final report

NATURAL RESOURCE MANAGEMENT

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Development of new biocontrol agents of Bellyache bush and Parkinsonia

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Abstract

This project pushed the prospects for biocontrol of the weeds bellyache bush and parkinsonia to a higher level. 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. Our MLA funded project surveyed new areas of South America, identified the entire insect fauna and assessed their potential for release in Australia. Before this project, we had not identified any high potential agents, now we have three agents for parkinsonia that have a high probability of being suitable for introduction into Australia. We have been less successful with bellyache bush but at least we now know with certainty that this target weed has few prospects. The techniques we developed in this project will assist future biocontrol projects to achieve their objectives more quickly. We are now beginning the next phase of this project which is to complete the evaluation of the insect species in Australian quarantine. 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.

Executive summary

This project significantly advances the development of new biocontrol agents for two of northern Australia's more invasive and damaging plant species, bellyache bush (*Jatropha gossypiifolia*) and parkinsonia (*Parkinsonia aculeata*). These plants species have been the subjects of biocontrol previously. Both have agents released against them but all are ineffective. Hence searches for new agents had commenced 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. This project surveyed in new areas in South America, identified the entire insect fauna, which consisted of hundreds of species for each plant, and assessed their potential for release in Australia.

Before this project, we had not identified any high potential agents, now we have three agents for parkinsonia that have a high probability of being suitable for introduction into Australia. This short-list was determined after the careful screening of a large number of organisms both in the field and in the laboratory in Mexico and Argentina. We have been less successful in identifying any high potential agents for bellyache bush but at least we now know with certainty that this target weed has few prospects.

In this project we developed techniques to determine whether the extent of sampling was sufficient. We showed that the sampling of the arthropod species using *Parkinsonia aculeata* in both space and time, across its entire native range was not complete but that the species not yet found were probably not sufficiently abundant or closely associated with the plant to be of interest to us. Several biogeographic areas remain to be sampled intensively including Peru and NE Brazil. Surveying of *J. gossypiifolia* was closer to completion and no biogeographic areas remain to be sampled. The techniques we developed in this project will assist future biocontrol projects to achieve their objectives more quickly.

The project demonstrated the application of molecular biology to the search for insect biocontrol agents. We determined that DNA bar-coding is of limited application as the quality of the DNA in older specimens is not adequate for sequencing the gene region used for bar-coding. But we used shorter fragments to search for cryptic speces of seed feeding bruchids and to solve a taxonomic problem with a tip moth, *Calosima*. We make recomendatios for the future with regard to preserving specimens to make them more amenable to molecular studies.

We are now ready to begin the next phase of this project which is to complete the evaluation of the insect species in Australian quarantine. The highest priority agent, the looper *Eueupithecia cisplatensis*, has been exported by collaborators based at the USDA station in Argentina. The material has been received into the CSIRO quarantine facility in Brisbane and is being successfully reared and tested. The basic biology, the methodology for rearing and testing, and the preliminary host tests have already been completed for this species. Plans have been made for export of the second species, the stem borer, *Ofatulena luminosa* from the CSIRO station in Mexico and the third, the gall fly *Neolasioptera* sp. from Argentina. The methodologies are currently being developed in the native range stations for these insect species. Plants of Parkinsonia and various test plant species have been grown in readiness for the trials. A host specificity test list of about 50 plant species is being finalised. When the tests have been completed, a proposal for the release will be made based on the results of this research. The assessment process by the Federal Governemnt departments takes 1-2 years.

No immediate impact will result from the work covered in this project as the time frame for biocontrol projects is very long. However, in five years time we expect impacts will start to accrue. We expect that two to three new biocontrol agents of parkinsonia will be released into the

Australian environment. It may take many years for the full impact of the released agent to be realized. However, once in place, the benefits are self-sustaining, permanent, ecologically non-damaging, economically beneficial and do not require the continuous input of land managers.

We make the following recommendations for future work:

1. Complete the evaluation of the top three agents of parkinsonia including an application for their release. Funding for this is provided in the already commenced MLA funded project B.NBP.0620.
2. Upon receiving approval, mass-rear and release those parkinsonia agents widely in the Australian environment to gain maximum probability of establishment and rapid spread. We expect that various agencies such as state government departments and land-care groups will participate in this process
3. Complete evaluation of the last of the bellyache bush agents, although these prospects have a low probability of success. We will apply for funding to do this through the AWRC round of funding.
4. Survey the last areas not completed for parkinsonia which is NE Brazil and Peru. These areas are isolated from other areas surveyed and may yield further agents. We will apply for funding to do this through the AWRC round of funding.

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1 Background

MLA had identified the useful role of biological control in the management of weeds that impact on the northern beef industry. MLA had also prioritised bellyache bush and parkinsonia as two of the most serious of these weeds and two with the best prospects for biocontrol [1]. Agents had been released on both these target weeds in the past but they had either not established or were not effective. In 2006, MLA has made a call, through the Weeds CRC, for projects on this topic. A proposal was developed and was accepted for funding. The original contract was signed with the Weeds CRC. When this CRC failed in its rebid, a new contract was developed directly with CSIRO.

This project was developed as part of a package, co-funded by MLA (bellyache bush and parkinsonia), Qld Govt then NRMW now DEEDI (bellyache bush agent evaluation), Australian Government Defeating the Weeds Menace (DWM) (parkinsonia) and CSIRO (Brisbane and Mexico, bellyache bush and parkinsonia). It planned to draw on a range of modern techniques that will help identify the most efficacious agents, and assist in fast-tracking native-range survey work, and the processing of potential agents through quarantine.

The MLA funds were allocated to the task of synthesizing the results from extensive native range surveying of bellyache bush agents (6 years of collections) and parkinsonia agents (5 years) in Central America and Venezuela, and developing a prioritised list of agents. This included the use of molecular techniques to help sort and identify the large suite of unidentified insects already recorded from both target weeds, and the preliminary host specificity testing of potential bellyache agents at the Mexican field station. MLA funds were also allocated to survey for potential agents of bellyache bush in South America (including Peru, Ecuador, Brazil, Argentina, Paraguay), areas not previously covered. Surveys are costly, but were optimised using data on plant distributions, biogeography, climate and other variables. This survey work was done in tandem with DWM-funded parkinsonia surveys conducted in the same areas. Efficiency and hence cost savings were gained by working on both weeds simultaneously as their native ranges overlap, hence work on both species were made on single trips, using single permits, etc. Queensland government contributed by conducting host-specificity testing of potential bellyache bush agents in quarantine in Australian and funded work by CABI in the UK. They also provided additional funds to CSIRO to provide test insects, culturing techniques and relevant native-range data.

This is phase one of the project. Phase two commenced in April 2010 and is focussed on the next stage of biocontrol research which is processing the agents through Australian quarantine and assessing their suitability for release into the Australian environment.

[1]Grice, A.C. (2002) Weeds of significance to the grazing industries of Australia, Final report. The Co-operative Research Centre for Australian Weed Management report to Meat and Livestock Australia

2 Project objectives

By 1 August 2010 the project will have:

- Produced a comprehensive list of natural enemies, identified to “species” that are present on bellyache bush and parkinsonia in the Americas (North, Central and South)
- Identified the top ten potential biocontrol agents for bellyache bush and parkinsonia, prioritised on the basis of likely efficacy and likely host-specificity

- Conducted preliminary screening (in either the Mexican Field Station or Australian quarantine) of five of those potential agents for each weed and identified which are most likely to pass comprehensive host-specificity testing in quarantine
- Initiated testing of at least two bellyache bush agents, and for those that were host specific, sought approval (from AQIS and EA) to commence mass rearing and release activities (NRM&W)
- Completed preparations to commence detailed host specificity testing of the two best potential biocontrol agents for parkinsonia in quarantine (with approval sought for release of the first agent by 2010). Note this objective will be dependent on NHT funding to commence quarantine work by July 2008.
- Made progress towards halving the time required to comprehensively survey native enemies for new tropical weed targets, through scientific improvements in the way surveys are designed, conducted and analysed
- Made progress towards demonstrating the value of a technique that combines morphological characters and DNA bar-coding to rapidly characterise surveyed natural enemies

3 Methodology

3.1 Objective 1: Produce a comprehensive list of natural enemies present on bellyache bush and parkinsonia in the Americas

3.1.1 Bellyache bush

Surveys for natural enemies were primarily conducted by staff at the CSIRO Mexican Field Station particularly Ricardo Segura, Moisés Martínez, Manuel Juárez, Carlos Pascacio and Quiyari Santiago in the period 1996 to 2010. Occasionally Australian based scientists participated in the surveys especially Wendy Forno and Tim Heard. In the search for potential agents against *J. gossypiifolia*, we have made over 500 collections in approximately 270 locations in 14 countries (Mexico, Guatemala, Nicaragua, Costa Rica, Dominican Republic, Puerto Rico, Netherlands Antilles, Trinidad, Cuba, Venezuela, Ecuador, Peru, Bolivia and Brazil) (Heard *et al.* 2011).

