis	4	0	0
Tribe Millettieae			
Lonchocarpus nitidus	10	0	0

3.11.5 Tests of larval development on living plants in Australian quarantine

3.11.5.1 Details on the quarantine facility and methods of containment

Initial studies were conducted in the Quarantine Insectary at CSIRO Long Pocket Laboratories, Indooroopilly, Brisbane. This was an AQIS approved facility (Approval Number is: Q0174, with classes of goods 95.4 Quarantine Insectary and 6.1 Closed Quarantine Facility for Medium Risk Nursery Stock). Precautions included HEPA air filtering, negative air pressure, filtering and chlorine treatment of waste water, air lock entrances, autoclaving or fumigation of waste materials.

On 2 March 2011, the colony was moved to our new premises, 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: Q2140, QC level: 5.3 and QIC level 7.3. All necessary movement permits were obtained. 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.

3.11.5.2 Materials and Methods

A shipment of approximately 200 eggs was received in Australian quarantine from a mix of locations in Argentina in February 2010. A colony was established which prospered for four generations from May until July 1010, providing adults which were used for host specificity testing in which between 1 and 4 replicates of 22 plant species were completed. The fifth generation of the lab colony was heavily affected by a *Nosema*-like microsporidian pathogen in August 2010. This invalidated the tests undertaken with this generation. The disease was severe and damaging to the colony, which took until December 2010 to recover following a strict hygiene regimen. The majority of remaining tests were done with the recovered colony in 2011. In April 2011, another shipment of 20 pupae from Argentina was imported and integrated into the quarantine colony to boost the genetic diversity generally and especially of genetic material from the north which was more closely climatically similar to the areas of *P. aculeata* infestation in Australia. The final tests done between June and August of 2011 used this mixed colony.

Laboratory no-choice larval survival was evaluated on 40 species of Leguminosae (Table 9). To obtain larvae for testing, eggs were collected from the colony and held in a petri dish until emergence of the neonate larvae. From these, 50 larvae were counted and placed on the

foliage of an individual test plant species growing in a pot (Figure 8). The plants were held for larval development in an aluminium frame cage lined with gauze and measuring 250 x 250 x 800 mm or 250 x 250 x 500 mm depending on the size of the plant. The cages were kept in a quarantine glasshouse to allow plants to maintain good condition. Plants were monitored regularly and extra plants of the same species were added if the larval feeding depleted the original 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.

One *P. aculeata* control plant and a variable number of plants for each other test species were used in each trial. For validity, the survival and development of the immature stages to adult on the control plant had to be confirmed. For immature stage viability, the rate of the eggs that resulted in emerged adults on *P. aculeata* was set at 30%. This figure was somewhat arbitrarily set but allows the exclusion of the one trial where adult survival was low on the control plant. A total of 27 trials were done to complete the tests. For each plant species, different individual plants were used for each replicate throughout all trials. 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.

Figure 8. Andrew White transferring newly hatched larvae of *Eueupithecia cisplatensis* onto a plant during no-choice tests in an Australian quarantine

3.11.5.3 Results

The Australian no-choice tests showed a consistent failure of larvae of *E. cisplatensis* to develop on any plant species other than *P. aculeata* (Table 9). No feeding or damage was observed on any non-target test plant species.

Table 9. Results of laboratory no-choice larval survival tests on *Eueupithecia cisplatensis* in Australian quarantine. A replicate consisted of 50 larvae on one plant in one cage.

Subfamily / Tribe / Group / Section / Genus/species	Replicates	% adult emergence (range)
Caesalpinioideae		
Caesalpinieae		
Peltophorum		
Parkinsonia aculeata	**29**	**56% (34%-86%)**
Peltophorum pterocarpum	4	0
Colvillea racemosa	4	0
Caesalpinia		
Caesalpinia ferrea	4	0
Caesalpinia pulcherrima	4	0
Dimorphandra		
Erythrophleum chlorostachys	4	0
Caesalpinioideae		
Cassieae		
Cassia brewsteri	4	0
Ceratonia siliqua	4	0
Chaemacrista mimosoides	2	0
Chaemacrista nomane	4	0

Subfamily	Replicates	% adult emergence (range)
Tribe		
Group		
Section		
Genus/species		
Labichea lanceolata	4	0
Petalostylis labicheoides	4	0
Senna artemisioides	4	0
Senna glutinosa	4	0
Senna notabilis	4	0
Cercideae		
Barklya syringifolia	4	0
Bauhinia hookeri	4	0
Detarieae		
Cynometra ramiflora	4	0
Intsia bijuga	3	0
Maniltoa lenticillata	4	0
Schotia brachypetala	4	0
Tamarindus indica	4	0
Faboideae		
Aeschynomeneae		
Aeschynomene americana	4	0
Bossiaeeae		
Hovea acutifolia	4	0
Desmodieae		
Desmodium tortuosum	4	0
Mirbelieae		
Pultenaea villosa	4	0

Subfamily / Tribe / Group / Section / Genus/species	Replicates	% adult emergence (range)
Phaseoleae		
Cajanus cajan	4	0
Robinieae		
Sesbania cannabina	4	0
Tephrosieae		
Millettia sp. McIlwraith	4	0
Vicieae		
Vicia faba L.	4	0
Mimosoideae		
Acaciae		
Acacia		
Acacia bidwillii	4	0
Botrycephalae		
Acacia decurrens	4	0
Acacia oshanesii	4	0
Juliflorae		
Acacia disparrima	4	0
Acacia julifera	4	0
Plurinerves		
Acacia melanoxylon	4	0
Phyllodineae		
Acacia salicina	3	0
Ingeae		

Subfamily Tribe Group Section Genus/species	Replicates	% adult emergence (range)
Archidendron lucyi	4	0
Pararchidendron pruinosum	4	0
Mimoseae		
Dichrostachys cinerea	4	0
Leucaena leucocephala	4	0

3.11.6 Discussion

Three types of methods were applied to evaluate the specificity of this agent. 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 and is hence very accurate.

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. This is a conservative test in the sense that it is extremely unlikely to under-estimate the host range. 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.

4 Where, when and how initial release will be made

4.1 Release from quarantine

Eueupithecia cisplatensis is currently being cultured within the quarantine facility at the EcoSciences Precinct, Dutton Park, Brisbane. Specimens of this culture will be deposited with AQIS and the Australian national Insect collection as voucher specimens. 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.

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 and the identity of the collected material will be confirmed by an authority on the group.

4.2 Distributing in the field

Eueupithecia cisplatensis 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" packages and starter colonies to interested parties.

4.3 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.

5 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.

6 Acknowledgements

I wish to sincerely thank the following people for their efficient and meticulous lab work in Australian quarantine: Andrew White, Gio Fichera, Areli Mira and Alexandre Marchal.

Fernando Mc KayMckay, Alejandro Sosa, Marcelo Parisi and Juan Briano of the USDA-ARS-SABCL, Buenos Aires, 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, John Scott and Louise Morin commented on drafts of the application.