3.1.2 Parkinsonia

In the search for potential agents against *J. gossypiifolia*, we have made 443 collections in approximately 251 locations in 12 countries (Mexico, Guatemala, Nicaragua, Costa Rica, Dominican Republic, Puerto Rico, Venezuela, Ecuador, Peru, Paraguay, Brazil, Argentina). Approximately half of these collections have taken place in the period of this MLA funded project.

3.1.3 Identifications

Initially we intended to overcome the existing taxonomic impediment that has prevented identification of ca 90% of fauna collected since 1998, relying on a combination of expert taxonomists and DNA bar-coding to assist in species delineation of material collected throughout the Americas. However, the DNA bar-coding aspect of this project has not proven to be the valuable tool that we expected. This was largely due to the poor quality of the DNA in our preserved specimens. But identifications using traditional morphological techniques have proved adequate in most cases. We recruited an insect taxonomist in Mexico to work full time on sorting the collected material into families, finding the most appropriate taxonomist in the world, sending

the material to them, receiving the identifications, and re-sorting the specimens based on the specimens and keys provided. All data was entered into a database for later ease of access.

3.2 Objective 2: Identify the top ten potential biocontrol agents for bellyache bush and parkinsonia

The process of prioritising biocontrol agents is an iterative one that involves scanning lists of identified specimens and first doing literature searches on those species. This is sometimes adequate to eliminate species that are known from the literature to be polyphagous (feeding on a broad range of plant species) or not even phytophagous (eating for example dead plant material, or predators or parasites). However, for most specimens either the species identity is not certain and so a literature review does not help or there is no information available in the literature. In this case, it is necessary to conduct basic biological and host specificity studies. These studies cannot be conducted on all species and so first a screen is done to determine which species are sufficiently abundant for further work. If a species has only been collected once or twice, it is not a likely to be a useful candidate. Similarly we prioritise agents to determine their damage to the plant, with only damaging species passing this screen. If a species is determined to be abundant and damaging, then they may be selected for further studies. This is the subject of the next objective.

3.3 Objective 3: Conduct preliminary screening of five of those potential agents for each weed

The nature of these studies depends on the biology of the species involved and has to be designed for each one. It typically consists of a study into its life-cycle, whether it can be reared in vitro or needs to be studied in the field, and preliminary studies into its host specificity. The host specificity studies are conducted using the plant species available in the native range, which may not be representative of the species that are most at risk in Australia. That is why the next step is to import them into Australia for complete testing, see next section.

3.4 Objective 4: Initiate testing of at least two bellyache bush agents, and for those that were host specific, seek approval to commence mass rearing and release activities

This work was conducted by QDEEDI and took place in the quarantine facility is at the Queensland government's Alan Fletcher Research Station. This facility is not approved for pathogen agents, and so work on the pathogen was commissioned by DEEDI to be done at the quarantine facility of CABI, UK.

Testing methods are then designed and conducted against an approved list of plant species that are representative of the Australian flora (Heard et al. 2009). At the completion of these tests, an application was to be made to release the agent to the Australian government departments of Agriculture and Environment, currently DAFF and DEWHA. However, this stage was not reached due to the lack of host specificity of these agents.

3.5 Objective 5: Complete preparations to commence detailed host specificity testing of the two best potential biocontrol agents for parkinsonia in quarantine

Permits are gained from Australian authorities to import into an approved quarantine. In the case of parkinsonia agents, the quarantine facility is at the CSIRO's long Pocket Labs.

Testing methods are then designed and conducted against an approved list of plant species that are representative of the Australian flora. At the completion of these tests, an application is made to release the agent to the Australian government departments of Agriculture and Environment, currently DAFF and DEWHA.

3.6 Objective 6: Make progress towards halving the time required to comprehensively survey native enemies for new tropical weed targets

We examined data from surveys of the phytophagous arthropod fauna of the *P. aculeata* to test for survey completeness. Across geographic space, we used survey gap analysis, to determine to what extent existing arthropod surveys on *P. aculeata* sample the complete environmental diversity covered by the plant. Within biogeographic areas, we determined survey completeness through time based on species accumulation curves and comparisons of predicted species-richness to sampled species-richness (Bell *et al*. 2010).

3.7 Objective 7: Make progress towards demonstrating the value of a technique that combines morphological characters and DNA bar-coding to rapidly characterise surveyed natural enemies

We attempted barcoding in an attempt to get through the backlog of specimens that couldn't be identified by taxonomists. Barcoding involved sequencing a specific section of mitochondria DNA from the CO2 gene region and comparing it to other related species. The DNA was extracted and amplified at the CSIRO labs and sent for sequencing overseas.

4 Results and discussion

4.1 Objective 1: Produce a comprehensive list of natural enemies present on bellyache bush and parkinsonia in the Americas

4.1.1 Bellyache bush

Jatropha gossypiifolia occurs from Florida, USA south to Brazil. Due to its widespread dispersal by humans, it is difficult to know what is the native range and centre of origin. It thrives in human disturbed areas facilitating its spread and further obscuring its origin. It is common in Mexico, Central America and the Caribbean. In South America it occurs predominantly in drier areas around the Amazon basin as far south as Argentina. However, we believe that it is not native to most of South America as it appears to be strictly associated with human disturbance there, with no populations found in natural areas. Furthermore the fauna is very poor indicating recent establishment in this area. Limited genetic differentiation occurs across the native range, a fact which is attributed to human translocation. We speculate that its native range is the countries surrounding the Caribbean Sea, including Mexico, Central America, the northern coast of South American and the Caribbean Islands.

Several thousand specimens resulting from the surveys for natural enemies have been collected, curated, databased and sent for identification. Species were classified as phytophagous or non-phytophagous based on field observations, rearing from plant material and information in the literature. A total of 272 probable herbivore species or morphospecies have been recorded, with 212 species likely to be feeding on bellyache bush (that is, not just visiting the plant) (Appendix 1). Most of these are rare with only 73 species collected more than twice.

4.1.2 Parkinsonia

The total phytophagous fauna of *P. aculeata* can be estimated from the results of the unpublished USDA surveys by Hugo Cordo in Argentina and Paraguay, the surveys of Woods (1988) in USA, Mexico and Costa Rica, and surveys done as part of this project. Hugo Cordo collected 36 insect species in Argentina, William Woods collected 142 insects and two mites, and CSIRO collected at least 250 insect and one fungus species (Appendix 2). The total identified fauna is at least 353 species. However, most species (*c*. 53%) were rare on parkinsonia, with only one or two specimens collected. This rarity is reflected by field observations that most parkinsonia plants surveyed had few natural enemies present, and they rarely showed heavy damage to any plant parts. Analyses using species accumulation curves suggest 48% of all natural enemies on parkinsonia have been collected, but 90% of the common species have been and these are the ones most likely to be useful biocontrol agents (Bell *et al*. 2010b),

4.2 Objective 2: Identify the top ten potential biocontrol agents for bellyache bush and parkinsonia

A "top ten" list of potential biocontrol agents should never be regarded as static but as work-in-progress. As more is learnt about potential agents on and off these lists, they may be eliminated from or added to the list.

4.2.1 Bellyache bush

Table 1. List of the top potential biocontrol agents of Bellyache bush

Species	Notes	Status
Phakopsora jatrophicola	A rust fungus	Testing in UK
Euxestha abdominalis and *E.* aff. p*anamena*	Leaf & stem tip mining larvae	Seeking population for further testing
Pityophthorus sp.	A tip borer	Seeking population for further testing
Pachycoris prob. *fabricii*	A seed sucker	Seeking population for further testing
Ormiscus/Eusphyrus	Tip borer	Seeking population for further testing
Cerambycidae spp.	Stem borer	On hold
No other potential agents known		

4.2.2 Parkinsonia

In this project, considerable effort has gone into short-listing those species with most potential as biological control agents. Very rare species were not considered further as they are unlikely to be effective biocontrol agents for both practical and theoretical reasons. Review of the literature and host data from insect labels or records of taxonomists identified species that are generalists or lacking adequate host specificity and these were excluded from further consideration. Of the 90 arthropod species for which we had good data, only 3.3% were considered specific to the genus *Parkinsonia*, significantly lower than on other host plants examined (Bell et al 2011). This host-specificity data has been supplemented by detailed surveys on *P. aculeata* and related co-occurring species at a field site near Tampico (Mexico, 2007-2010). In addition, some species have been studied in the laboratory and associated gardens and subsequently rejected (see “Tested and rejected” below). Finally, some species were ranked as low priorities as they were never observed to cause significant damage. The current “top ten” is shown in Table 2.

Table 2. List of the top potential biocontrol agents for parkinsonia

Species	Notes	Status	*Origin
Eureupithecia cisplatensis	Defoliating looper caterpillar	Testing in Argentina	8
Ofatulena luminosa	Stem borer	Testing in Mexico	2
Neolasioptera sp.	A gall fly that attacks growing tips	Testing in Argentina	8
Agrilus parkinsoniae	Stem borer	Testing in Mexico	2
Nr *Rudenia leguminana*	A defoliating / flower bud feeding caterpillar	Testing in Mexico	2,3,5
Glyptoscelis sonorensis	Defoliating leaf beetle	Testing in Mexico	3
Cerambycidae spp.	Stem borers	On hold	
Eulophidae spp.	Flower feeding wasp	Testing in Mexico	3
Tetrastichus sp.	Flower feeding wasp	Testing in Mexico	2,3
Septoria sp.	Leaf and stem fungal cankers	Testing in Mexico	3,5

* 1 = Northern and western Mexico; 2 = Southern and eastern Mexico; 3 = Central America; 4 = Caribbean Islands; 5 = Venezuelan Coast; 6 = South American Pacific Coast; 7 = Brazilian Caatinga; 8 = Argentinean Chaco.

4.3 Objective 3: Conduct preliminary screening of five of those potential agents for each weed

Below we describe the work done on the more studied species.

4.3.1 Bellyache bush

1. *Colaspis musae* Bech. or near (Coleoptera: Chrysomelidae). This leaf beetle from Venezuela originally appeared promising. Heavy adult feeding damage has been observed in the field. Observations on surrounding plant species indicated specificity. Data on the host specificity of this species was obtained from a garden trial in Mexico, from adults colleted in Venezuela in 2008. Adult damage was found only on *J. gossypiifolia*, not *Cnidoscolus aconitifolius*, *Ricinus communis*, and *Jatropha curcas*. One attempt at rearing in Australian quarantine by breeding larvae on roots of potted plants was partially successful in 1999 with the rearing of three adults from one potted plant but this success was not repeated with a second shipment in 2001. Wills Flowers then determined the species to be *Colaspis musae* although there is disagreement on the species in this group and so the possibility remained that it could be a related species. *Colaspis musae* is a pest of bananas, and is thought to breed on grasses. The next step would be to test specificity in young banana leaves, and larval development in grass roots. However, this is a low priority due to the many difficulties in testing and developing this species, including difficulty in rearing and testing a root breeding species, taxonomic uncertainty and the expense of obtaining material from Venezuela.

2. *Cylindrocopturus imbricatus* Champion (Coleoptera: Curculionidae). *Cylindrocopturus imbricatus* is a damaging stem borer of *Jatropha gossypiifolia* in its natural habitat. Populations have been found in Mexico, Guatemala and the Dominican Republic. Damage by larvae weakens the stems and causes death of the host plant when the infestation is severe. Given the damage of *C. imbricatus* to *J. gossypiifolia* in the field at Mexico, it was prioritized as a potential biocontrol agent. It was first identified as *C. jatrophae*, but later as *C. imbricatus*. Eggs are laid in the bark. The larva bores into the stem making a large tunnel in the fresh living material. The prepupa makes its cocoon with stem fibres in the stem, pupates and adults emerge from holes chewed through the stem. The adults probably graze on the outer surfaces of plant parts. A first attempt to

rear this insect at the Mexican Field Station in 2002 was partially successful. In the search for ideal conditions, we tried a total of 19 different rearing systems. Of the rearing systems used, none gave an outstanding result. Overall, a larger number of adult insects were added than emerged in the next generation showing a population decline. Often the same system produced variable results. Rearing of *C. imbricatus* is possible, but a more reliable method is needed. Because of the variable results with rearing this species, host specificity testing based on larval development did not proceed. Instead we turned our attention to surveys of natural host plant use. In these trials, *C. imbricatus* emerged from *Xanthium strumarium* (Asteraceae), poss. *Simsia* sp. (Asteraceae) and *J. curcas* (Euphorbiaceae) proving that these species are hosts of *C. imbricatus*. This weevil is clearly not adequately specific to be useful as a biocontrol agent. It may be possible that *J. gossypiifolia* is a poor host of this insect which would explain the variable results in the rearing trials.

3. *Phakopsora jatrophicola* (Arth.) Cumm. (Uredinales). The rust fungus *Phakopsora jatrophicola* commonly causes lesions on leaves of *J. gossypiifolia* in many parts of tropical America. It is very prolific in spore production. CABI BioScience, UK, and CSIRO MFS staff have conducted preliminary studies on this species. In 2008 host range testing by CABI UK was initiated (see section 4.4.2). Work may also be required to determine if the rust is autoecious or heteroecious. If heteroecious it may have other hosts in the sexual stage of its life cycle and testing would be more difficult. It would also be useful to test the effectiveness of this rust pathogen in the native range to determine whether expenditure on fully testing this fungus is justified.

4. *Euxesta abdominalis* Loew and *Euxesta* aff. *panamena* Curran (Diptera: Ulidiidae). The *Euxesta* were recently confirmed as *Euxesta abdominalis* and *Euxesta* aff. *panamena*. Larvae mine leaves and stems, causing significant damage to the plant. These species are common with a wide distribution including many sites in Mexico but it is difficult to collect adults. We have established a relationship with Vicente Hernandez who is a specialist in this group and is interested in working with us on the taxonomic and biological aspects of these species.

5. *Pachycoris* prob. *fabricii* (Burmeister) (Heteroptera: Scutelleridae). This insect was collected in the Dominican Republic. Host specificity testing was carried out at Mexico on *Cnidoscolus aconitifolius* and *Ricinus communis*. Tests were not completed due to lack of fruiting plants.

6. *Pityophthorus* sp. (Coleoptera: Scolytidae). *Pityophthorus* is likely to be a host specific phytophage capable of killing tips, unlike *Hypothenemus*, the other scolytid found on *J. gossypiifolia*. However, it is an uncommon insect, only having been found at one site in Mexico. Recent collections of 110 dead tips resulted only in *Hypothenemus*, no *Pityophthorus*.

7. *Ormiscus/Eusphyrus* (Coleoptera: Anthribidae). This insect is locally common at sites in Mexico where it emerges from stems. Larvae have been reared from artificial diet. More specific identification is not yet available. Also we need to confirm that it is indeed a phytophage and not a fungus feeder like most members of its family.

4.3.2 Parkinsonia

1. *Eueupithecia cisplatensis* (Lepidoptera: Geometridae) is a multi-voltine looper that is abundant and widespread in Argentina. Adults are relatively short-lived (females survived for up to seven days). Larvae tend to be evenly dispersed in the field and so spectacular

damage is not observed, but free of its natural enemies, it may cause heavy damage to both pinnules and rachises. Both field and laboratory studies by USDA collaborators in Argentina have provided convincing evidence of the host specificity of this species. A total of 28 legume species have been tested in replicated experiments and showed that it is incapable of developing on any species other than *P. aculeata*. Field surveys of host plant use confirmed that the species only occurs naturally on the target weed. This insect was imported into Australian quarantine for final testing in 2010.