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

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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

Table of 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 5). 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 5. 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.

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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 6). Adults typically grow to 5-7 m tall and wide (van Klinken et al. 2009a).

a)

b)

c)

d)

e)

Figure 6. *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 7). 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.

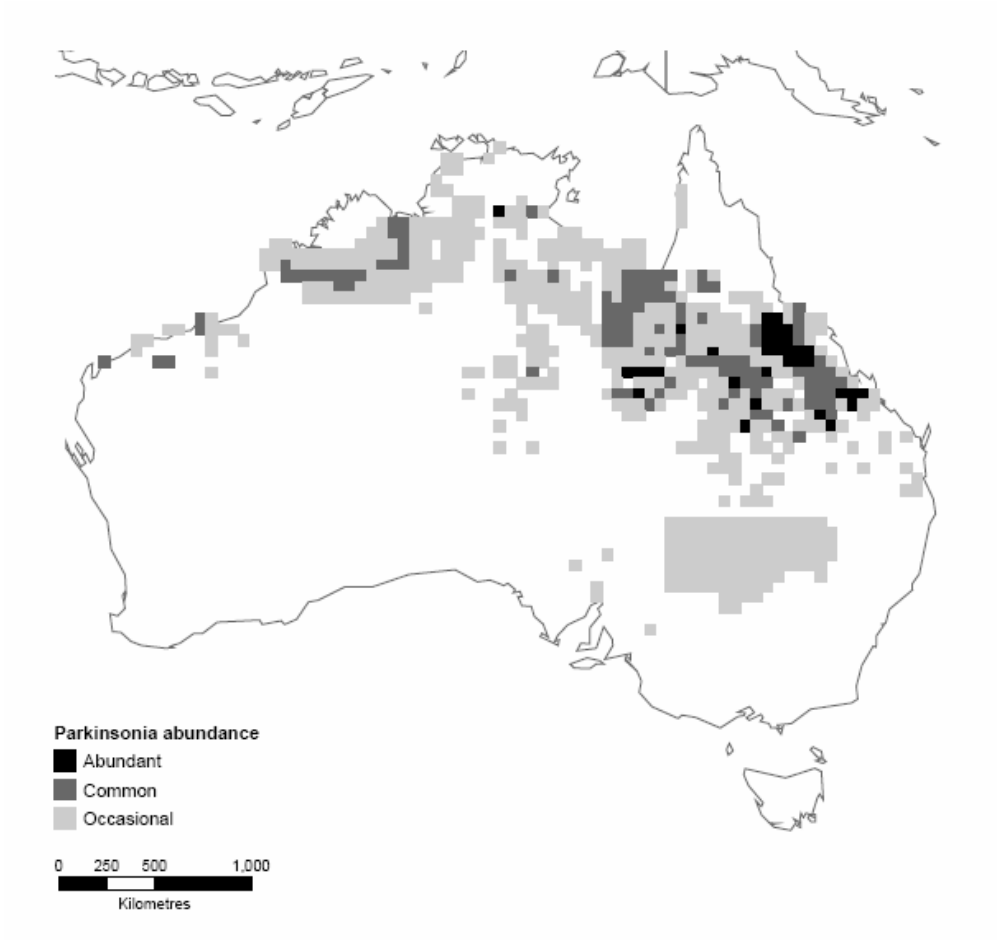

Figure 7. Current distribution and abundance of *P. aculeata* in Australia. Source: Queensland Biosecurity

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 8

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

Figure 8. *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 2, Figure 9, Figure 10). 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 11).

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

Table 2. Differential features between the two *Eueupithecia* species collected on *Parkinsonia aculeata*

	E. cisplatensis	***Eueupithecia* species 2**
Female genitalia (Figure 9)	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 10)	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 11)	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

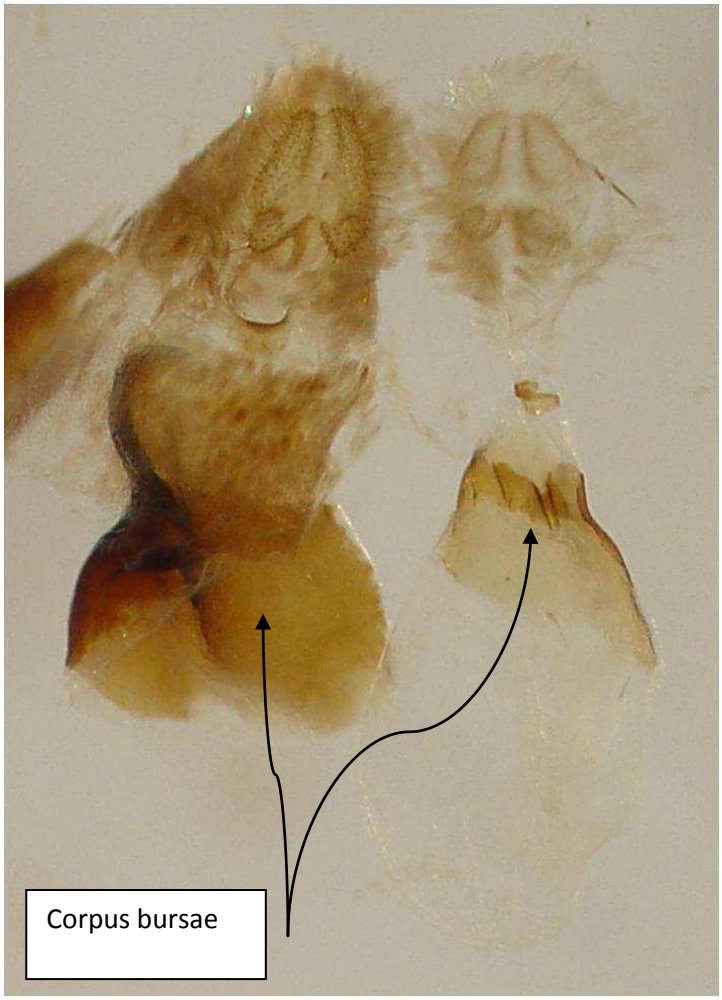

Figure 9. Female internal genitalia of *Eueupithecia* sp.2 (left) and *Eueupithecia cisplatensis* (right). Note the different sclerotisation of the corpus bursa