2. *Ofatulena luminosa* (Lepidoptera: Tortricidae) is a multi-voltine species that is consistently common 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 (Woods 1992). No success has yet been achieved with rearing this species for a full generation as adults are fragile and short-lived (several days). In a field survey of potential host plants, this species was not collected from any other legume (>20 species surveyed) except from pods of *Parkinsonia florida.* Nor was any damage caused to other legumes in no-choice host-specificity tests conducted in garden plots (Brown *et al*. 2010).

3. *Neolasioptera* n. sp. (Diptera: Cecidomyiidae) is a gall fly that causes damage to growing tips of *P. aculeata* in Argentina and Paraguay, where it can be very abundant. *Neolasioptera* species are generally specific and tied to host biology. A formal description of this species is being conducted by Dr. Raymond Gagné (USDA-ARS Systematic Entomology Laboratory). Preliminary work shows that this species is culturable in the laboratory.

4. Adult *Agrilus parkinsoniae* (Coleoptera: Buprestidae) are relatively common on *P. aculeata* in Oaxaca State (Mexico). Although only adults have so far been collected, taxonomy suggests that it is a stem-borer and may therefore have potential as a biological control agent. This species has no other recorded host plants. However, at this stage its host range hasn't been examined thoroughly. *Parkinsonia aculeata* biological control agents previously released in Australia do not include any stem boring insects, so such species may have high potential as agents.

5. *Rudenia leguminana* complex sp. B (Lepidoptera: Tortricidae) 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 rachis of leaves. Larvae can also develop in flowers and occasionally pods. It could potentially be a species complex, as it has an unusually broad geographic range for Neotropical tortricids, and analyses of two molecular markers strongly suggest that the individuals examined belong to more than one species (Brown *et al*. 2010). Host specificity studies at MFS 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 may therefore refer to other species in this complex.

6. Three leaf beetles (Coleoptera: Chrysomelidae) appear to have potential as biological control agents although their host range is not yet known. *Glyptoscelis sonorensis* is widespread in Mexico and locally abundant, *Myochrous melancholicus* Jacoby is known

from southern Mexico (Oaxaca), and *Myochrous austrinus* Blake (Coleoptera: Chrysomelidae: Eumolpinae) is known from southern Mexico (Oaxaca), Panama and Colombia. All three species have only been recorded on *P. aculeata*. Larvae of all three species may be root-feeders as they all belong the subfamily Eumolpine which typically feed on plant roots, and larvae have never been found on above ground parts. However, larval feeding habits would need to be understood before their potential to significantly damage *P. aculeata*, which has an extensive root system, can be assessed.

7. At least one undescribed *Calosima* sp. (Lepidoptera: Coleophoridae) was found on *P. aculeata* at several sites in Central America and northern South America. It is a stem-borer with a similar habit to *O. luminosa*, although adults are larger. Formal taxonomic description of these species is being carried out by David Adamski (USDA-ARS Systematic Entomology Laboratory). It is possibly host-specific, but may not be sufficiently common to establish cultures for host-specificity testing.

8. The seed-feeder *Atrypanius irrorellus* (Cerambycidae) is common in the gulf coast of Mexico. Its host specificity was tested in the field and laboratory garden in Mexico and was found to develop on various legume species (R. Segura and T.A. Heard, unpublished data).

9. The leaf-feeder *Iridopsis aglauros* Shaus (Geometridae) is relatively abundant in Mexico and Central America. There were no previous host-plant records in the literature, so field host-specificity tests were carried out in Mexico, which found that the insect could complete its lifecycle on several other legume species (*Caesalpinia pulcherrima*, *Leucaena leucocephala*, *Acacia cornigera*, *Mimosa asperata*, *Acacia farnesiana*, *Desmanthus virgatus* and *Prosopis tamaulipana*), making it unsuitable as a biocontrol agent.

10. Similarly the leaf-feeder *Tolype nanus* (Lasiocampidae) is relatively abundant in Mexico. It was tested at the Mexican Field Station garden and found to develop on a range of legume species (*Mimosa asperata*, *Tamarindus indica* and *Delonix regia*).

4.4 Objective 4: Initiate testing of at least two bellyache bush agents, and for those that were host specific, seek approval to commence mass rearing and release activities

1. *Cylindrocopturus imbricatus* Champion (Coleoptera: Curculionidae). *Cylindrocopturus imbricatus* is a damaging stem borer of *Jatropha gossypiifolia* in its natural habitat (see section 3.4.1 above on work done in the Mexico). In 2008, three shipments were made to the Queensland government's Alan Fletcher Research Station. All attempts to rear and test the specificity of this insect in Australia failed, as adults did not copulate or lay eggs; the reasons for this have not been determined. We did prove in Mexico that the green and bronze "varieties" of Australian *J. gossypiifolia* are suitable to rear this insect species so the failure in Australia is not due to incompatible plant biotypes. Later, work in Mexico confirmed that this species is not host specific and so further work ceased.

2. *Phakopsora jatrophicola* (Arth.) Cumm. (Uredinales). CABI BioScience, UK, and CSIRO MFS staff have conducted preliminary studies on this species. First plants of *J. gossypiifolia* were established in the CABI glasshouse, then the fungus sent from Mexico was successfully reared in the lab. In 2008 host range testing by CABI UK was initiated and showed that the rust can also attack *Jatropha multifida* and the potential biofuel crop species *Jatropha curcas*. The current focus is to find the most suitable and virulent

biotype of this rust fungus prior to commencing detailed host specificity testing. Staff at the Mexican field station have contributed to this project by the collection and shipment of biotypes from Mexico and Nicaragua. E.g. the trip to the pacific coast in 2010 of Mexico resulted in the collection of 197 infected leaves from 10 sites from *J. gossypiifolia* and 10 infected leaves from one site from *J. curcas*. The trip to Nicaragua resulted in the shipment on March 2010 of 171 leaves infected with the pathogen from 17 sites in that country. Both shipments arrived safely in the UK.

4.5 Objective 5: Complete preparations to commence detailed host specificity testing of the two best potential biocontrol agents for parkinsonia in quarantine

The highest priority agent, the looper *Eueupithecia cisplatensis*, has been exported by collaborators based at the USDA station in Argentina. Plans have been made for export of the second species, *Ofatulena luminosa* from the CSIRO station in Mexico and the third, *Neolasioptera* sp. from Argentina. The necessary permits have already been obtained for *Eueupithecia cisplatensis*. The material has been received into the CSIRO quarantine facility in Brisbane and is being successfully reared. The basic biology, the methodology for rearing and testing, and the preliminary host tests have already been completed for this species. The methodologies are currently being developed in the native range stations for other insects such as the gall fly, *Neolasioptera* sp. and the stem borer, *Ofatluena luminosa*.

Plants of Parkinsonia and various test plant species have been grown in readiness for the trials. A host specificity test list of about 50 plant species is being finalised. Tests will be conducted against these species. A proposal for the release will be made 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 will be made on the proposal, rather than the use of the current system of assessors (co-operators) spread across many federal and state department. The impact of this is expected to be an extension of the assessment process from 6-12 months to 1-2 years.

4.6 Objective 6: Make progress towards halving the time required to comprehensively survey native enemies for new tropical weed targets

Surveying of natural enemies of widely distributed species across their native range is expensive and can take a long time, over ten years in the case of parkinsonia. We used databases from the parkinsonia and bellyache bush native range survey work to develop and test new analytical approaches that will help future surveys be more systematic and efficient.

Survey data within geographic regions were examined for survey completeness using species accumulation curves (Bell *et al*. in prep.). A predominance of rare species meant that all but the most intensively sampled regions remained undersampled (less than 70% of predicted species-richness documented). However, most of the ecologically dominant species had probably been sampled (89% overall). Based on this information, we conclude that further surveying in most regions would reveal more arthropod species, but that most new records are likely to be rare and therefore to be poor prospects for biocontrol. This is typical of many biodiversity surveys of the arthropod fauna of an individual plant species, where many rare species continue to be sampled, even after extensive surveying, and may be due to species occasionally using the plant as a secondary host (Bell *et al*. in prep.). Surveying of *J. gossypiifolia* seems to be closer to completion than that on *P. aculeata*. This is likely to be because there is limited variation in the composition of natural enemy communities across the geographic distribution of *J. gossypiifolia*.

A very different analytical tool, survey gap analysis, was used for the first time on the survey data. This analysis identified new sites across the geographic distribution of parkinsonia where additional surveys are most likely to yield new natural enemies that haven't been detected anywhere else. This analysis identified sites in northern Argentina, north-western South America (including Peru) and north-west Mexico (previously surveyed by Woods in the 1990s) as ones that are most likely to yield new agents.

It is anticipated that application of such approaches, together with the genetic approaches already developed to better characterise the native-range and biogeography of the target plant, has the potential to halve the time to comprehensively survey natural enemies. This is especially the case for with widely distributed species such as parkinsonia and bellyache bush. In addition, application of these approaches allows for a proper assessment of "survey completeness". A measure of survey completeness has previously been lacking, even for extensively studies species such as Noogoora burr, *Mimosa pigra* and *Lantana camara,* but is invaluable when, for example, determining whether historical biocontrol projects should be "reopened".

4.7 Objective 7: Make progress towards demonstrating the value of a technique to rapidly characterise surveyed natural enemies

Overall, we found that barcoding wasn't particularly successful, mostly due to the timescales of a biocontrol project and the breakdown of DNA over time in dried, pinned insects stored at room temperature, often in tropical climates. These results were generally consistent with other studies not related to biocontrol, in terms of percentage of specimens that were successful and the length of PCR product obtained. From this we came up with recommendations of how to modify insect sampling so that future DNA work has more chance of success, and made estimates of what rate of success can be expected (Appendix 3)

We also overcame the limitations of poor DNA quality due to age and preservation of specimens, for selected groups of potential biocontrol agents, by using shorter fragments of DNA. The species that we chose for this work were those where we expected to find cryptic species which may not be obvious using traditional morphological techniques and which could represent host specific natural enemies. The two groups chosen were Bruchidae and *Calosima*. We were unable to identify host races of either of these taxa and so no opportunities for host specific cryptic species. In Appendix 4, we present the detailed results of this work.

5 Success in achieving objectives

5.1 Objective 1: Produce a comprehensive list of natural enemies present on bellyache bush and parkinsonia in the Americas

This objective was completed following a solid effort on the part of insect taxonomist Quiyari Santiago employed in Mexico. The complete list is shown in appendices 1 and 2 and a summary report is presented in section 4.1.

5.2 Objective 2: Identify the top ten potential biocontrol agents for bellyache bush and parkinsonia

This objective has been achieved, although it proved to be ambitious to aim for the ten top agents for each weed. Indeed for bellyache bush, only 6 species (or groups of species) were identified.

5.3 Objective 3: Conduct preliminary screening of five of those potential agents for each weed

We exceeded this objective in the sense that more than five species were preliminarily screened for each weed.

5.4 Objective 4: Initiate testing of at least two bellyache bush agents, and for those that were host specific, seek approval to commence mass rearing and release activities

We initiated testing on two bellyache bush agents, the weevil, *Cylindrocopturus imbricatus* and rust fungus, *Phakopsora jatrophicola*. However neither has proved to be sufficiently specific to apply for release.

5.5 Objective 5: Complete preparations to commence detailed host specificity testing of the two best potential biocontrol agents for parkinsonia in quarantine

This was fully achieved with detailed host specificity testing in Australian quarantine already underway for one agent, the looper, *Eueupithecia cisplatensis*, and plans well advanced for the second, the stem boring moth *Ofatulena luminosa*.

5.6 Objective 6: Make progress towards halving the time required to comprehensively survey native enemies for new tropical weed targets

This was achieved. It is anticipated that the new analytical approaches developed during this project will allow us to greatly improve the efficiency and comprehensiveness of future survey efforts both on species that have already been extensively surveyed (e.g. parkinsonia) and new targets. Specifically, these analytical tools help us to direct survey effort spatially across the targets native-range distribution so as to find the most new species, and to help us determine when further surveys within a particular region are unlikely to yield additional potential agents. These approaches complement other developments, including in databasing and with the use of genetic tools.

5.7 Objective 7: Make progress towards demonstrating the value of a technique to rapidly characterise surveyed natural enemies

The original intention of this project was to barcode all the collected specimens as an aid to solving taxonomic impediments. The objective proved to be impossible due to the poor quality of the DNA in the majority of older specimens. Instead we turned our attention to searching for cryptic species (Bruchidae) and solving specific taxonomic problems (*Calosima*).

6 Impact on meat and livestock industry – now and in five years time

No immediate impact will result from the work covered in this project. The time frame for biocontrol projects to bear fruit needs to be measured in decades rather than triennia. However, in five years time we expect impacts will start to accrue. We expect that two to three new biocontrol agents of parkinsonia will be released into the Australian environment. The explicit aim of releasing these agents are to reduce patch density and size, to reduce spread and in-fill rates and to reduce management costs by decreasing regrowth and recruitment rates and increasing

time to reproduction (van Klinken 2006). It may take many years for the full impact of the released agent to be realized. However, once in place, the benefits are self-sustaining, permanent, ecologically non-damaging, economically beneficial and do not require the continuous input of land holders.

7 Conclusions and recommendations

This project has made enormous progress in advancing our knowledge of the natural enemies of bellyache bush and parkinsonia and application of this information towards the development of new biocontrol agents. We now have at least three agents for parkinsonia that have a high probability of being suitable for introduction into Australia. We have been less successful with bellyache bush but at least we now know with certainty that this target weed has few prospects. We are now ready to begin the next phase of this project which is to complete the evaluation of the insect species in Australian quarantine. We recommend the following.

7.1 Recommendations

1. Completion of the evaluation of the top three agents of parkinsonia including an application for their release. Funding for this is provided in the already commenced MLA funded project B.NBP.0620.
2. Upon receiving approval, mass-rear and release those parkinsonia agents widely in the Australian environment to gain maximum probability of establishment and rapid spread. We expect that various agencies such as state government departments and land-care groups will participate in this process
3. Complete evaluation of the last of the bellyache bush agents. Although these prospects have a low probability of success. We will apply for funding to do this through the AWRC round of funding.
4. Survey the last areas not completed for parkinsonia which is NE Brazil and Peru. These areas are isolated from other areas surveyed and may yield further agents. We will apply for funding to do this through the AWRC round of funding.

8 Bibliography

The following list is a compilation of publications resulting from this study that are already published or are at an advanced stage of preparation.

Bell, K.L. Heard, T.A., Manion, G., Ferrier, S. and van Klinken, R.D. (2010) Bioclimatic and biogeographic variation in the herbivorous insect assemblages on a neotropical legume. Journal of Biogeography (In preparation)

Bell, K.L. Heard, T.A. Manion, G., Ferrier, S. and van Klinken, R.D. (2010) Survey completeness of the phytophagous insect fauna on *Parkinsonia aculeata* L. (Leguminosae: Caesalpinioideae) through time and space. Diversity and Distributions (In preparation)

Bell, K.L. Heard, T.A. and van Klinken, R.D. (2011) Host specificity, diversity, and ecological guilds of arthropod natural enemies of *Parkinsonia aculeata* L. (Leguminosae: Caesalpinioidea). Ecological Entomology (In preparation).

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 (accepted).

Bebawi, FF, Vitelli, J, Campbell, SD, Vogler, WD, Lockett, CJ, Grace, BS, Lukitsch, B & Heard, TA 2009, '*Jatropha gossypiifolia* L.', in FD Panetta (ed), *The biology of Australian weeds: volume 3*, RG and FJ Richardson, Melbourne, pp. 102–127.

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.

Heard, T.A., Chan, R.R., Wilmot Senaratne, K.A.D., Palmer, W.A., Lockett C. and Lukitsch B. (2009) *Agonosoma trilineatum* (Heteroptera: Scutelleridae) a biological control agent of the weed bellyache bush, *Jatropha gossypiifolia* (Euphorbiaceae). Biological Control 48: 196-203.