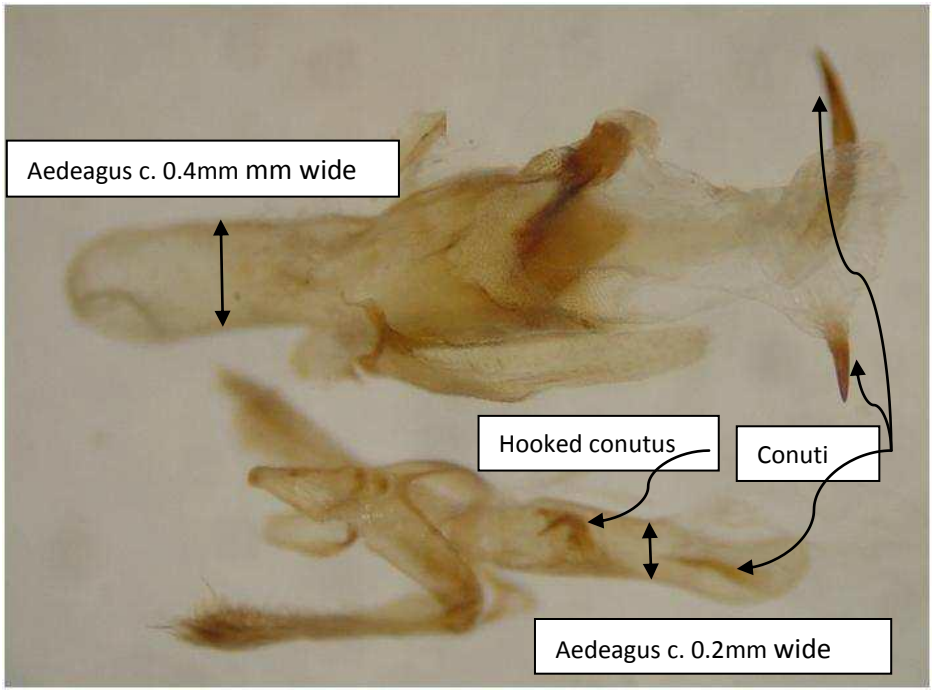

Figure 10. Male internal genitalia of *Eueupithecia* sp.2 (top) and *Eueupithecia cisplatensis* (below). Note the wider aedeagus and lack of a hook shaped cornutus in *Eueupithecia* sp.2.

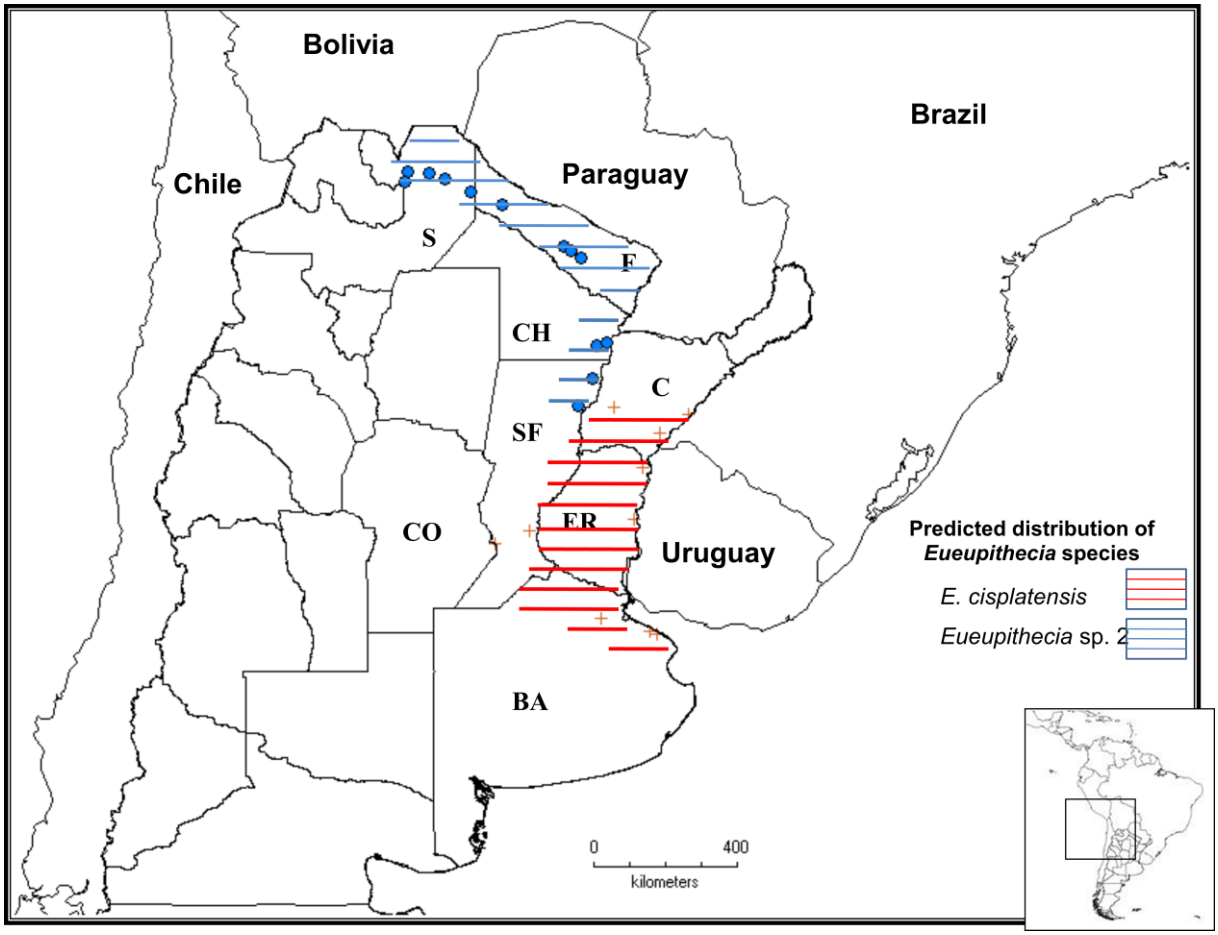

Figure 11. Distribution of *Eueupithecia* species in Argentina confirmed by genitalia dissections. Red crosses: *E. cisplatensis* localities. Blue dots: *Eueupithecia* sp.2 localities. BA, Buenos Aires; CH, Chaco; CO, Córdoba; C, Corrientes; ER, Entre Rios.

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 12). The eggs hatch and larvae begin to feed about 5 days after eggs were laid. Body colour of larvae ranges from green (Figure 13) to brown (Figure 14). 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 12. Strings of eggs of *Eueupithecia* sp.2 laid on paper

Figure 13. Green larva of *Eueupithecia* sp.2 on *Parkinsonia aculeata* leaf

Figure 14. 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 8). 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 11) 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 7). The climate of Emerald is closely matched to northwest Argentina where this agent was sourced (Figure 15).