Heard, T.A. Segura R., Dhileepan K, (2011) Jatropha gossypiifolia L. bellyache bush In *Biological control of weeds in Australia* (Eds. M Julien, R Mcfadyen and J Cullen). CSIRO, Canberra (submitted)

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., Bell, K. Segura, R., Santiago Q. and Chan, R.R. (2011) The natural enemies of *Jatropha gossypiifolia* and their potential for biocontrol. *Biological Control* (In preparation).

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

Lawson, BE, van Klinken, R.D., Day, MD, Heard TA and Zalucki MP. (2008) Improving the agent selection, release and evaluation process: the role of bioclimatic modelling. Proceedings of the 16th Australian Weeds Conference, eds R.D. van Klinken, V.A. Osten, F.D. Panetta and J.C. Scanlan. (Queensland Weeds Society, Brisbane), pp. 510-512.

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). (submitted)

9 Appendices

9.1 Appendix 1. Comprehensive list of natural enemies on bellyache bush

A=Abundant; O=Occasional; R=Rare.
FR=fruit; LE=leaf; RO=root; SE=seed; ST=stem; UN=unknown
J=restricted to Jatropha; E=restricted to Euphorbiaceae; W=wide host range; U=unknown

Appendix 1. Comprehensive list of natural enemies on bellyache bush

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
Acrolophus sp.	Acrolophidae	Lepidoptera	UN	UN	W	R
Agonosoma trilineatum	Scutelleridae	Hemiptera	SE	SE	J	A
Allocolaspis sp.	Chrysomelidae	Coleoptera	UN	UN	W	R
Amorbia depicta	Tortricidae	Lepidoptera	LE	UN	U	O
Amorbia emigratella	Tortricidae	Lepidoptera	LE	UN	W	A
Amorbia near concavana	Tortricidae	Lepidoptera	LE	UN	U	O
Amorbia prob. concavana	Tortricidae	Lepidoptera	LE	UN	U	O
Amorbia sp.	Tortricidae	Lepidoptera	LE	UN	U	A
Amorbia sp. near emigratella	Tortricidae	Lepidoptera	LE	UN	U	O
Anasa scorbutica	Coreidae	Hemiptera	UN	UN	W	R
Anthonomus sp. 1	Curculionidae	Coleoptera	ST	UN	U	R
Anthribinae sp. 1	Anthribidae	Coleoptera	ST	UN	U	O
Apinocis sp. 1	Curculionidae	Coleoptera	UN	UN	U	R
Araecerus fasciculatus	Anthribidae	Coleoptera	ST	UN	W	A
Arctiidae sp.	Arctiidae	Lepidoptera	UN	UN	U	R
Baridinae sp.	Curculionidae	Coleoptera	UN	UN	U	R
Blastobasiinae sp.	Coleophoridae	Lepidoptera	ST	UN	U	A
Branchus opatroides	Tenebrionidae	Coleoptera	UN	UN	W	R
Bulia confirmans	Noctuidae	Lepidoptera	ST	UN	U	O
Cassidinae sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Catolethrus longulus	Curculionidae	Coleoptera	ST	UN	U	A
Catolethrus sp.	Curculionidae	Coleoptera	ST	UN	U	A
Catorintha sp.	Coreidae	Hemiptera	UN	FR	U	A
Cephalalges murinus	Curculionidae	Coleoptera	UN	UN	U	R
Ceresini sp.	Membracidae	Hemiptera	UN	UN	U	O
Cicadellidae sp.	Cicadellidae	Hemiptera	LE	UN	U	O
Clytrini sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Colaspis musae	Chrysomelidae	Coleoptera	RO	LE	W	A
Colaspis nr. musae	Chrysomelidae	Coleoptera	RO	LE	U	A
Colaspis sp.	Chrysomelidae	Coleoptera	RO	LE	U	A
Colecerus sp. 1	Curculionidae	Coleoptera	UN	UN	U	R
Coleoptera sp. 1		Coleoptera	ST	UN	U	R
Coleoptera sp. 10		Coleoptera	ST	UN	U	A
Coleoptera sp. 2		Coleoptera	UN	UN	U	R
Coleoptera sp. 3		Coleoptera	UN	UN	U	R
Coleoptera sp. 4		Coleoptera	UN	UN	U	O
Coleoptera sp. 5		Coleoptera	UN	UN	U	O
Coleoptera sp. 6		Coleoptera	UN	UN	U	R

Appendix 1. Comprehensive list of natural enemies on bellyache bush

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
Coleoptera sp. 7		Coleoptera	UN	UN	U	R
Coleoptera sp. 8		Coleoptera	UN	UN	U	R
Coleoptera sp. 9		Coleoptera	UN	UN	U	O
Colpoptera sp.	Issidae	Hemiptera	UN	UN	U	R
Corythucha gossypii	Tingidae	Hemiptera	LE	LE	W	A
Coscinoptera mucida	Chrysomelidae	Coleoptera	UN	UN	W	R
Cosmopterygidae sp.	Cosmopterygidae	Lepidoptera	ST	UN	U	O
Cryptocephalinae sp. 1	Chrysomelidae	Coleoptera	UN	LE	U	A
Cryptocephalinae sp. 2	Chrysomelidae	Coleoptera	UN	UN	U	R
Cryptocephalus irroratus	Chrysomelidae	Coleoptera	UN	UN	W	R
Cryptocephalus sp.	Chrysomelidae	Coleoptera	UN	UN	U	O
Cryptocephalus trizonatus	Chrysomelidae	Coleoptera	UN	UN	W	R
Cryptorhynchus sp. 1	Curculionidae	Coleoptera	UN	UN	U	R
Cryptorhynchus sp. 2	Curculionidae	Coleoptera	UN	UN	U	O
Cylindrocopturus imbricatus	Curculionidae	Coleoptera	ST	ST	W	A
Cylindrocopturus sp.	Curculionidae	Coleoptera	ST	UN	W	R
Chalcosicya aptera	Chrysomelidae	Coleoptera	UN	UN	U	O
Chelysomidea variabilis	Scutelleridae	Hemiptera	UN	ST, LE	W	A
Chinavia marginata	Pentatomidae	Hemiptera	UN	FR	W	A
Chrysobothris haitiensis	Buprestidae	Coleoptera	UN	UN	U	O
Chrysomelinae sp. 1	Chrysomelidae	Coleoptera	UN	LE	U	R
Dermestidae sp.	Dermestidae	Coleoptera	UN	UN	U	A
Derodontidae sp.	Derodontidae	Coleoptera	UN	UN	U	O
Diabrotica flaviventris	Chrysomelidae	Coleoptera	UN	UN	U	R
Diptera sp. 1		Diptera	ST	UN	U	R
Diptera sp. 2		Diptera	UN	UN	U	R
Diptera sp. 3		Diptera	UN	UN	U	O
Diptera sp. 4		Diptera	ST	UN	U	A
Diptera sp. 5		Diptera	UN	UN	U	A
Disonycha collata	Chrysomelidae	Coleoptera	UN	UN	W	R
Disonycha? glabrata	Chrysomelidae	Coleoptera	UN	UN	W	R
Dysdercus minor	Pyrrhocoridae	Hemiptera	UN	UN	W	A
Elateridae sp. 1	Elateridae	Coleoptera	UN	UN	U	R
Elateridae sp. 2	Elateridae	Coleoptera	UN	UN	U	R
Elateridae sp. 3	Elateridae	Coleoptera	UN	UN	U	A
Embioptera sp.		Embioptera	UN	UN	U	R
Empoasca near sp.	Cicadellidae	Hemiptera	UN	UN	U	A
Empoasca sp.	Cicadellidae	Hemiptera	UN	UN	U	A
Epicaerus sp. 1	Curculionidae	Coleoptera	UN	UN	U	R
Epitragus aurulentus	Tenebrionidae	Coleoptera	UN	UN	W	O
Epitragus sp.	Tenebrionidae	Coleoptera	UN	UN	U	A
Estigmene acrea	Arctiidae	Lepidoptera	LE	UN	W	A
Eudiagogus maryae	Curculionidae	Coleoptera	UN	UN	W	R
Eumolpinae sp. 1	Chrysomelidae	Coleoptera	UN	UN	U	R
Eumolpinae sp. 2	Chrysomelidae	Coleoptera	UN	UN	U	O
Eupogonius sp.	Cerambycidae	Coleoptera	ST	UN	U	A