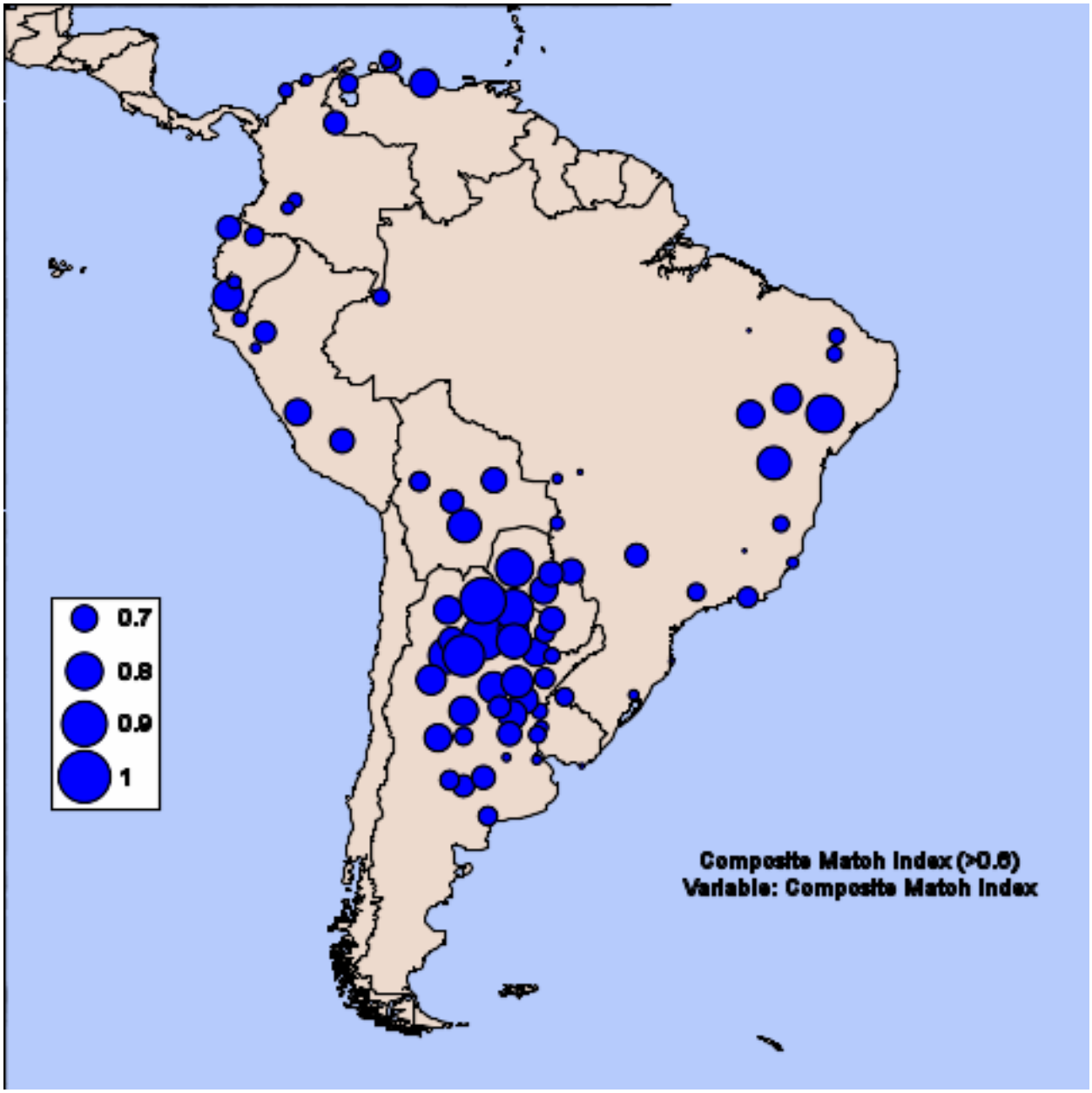

Figure 15. A climate match of South America with Emerald, Queensland, Australia, generated by the computer program Climex

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 3. 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 3).

- 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 3) 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 3. 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 16). 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 4). 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 16. FuEDEI researcher Fernando Mc Kay beating *P. aculeata* plants in northern Argentina

Table 4. 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 5) and Argentina (Table 6). 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 17). 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 5). No feeding occurred on any test plant species and hence no damage was observed on non-target species.

Figure 17.Test of larval development on cut plant material

Table 5. 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 3.

	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 6. 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 3.

Plant species	No. Replicates	%Survival to pupae	%Survival to adult
Parkinsonia aculeata	**11**	**76.4 (50-90)**	**67.3 (50-80)**
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 5). 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 18). When day lengths decreased, trials were conducted in quarantine controlled environment rooms under artificial lighting (Figure 19). 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 5). No feeding or damage was observed on any non-target test plant species.

Figure 18. Andrew White transferring newly hatched larvae of *Eueupithecia* sp.2 onto a plant during no-choice tests in an Australian quarantine glasshouse

Figure 19. 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.

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4 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

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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

8.3 A draft manual on the methods for rearing and release *Eueupithecia* spp.

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A manual for the Rearing and Releasing the parkinsonia looper, *Eueupithecia cisplatensis* (Lepidoptera: Geometridae), for biological control of the weed parkinsonia, *Parkinsonia aculeata* (Leguminosae: Caesalpinioideae) in Australia

Tim A. Heard, Andrew White, Gio Fichera, Ryan Zonneveld

CSIRO Ecosystem Sciences

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

Contact: Dr Tim A. Heard

Phone: 07 3833 5730

Mobile: 0434 416 053

tim.heard@csiro.au

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Tables

Summary

In this document, we provide the information needed to rear, release and monitor the new biocontrol agent for parkinsonia. Related topics such as plant propagation and insect disease identification and management are covered. In addition we provide some background and references to this topic.

Acknowledgments

We gratefully acknowledge the funding of Meat and Livestock Australia for their support for the search for biocontrol agents of parkinsonia in South America and their development in Australian quarantine. Researchers and field workers based at the Foundation for the Study of Invasive Species (Fundación para el Estudio de Especies Invasivas – FUEDEI) Buenos Aires, Argentina provided essential support in the native range.

1 Introduction

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

Three insect species have previously been released in Australia for biocontrol of *P. aculeata*. But only one, the seed-feeding bruchid *Penthobruchus germaini* Pic., from Argentina, established and dispersed readily. However, seed consumption rates are relatively low, and the agent is therefore unlikely to be causing any population-level impacts (van Klinken 2005, van Klinken and Flack 2008).

The defoliating looper caterpillar, *Eueupithecia cisplatensis*, nicknamed "uu", has been identified as a potential biocontrol agent of *P. aculeata* (Figure 20). Preliminary studies on its biology and host specificity made in Argentina, in the field and in laboratory conditions, strongly indicated specificity to *P. aculeata*. It was imported into an Australian quarantine where testing was completed on a broad range of plant species (Heard 2012). We concluded that the level of risk associated with releasing *Eueupithecia cisplatensis* into the Australian environment is acceptable and that it will potentially be an effective biological control agent for *P. aculeata*. We applied for and gained permission for its release in Australia in 2012. In 2013, we enter the release stage of the project.

A second agent, the closely related *Eueupithecia* sp.2, , nicknamed "u2", has been tested and also proved to be specific. An application has been made for its release. If successful, we expect it to be available for release in 2014.