Appendix 1. Comprehensive list of natural enemies on bellyache bush

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
Euschistus crenator orbiculator	Pentatomidae	Hemiptera	UN	UN	W	O
Euschistus sp.	Pentatomidae	Hemiptera	FR	FR	U	A
Eusphyrus sp.	Anthribidae	Coleoptera	ST	ST	U	A
Euxesta abdominalis	Ulidiidae	Diptera	ST	UN	U	A
Euxesta aff. Panamena	Ulidiidae	Diptera	ST	UN	U	A
Flatidae sp.	Flatidae	Hemiptera	UN	UN	U	R
Fulgoroidea sp.		Hemiptera	UN	UN	U	A
Galerucinae sp. 1	Chrysomelidae	Coleoptera	UN	UN	U	R
Galerucinae sp. 2	Chrysomelidae	Coleoptera	UN	UN	U	O
Galgupha sp.	Thyreocoridae	Hemiptera	UN	UN	U	O
Gynandrobrotica lepida	Chrysomelidae	Coleoptera	UN	UN	W	O
Hapalips sp.	Languriidae	Coleoptera	UN	UN	U	A
Harmostes serratus	Rhopalidae	Hemiptera	UN	UN	W	R
Hemiptera sp.		Hemiptera	ST	UN	U	A
Hemiptera sp. 2		Hemiptera	UN	UN	U	R
Hemiptera sp. 3		Hemiptera	UN	UN	U	R
Hylocrinus sp.	Tenebrionidae	Coleoptera	UN	UN	U	R
Hypothenemus hampei	Curculionidae	Coleoptera	ST	UN	W	A
Hypothenemus sp.	Curculionidae	Coleoptera	ST	UN	U	R
Hypothenemus sp. 1	Curculionidae	Coleoptera	ST	UN	U	A
Hypothenemus sp. 2	Curculionidae	Coleoptera	ST	UN	U	O
Hypselonotus sp.	Coreidae	Hemiptera	UN	UN	U	R
Insara tolteca	Tettigoniidae	Orthoptera	UN	UN	U	R
Iridopsis sp.	Geometridae	Lepidoptera	LE	UN	U	O
Isorhinus undatus	Curculionidae	Coleoptera	UN	UN	W	R
Issidae sp.	Issidae	Hemiptera	UN	UN	U	A
Lachnopus inconditus	Curculionidae	Coleoptera	UN	UN	W	R
Lachnopus sp.	Curculionidae	Coleoptera	UN	UN	U	O
Lagocheirus araneiformis	Cerambycidae	Coleoptera	ST	UN	W	A
Lagocheirus araneiformis ypsilon	Cerambycidae	Coleoptera	ST	UN	W	O
Lagocheirus obsoletus	Cerambycidae	Coleoptera	ST	UN	W	A
Lagocheirus sp.	Cerambycidae	Coleoptera	ST	UN	U	A
Lagocheirus undatus	Cerambycidae	Coleoptera	ST	UN	W	A
Lampetis (Spinthoptera) sp.	Buprestidae	Coleoptera	ST	UN	U	A
Langurinae sp. 1	Erotylidae	Coleoptera	ST	UN	U	O
Langurinae sp. 2	Erotylidae	Coleoptera	ST	UN	U	O
Largus sp.	Largidae	Hemiptera	UN	LE	U	R
Lasiocampidae poss. sp.	Lasiocampidae	Lepidoptera	UN	UN	U	R
Lepidoptera sp. 10		Lepidoptera	UN	UN	U	O
Lepidoptera sp. 7		Lepidoptera	LE	UN	U	R
Leptinotarsa decemlineata	Chrysomelidae	Coleoptera	LE	LE	W	R
Leptostylus albicinctus	Cerambycidae	Coleoptera	ST	UN	U	A
Leptostylus cretatellus	Cerambycidae	Coleoptera	ST	UN	W	O
Leptostylus decipiens	Cerambycidae	Coleoptera	ST	UN	W	R
Leptostylus hispidulus	Cerambycidae	Coleoptera	ST	UN	W	A
Leptostylus ochropygus	Cerambycidae	Coleoptera	ST	UN	U	A

Appendix 1. Comprehensive list of natural enemies on bellyache bush

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
Leptostylus sp. 1	Cerambycidae	Coleoptera	ST	UN	U	A
Lepturges sp.	Cerambycidae	Coleoptera	ST	UN	U	R
Longitarsus sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Melolonthinae sp.	Scarabaeidae	Coleoptera	UN	UN	U	R
Membracidae sp. 1	Membracidae	Hemiptera	UN	UN	U	A
Membracidae sp. 2	Membracidae	Hemiptera	UN	UN	U	R
Metachroma sp. nr. convexum	Chrysomelidae	Coleoptera	UN	UN	U	O
Metaponpneumata rogenhoferi	Noctuidae	Lepidoptera	ST	UN	W	A
Micrapate sp.	Bostrichidae	Coleoptera	ST	UN	U	A
Miridae sp. 1	Miridae	Hemiptera	UN	UN	U	A
Miridae sp. 2	Miridae	Hemiptera	UN	UN	U	R
Monoxia sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Mormidea sp.	Pentatomidae	Hemiptera	UN	UN	U	R
Naupactina sp.	Curculionidae	Coleoptera	UN	UN	U	R
Nitidulidae sp.	Nitidulidae	Coleoptera	UN	UN	U	R
Noctuidae sp.	Noctuidae	Lepidoptera	UN	UN	U	R
Norape sp.	Megalopygidae	Lepidoptera	LE	UN	U	R
Notozona histrionica	Chrysomelidae	Coleoptera	UN	LE	W	O
Nyssodrysina haldemani	Cerambycidae	Coleoptera	ST	UN	W	O
Omophoita abbreviata	Chrysomelidae	Coleoptera	UN	LE	W	R
Oncometopia sp.	Cicadellidae	Hemiptera	UN	UN	U	A
Orthoptera sp. 1		Orthoptera	UN	LE	U	R
Orthoptera sp. 2		Orthoptera	UN	FR, L	U	A
Ozineus sp.	Cerambycidae	Coleoptera	ST	UN	U	O
Pachycoris fabricii	Scutelleridae	Hemiptera	FR	FR	W	A
Pachycoris klugii	Scutelleridae	Hemiptera	UN	UN	E	O
Pantomorus sp.	Curculionidae	Coleoptera	UN	UN	U	R
Papilionoidea sp.		Lepidoptera	UN	UN	U	O
Paragrilus leseueri	Buprestidae	Coleoptera	UN	UN	W	O
Paragrilus sp.	Buprestidae	Coleoptera	UN	UN	U	O
Paraulacizes panamensis	Cicadellidae	Hemiptera	UN	UN	U	R
Parmenonta sp.	Cerambycidae	Coleoptera	ST	UN	U	A
Parmenonta valida	Cerambycidae	Coleoptera	ST	UN	W	A
Pentatomidae sp. 1	Pentatomidae	Hemiptera	UN	UN	U	R
Phakopsora jatrophicola	Pleosporaceae	Uredinales	NO	NO	J	O
Phegoneus sp.	Tenebrionidae	Coleoptera	UN	UN	W	O
Phyllotreta pusilla	Chrysomelidae	Coleoptera	UN	UN	W	R
Piestus sp.	Staphylinidae	Coleoptera	UN	UN	U	O
Pityophthorus sp. 1	Curculionidae	Coleoptera	UN	UN	U	O
Platynota near labiosana	Tortricidae	Lepidoptera	LE	UN	U	O
Platynota near subargentea	Tortricidae	Lepidoptera	LE	UN	W	A
Platynota nr. rostrana	Tortricidae	Lepidoptera	LE	UN	U	O
Platynota rostrana	Tortricidae	Lepidoptera	LE	UN	W	A
Platynota rostrana complex	Tortricidae	Lepidoptera	LE	UN	U	A
Platynota sp.	Tortricidae	Lepidoptera	LE	UN	U	O
Platynota sp. poss. flavedana	Tortricidae	Lepidoptera	LE	UN	U	R