Figure 20 Eight larvae, seven green one brown, of *Eueupithecia cisplatensis* on a damaged Parkinsonia leaf, most of the pinnules have been removed from the leaves and rasping of the leaf surface is visible on the leaf at the bottom

2 Information on Eueupithecia cisplatensis

2.1 Name and classification

Eueupithecia cisplatensis Prout 1910 (family Geometridae) (Figure 21)

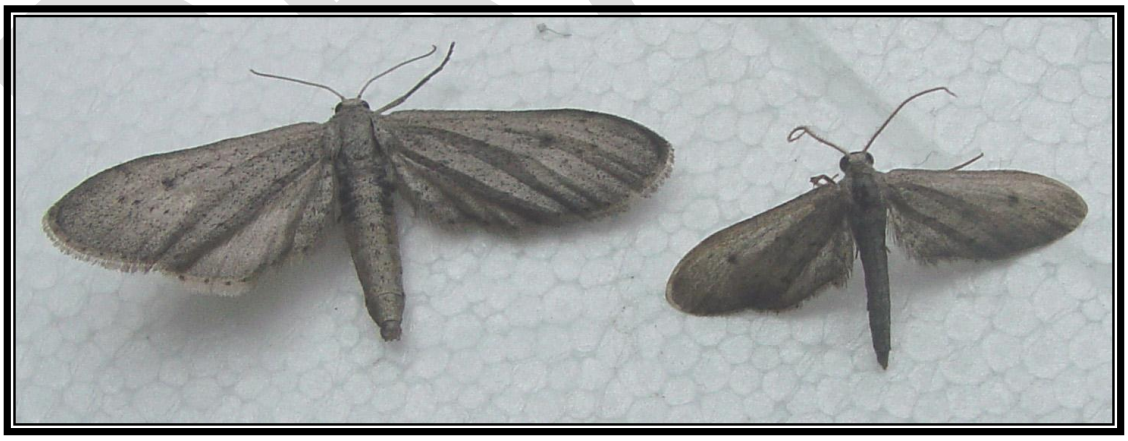

Figure 21 *Eueupithecia cisplatensis*, female left and male right

Eueupithecia is placed into subfamily Sterrhinae, tribe Sterrhini. Parsons et al. (1999) included only two species in the genus *Eueupithecia*. Geometridae specialist Dr. Axel Hausmann (Bavarian State Collection of Zoology, Munich, Germany) recently identified the second cryptic species. This species shows striking differences in internal genitalia of females (Figure 3) and males (Figure 4) and CO1 gene sequence (Table 1). The CO1 barcode gene differs by 4%, an amount that normally indicates a separate species. But no significant and

constant differential features in colour or pattern of adults or larvae have been found. The second species is distributed further to the north and west of *E. cisplatensis* (Figure 5). Both species are known from field surveys from Argentina.

Table 7 Differential features between the two *Eueupithecia* species collected on *Parkinsonia aculeata*.

	E. cisplatensis	*Eueupithecia* sp. 2
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

Figure 22. Female genitalia dissection of *Eueupithecia* spp. Left. *Eueupithecia* sp. 2. Right. *E. cisplatensis*. Note the difference in size and sclerotisation (darkening). The spermathecal sac is barely visible at the bottom of the image.

Figure 23 Male genitalia dissection of *Eueupithecia* spp. Top: aedeagus (to the left) of *Eueupithecia* sp. 2., Bottom: aedeagus (to the right) of *E. cisplatensis* specimen. Note the difference in size and armature.

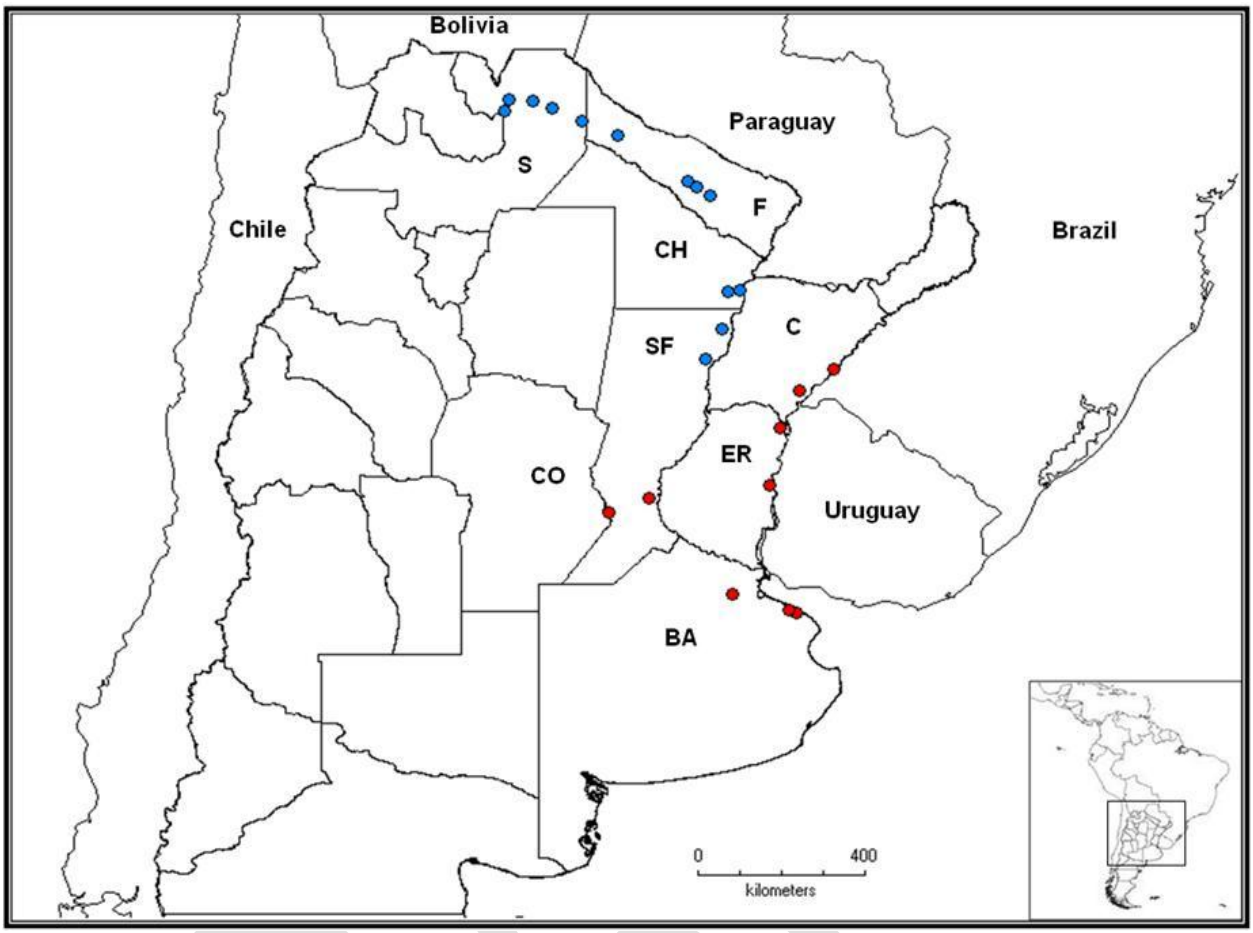

Figure 24 Distribution of *Eueupithecia* species in Argentina. ●: *E. cisplatensis* localities confirmed by genitalia dissections. ●: *Eueupithecia* n. sp. localities confirmed by genitalia dissections. BA, Buenos Aires; CH, Chaco; CO, Córdoba; C, Corrientes; ER, Entre Ríos; F, Formosa; S, Salta; SF, Santa Fé.

2.2 Biology of Eueupithecia cisplatensis

Brown or green cylindrical eggs, approximately 0.3 mm in length, are usually laid individually or in strings on the leaflets (Figure 25). Sometimes the green eggs are sterile (Figure 26). Fertile eggs hatch about 5 days after oviposition and larvae begin to feed immediatley. Body colour of larvae is variable but is usually brown or green (Figure 27). Larvae mimic leaf rachises and young shoots (Figure 28). As larvae develop, they eat most of the pinnules and parts of the rachises. The reduced number of prolegs results in the larvae progressing with a looping motion, hence the common name loopers.

Figure 25 Strings of brown eggs of *Eueupithecia cisplatensis* on *Parkinsonia aculeata* leaf.

Figure 26. Green egg of *Eueupithecia cisplatensis*

Figure 27. Green and brown larvae of *Eueupithecia cisplatensis*

Figure 28. Two young larvae of *Eueupithecia cisplatensis* on *Parkinsonia aculeata* leaf

Life stage duration. *E. cisplatensis* undergoes four larval instars. No overlap in head capsule width ranges was found, therefore they can be used to distinguish the instars (Table 2). Larval mortality was greater during the first and second instars and the survival to the adult stage was 42%. The duration of the stages was approximately 5 days for eggs, 17 days for larvae and 4 days for pupae.

Table 8. Life stage duration and larval head capsules width of *Eueupithecia cisplatensis* on *Parkinsonia aculeata*

Stage	n	Life stage duration (days)		Cumulative survival (%)	Head capsule width (mm)	
		Mean ± SD	Range		Mean ± SD	Range
Larva 1st instar	43	5 ± 0.24	2-8	100	0.26 ± 0.01	0.23-0.26
Larva 2nd instar	28	3 ± 0.46	1-14	65	0.42 ± 0.03	0.33-0.42
Larva 3rd instar	22	4 ± 0.21	2-7	51	0.68 ± 0.0	0.62-0.72
Larva 4th instar	21	5 ± 0.28	3-9	49	1.04 ± 0.06	0.91-1.11
Larva total	21	17 ± 3.1	13-27	49	-	-
Prepupa	21	2 ± 0.11	1-2	49	-	-
Pupa	21	3 ± 0.11	3-15	49	-	-
Adult	18	4 ± 0.11	1-13	42	-	-

Female longevity and fecundity. Preoviposition period is 1-2 days, fecundity is around 80 eggs per female and the longevity of females is about one week.

Adult females are bigger than male, with a wider abdomen. The morphology of the antennae also shows sexual dimorphism being bristly in the male and smooth in the female (Figure 29).

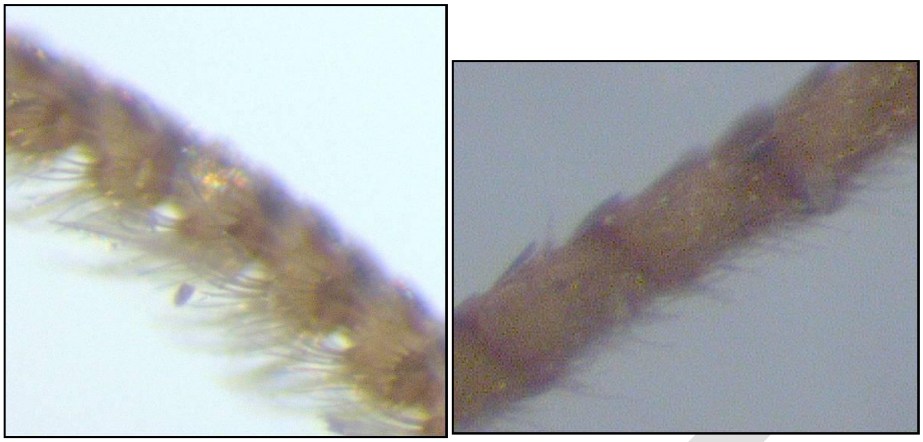

Figure 29. Left: pectinate and bristled antennae of males. Right, smoother antenna of female.

Natural enemies. In the native range, two species of *Conura* (Hymenoptera: Chalcidoidea) (Figure 30) emerged from cocoons, and probably parasitised the larvae. These were eliminated from the colonies in quarantine and so do not occur in Australia. It is however possible that parasitism of larvae will occur in Australia by native parasitoids such as braconids, chalcoids and tachinids.

Figure 30. Left: One of two species of *Conura* (Hymenoptera: Chalcidoidea); Right: Unidentified Braconidae parasitoid of *E. cisplatensis*.

3 Rearing

3.1 Rearing insects

Eueupithecia cisplatensis is currently being cultured at the EcoSciences Precinct, Dutton Park, Brisbane. Culturing is relatively easy. The following steps describe a rearing method.

Collect adults daily as they emerge from the larval colony. This avoids eggs being laid in cage which results in an unwanted uncontrolled next generation population in the cage. Sex the adults by observing their shape (Figure 31) or if uncertain, by observing their antennae under magnification (Figure 10).

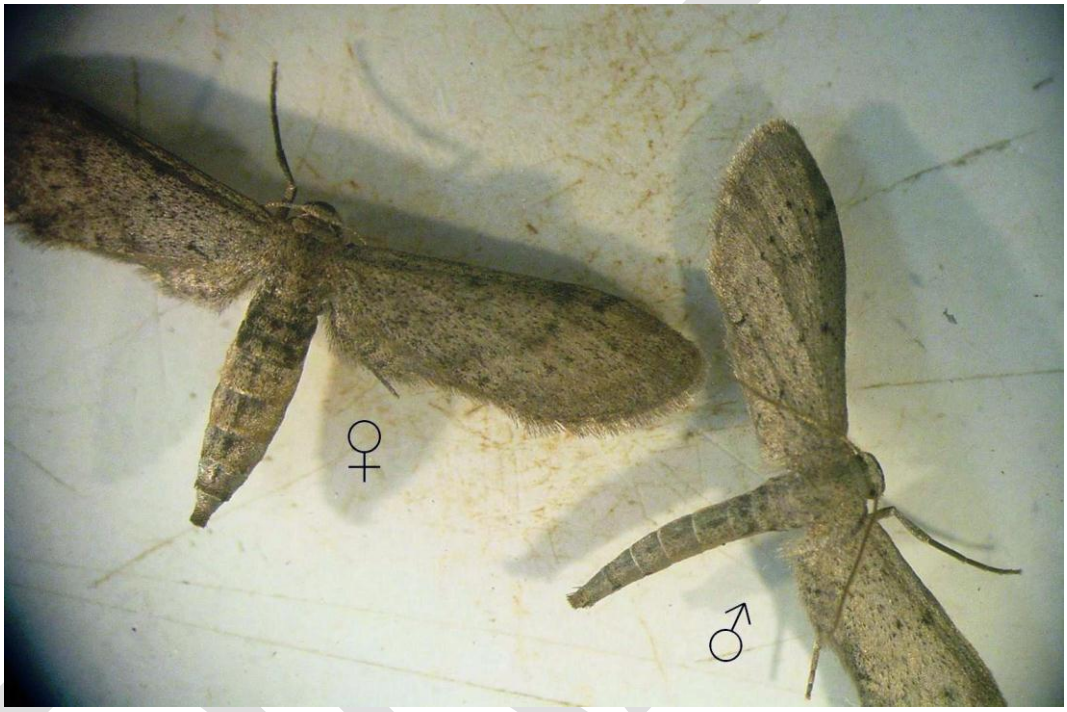

Figure 31. The two sexes of *Eueupithecia cisplatensis* showing the difference in abdomen shape and size.

Prepare mating and egg-laying containers. Place paper towel on base of container (e.g. take-away food container). Cut section out of lid. Place second piece of paper towel over the box and secure with lid (Figure 32). Place two to three pairs of adults into plastic containers. Eggs are laid mainly on top piece of towel. Allow eggs to incubate in containers.

Figure 32. Bottom and top views of food container used for mating, egg laying and incubation of larvae.

Watch the eggs daily and collect larvae when they hatch. If left more than 12 hours, they will die.

Using a fine brush, bristles moistened with water, transfer about 50 larvae onto a living parkinsonia plant (Figure 33).

Figure 33. Transferring newly hatched larvae onto a plant with a fine brush

Alternative method: Place a sprig of parkinsonia in the egg-laying container. When the eggs hatch the larvae will move to the sprig. They should live on the sprig for several days if the sprig is well set up in a vial of water (Figure 34). Then move the sprig to a cage. We recommend that you count the larvae on the sprig before moving and do not overload the cage with more than 50 larvae.

Figure 34. An egg laying container with sprig

Place plant in a cage to contain the larvae and emerging adults. We use an aluminium frame cage lined with gauze and measuring 450 x 450 x 900 mm (Figure 35). Add more plants to the cages as needed as the larvae develop. Larvae feed, grow, develop, pupate in the cage. The prepupae typically spin cocoons in folds in the gauze roofs of the cage and in concealed positions (Figure 36).

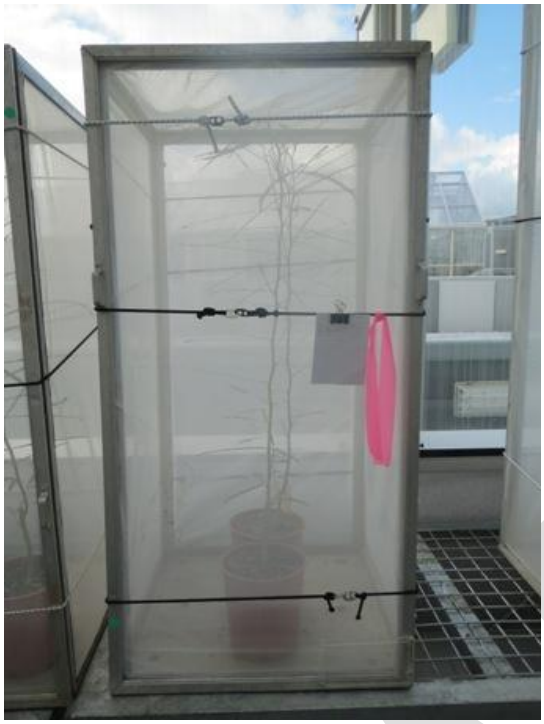

Figure 35. A rearing cage of *Eueupithecia cisplatensis*, the larvae have striped the plants of many of their leaves and the resulting adults will soon emerge.

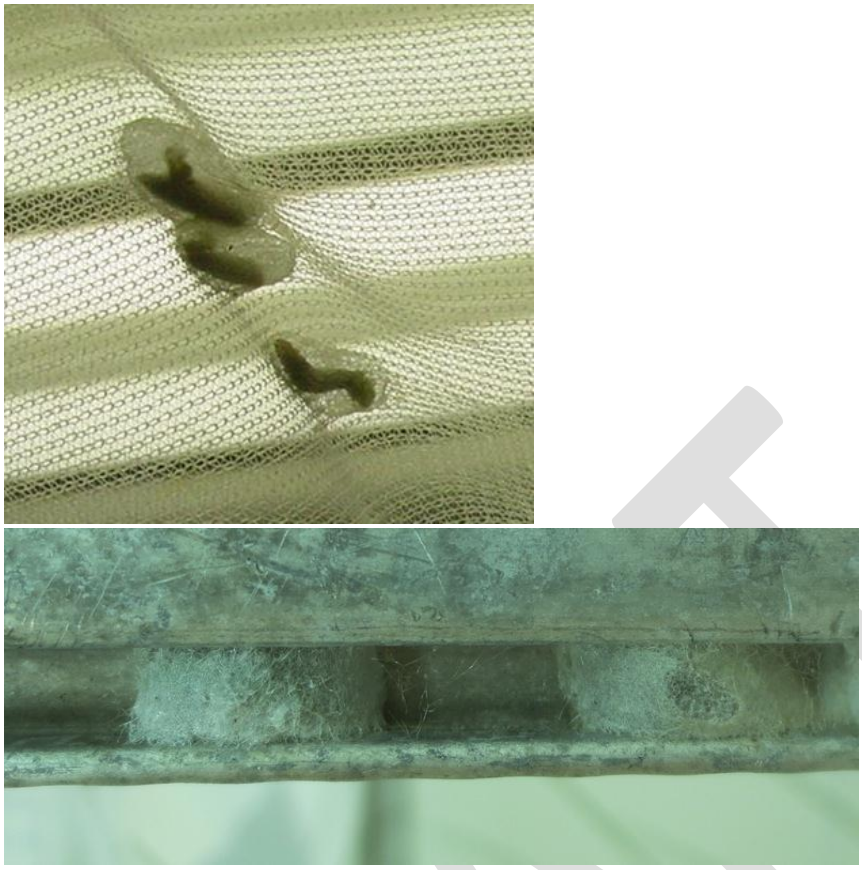

Figure 36. Top: Two pupae and a prepupa of *Eueupithecia cisplatensis* spinning cocoons in folds in the gauze roofs of the cage. Below: two cocoons in a gap in cage frame.

Adults emerge and the cycle begins again with pairs placed into food containers. It is important maximise genetic diversity and colony vigour by minimising inbreeding. This is achieved by pairing females and males, at each generation, that have emerged from different cages which further avoids mating between siblings. In addition, we can avoid potential laboratory adaption by making new importations and incorporating this fresh genetic material into lab colonies.

3.2 Plant propagation

The larvae are voracious feeders and completely strip potted plants of all foliage. Plants can be recycled after use by allowing a growth recovery period following pruning, pest treatment and re-fertilisation. However a large stock of plants (Figure 37) is required continuous culture of a large insect colony.

Figure 37 Benches of healthy parkinsonia plants ready to be used for insect production

3.3 Pathogens

Outbreaks of disease are occasionally observed in the larval stage. The larvae show symptoms of disease may die (Figure 38). These larvae are infested with a pathogen that resembles a microsporidium (Figure 39).

Figure 38 Dead and diseased *Eueupithecia cisplatensis* larvae.

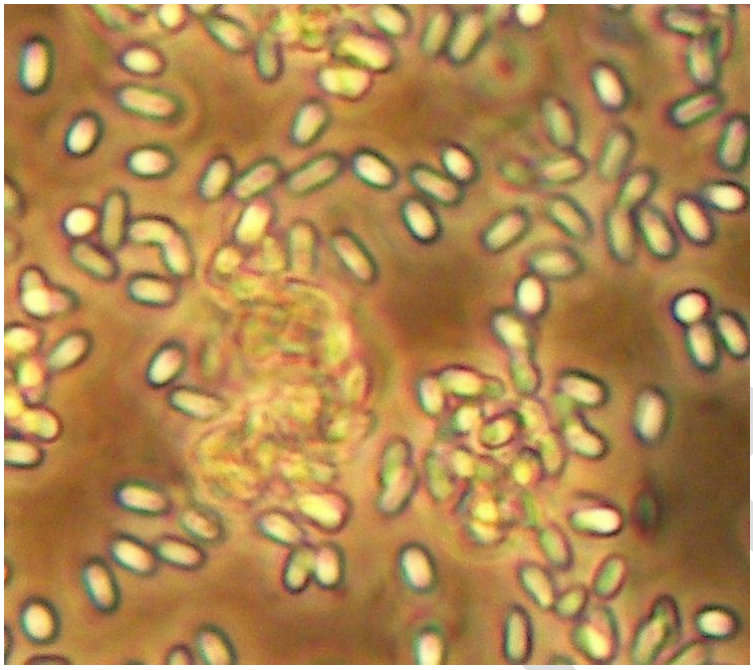

Figure 39 Pathogens observed in a squash of a diseased *Eueupithecia cisplatensis* larva.

The pathogen can be controlled by maintaining low densities of larvae, ample food (minimising contact with contaminated frass) and by high levels of hygiene. In particular, cages, containers and any other equipment must be thoroughly washed and regularly treated with a sterilizing agent such as bleach (sodium hydroxide??/or hypochlorite, 1% solution), before being reused. Hose the cages to remove all traces of plant material, excrement, soil, cocoons, etc. The cages should then be treated with the sterilizing agent. Hand held steam (**not** hot water vapour) sterilization is being evaluated as a suitable alternative to chemicals.

4 Field Release

4.1 Field release

THIS SECTION IS TO BE EXPANDED

Eueupithecia cisplatensis will be distributed to selected sites throughout the weed's range in Australia. It is expected that state and territory government departments, community groups such as Landcare, Bushcare, NRM and CMA groups and schools may contribute to this distribution.

Insects for release may be obtained from local colonies or from shipments from remote colonies such as Brisbane EcoSciences precinct.

Two release methods are proposed.

1. **Release young caterpillars**

   Containers of fresh leaf material are placed in the oviposition boxes in which adults have laid eggs. When the eggs hatch, the larvae move to the fresh plant material. They can survive on this material for several days. In this time, the leaves can be transported to the field and tied onto parkinsonia trees. The larvae will move from the old wilting leaves onto the surrounding living leaves and complete their development there. Any remaining adults can also be released at the same time. To further protect larvae, they can be placed on high branches pulled down and tied together with wire, rope, zip ties, etc. The sprig of larvae can then be inserted into the tied branches. By tying the leaves together you provide protection for the larvae from the elements, prevent the sprig from being dislodged from the tree and also give ready access for the larvae to fresh leaf material.

   This method requires more field time than the adults release method explained below.

2. **Release adult stage**

   Pupae remain in their non-feeding, immobile stage for approximately one week. They can be easily kept and transported in this stage, making them convenient to place in the field for adult emergence and release. Emerging adults can then find mates and the females find their host plants and lay eggs. The ideal containers for protecting the pupae and allowing adults to emerge is one that sheds water, provides shade and protection from predation and allows the escape of emerging adults. We recommend delta traps (illustrated). These are light and easy to transport and can be re-used. The traps need to be carefully placed in a tree. It does not need to be a parkinsonia tree, but another species within a patch of parkinsonia. The trap is attached to a branch of the tree with its wires allowing it to hang horizontally. The trap should be placed so that no branches will come into contact with it. Nearby branches may need to be removed to achieve this. A sticky material like Vaseline, stickum, or tanglefoot should be smeared onto the wire to prevent ants and other predators from reaching the traps. The trap should be placed high to avoid damage by animals or humans. The trap should be placed out of sight of the public.

   It is quicker to set up these devices in the field compared to larval releases. But it is time-consuming to find and remove the pupae in their cocoons in the rearing cage.

All releases should be made in areas with good numbers and density of parkinsonia trees. Releases should be made when the parkinsonia has young leaf growth as young leaves are the food that will give best survival and growth of larvae. Mark trees with flagging tape and record coordinates with GPS.

4.2 Evaluation of establishment

THIS SECTION IS TO BE EXPANDED

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.

The easiest way to determine the presence of the insect is to use a beating method. Lay a large beat sheet on the ground under a tree and beat the branch with a stout stick (Figure 40).

Figure 40 Researcher beating *P. aculeata* plants in northern Argentina to collect larvae of *E. cisplatensis*

References

Heard, T.A. (2011) Application to release the defoliating caterpillar Eueupithecia cisplatensis (Lepidoptera: Geometridae) for biological control of the weed Parkinsonia aculeata (Leguminosae: Caesalpinioideae). Unpublished proposal/report to AQIS, 2011-10-26.

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Dr Tim Heard

Phone: 07 3833 5730

Mobile: 0434 416 053

tim.heard@csiro.au

andrew.white@csiro.au

Gio Fichera

Phone: (07) 38335697;

Mobile: 0404957180

Email: Gio.Fichera@csiro.au