Appendix 1. Comprehensive list of natural enemies on bellyache bush

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
Ponometia exigua	Noctuidae	Lepidoptera	UN	UN	U	R
Promecosoma scutellare	Chrysomelidae	Coleoptera	UN	UN	U	R
Pronotacantha stusaki	Berytidae	Hemiptera	UN	UN	U	O
Proxys punctulatus	Pentatomidae	Hemiptera	UN	UN	U	R
Psapharochrus circumflexus	Cerambycidae	Coleoptera	UN	UN	U	R
Psychidae sp.	Psychidae	Lepidoptera	UN	UN	U	R
Pyralidae sp.	Pyralidae	Lepidoptera	UN	UN	U	O
Pyraloid sp.		Lepidoptera	UN	UN	U	R
Rothschildia lebeau lebeau	Saturniidae	Lepidoptera	LE	UN	W	A
Scaphytopius sp.	Cicadellidae	Hemiptera	UN	UN	U	R
Scarinae sp.	Cicadellidae	Hemiptera	UN	UN	U	A
Scopula cf. subquadrata	Geometridae	Lepidoptera	UN	UN	U	R
Sparganothini new genus	Tortricidae	Lepidoptera	UN	UN	U	O
Sphenarium purparascens purparascens	Pyrgomorphidae	Orthoptera	UN	UN	W	A
Spodoptera albula	Noctuidae	Lepidoptera	LE	UN	W	A
Spodoptera latifascia	Noctuidae	Lepidoptera	LE	UN	W	A
Spodoptera ornithogalli	Noctuidae	Lepidoptera	LE	UN	W	R
Sterrhinae sp.	Geometridae	Lepidoptera	UN	UN	U	R
Styloleptus laticollis	Cerambycidae	Coleoptera	ST	UN	U	A
Styloleptus nigrofasciatus	Cerambycidae	Coleoptera	ST	UN	U	A
Styloleptus sp.	Cerambycidae	Coleoptera	ST	UN	U	A
Synbrotica sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Synchlora frondaria	Geometridae	Lepidoptera	LE	UN	W	O
Tenebrionidae sp. 1	Tenebrionidae	Coleoptera	UN	UN	U	O
Thyanta sp.	Pentatomidae	Hemiptera	UN	UN	U	O
Tingidae sp. 1	Tingidae	Hemiptera	UN	UN	U	A
Tingidae sp. 2	Tingidae	Hemiptera	UN	UN	U	A
Trachyderes mandibularis	Cerambycidae	Coleoptera	ST	UN	W	R
Ulidiidae sp.	Ulidiidae	Diptera	ST	UN	U	A
Urgleptes sp.	Cerambycidae	Coleoptera	ST	UN	U	A
Utetheisa ornatrix ornatrix	Arctiidae	Lepidoptera	UN	UN	W	O
Xylesthia pruniramiella	Tineidae	Lepidoptera	ST	UN	W	A
Xylesthia sp.	Tineidae	Lepidoptera	ST	UN	U	A
Xystropus sp.	Tenebrionidae	Coleoptera	UN	UN	U	R
Yponomeutidae sp.	Yponomeutidae	Lepidoptera	UN	UN	U	O
Zicca sp.	Coreidae	Hemiptera	UN	UN	U	R

9.2 Appendix 2. Comprehensive list of natural enemies on parkinsonia

A=Abundant; O=Occasional; R=Rare.
FR=fruit; LE=leaf; RO=root; SE=seed; ST=stem; UN=unknown
J=restricted to Jatropha; E=restricted to Euphorbiaceae; W=wide host range; U=unknown

Appendix 2. Comprehensive list of natural enemies on parkinsonia

Genus species	Family	Order	Larval feeding site	Adult feeding site	Host range	Abundance
"Ormenis" sp.	Flatidae	Hemiptera	UN	UN	U	R
Acalymma sp.	Chrysomelidae	Coleoptera	UN	UN	W	R
Acanalonia sp.	Acanaloniidae	Hemiptera	UN	UN	U	O
Acanaloniidae sp. 1	Acanaloniidae	Hemiptera	UN	UN	U	A
Acanthoscelides vexatus	Chrysomelidae	Coleoptera	UN	UN	U	R
Acarina sp.		Acarina	ST	ST	U	A
Achryson surinamum	Cerambycidae	Coleoptera	ST	UN	W	A
Acrididae sp.	Acrididae	Orthoptera	UN	UN	U	A
Acyphus funicularius	Curculionidae	Coleoptera	UN	UN	L	R
Agrilus parkinsoniae	Buprestidae	Coleoptera	ST	UN	P	A
Agrilus sp. a	Buprestidae	Coleoptera	ST	UN	U	R
Agromyzidae sp.	Agromyzidae	Diptera	FL	UN	U	O
Alydidae sp. 1	Alydidae	Hemiptera	UN	UN	U	O
Alydidae sp. 2	Alydidae	Hemiptera	UN	UN	U	R
Amblycerus testaceus	Chrysomelidae	Coleoptera	SE	UN	U	O
Amorbia prob. concavana	Tortricidae	Lepidoptera	LE	UN	W	A
Amphicerus cornutus	Bostrichidae	Coleoptera	ST	UN	W	A
Anatinomma alveolatum	Cerambycidae	Coleoptera	ST	UN	W	A
Ancylocera amplicornis	Cerambycidae	Coleoptera	ST	UN	U	O
Anelaphus inermis	Cerambycidae	Coleoptera	ST	UN	W	O
Anelaphus moestus	Cerambycidae	Coleoptera	ST	UN	W	R
Anelaphus sp.	Cerambycidae	Coleoptera	ST	UN	U	O
Asphondylia websteri	Cecidomyiidae	Diptera	FL	UN	W	A
Atrypanius irrorellus	Cerambycidae	Coleoptera	SE	PO, FL	W	A
Atrypanius sp.	Cerambycidae	Coleoptera	PO	UN	U	O
Babia (Babia) sp.	Chrysomelidae	Coleoptera	UN	UN	U	A
Baridinae sp.	Curculionidae	Coleoptera	UN	UN	U	A
Blapstinus sp.	Tenebrionidae	Coleoptera	UN	UN	U	R
Blepyrus sp.	Encyrtidae	Hymenoptera	NO	NO	U	R
Brachyacma palpigera	Gelechiidae	Lepidoptera	PO, FL	UN	W	A
Bracmia? sp.	Gelechiidae	Lepidoptera	UN	UN	U	O
Braconidae sp. 1	Braconidae	Hymenoptera	UN	UN	U	A
Bruchinae sp.	Chrysomelidae	Coleoptera	SE	UN	U	A
Burtinus notatipennis	Alydidae	Hemiptera	UN	UN	W	R
Calosima n. sp.	Coleophoridae	Lepidoptera	ST, LE	UN	U	A
Catocalinae sp.	Noctulidae	Lepidoptera	LE	UN	U	O
Cecidomyiidae sp.	Cecidomyiidae	Diptera	FL	UN	U	A

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
Cerambycidae sp.	Cerambycidae	Coleoptera	UN	UN	U	R
Cerambycidae sp. 1	Cerambycidae	Coleoptera	ST	ST	U	R
Cerambycidae sp. 2	Cerambycidae	Coleoptera	UN	ST	U	R
Ceresa sp.	Membracidae	Hemiptera	UN	UN	U	R
Cerotoma sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Chinavia marginata	Pentatomidae	Hemiptera	PO	PO	W	A
Chrysomelidae sp.	Chrysomelidae	Coleoptera	UN	UN	U	O
Cicadellidae sp. 1	Cicadellidae	Hemiptera	LE	LE	U	O
Clastoptera sp.	Cercopidae	Hemiptera	UN	UN	U	O
Cochylis sp.	Tortricidae	Lepidoptera	FL	UN	U	O
Colecerus marmoratus	Curculionidae	Coleoptera	UN	UN	L	A
Colecerus sp.	Curculionidae	Coleoptera	UN	UN	U	O
Coleoptera sp. 1		Coleoptera	UN	UN	U	R
Coleoptera sp. 10		Coleoptera	UN	UN	U	R
Coleoptera sp. 11		Coleoptera	UN	UN	U	A
Coleoptera sp. 12		Coleoptera	UN	UN	U	R
Coleoptera sp. 13		Coleoptera	UN	UN	U	R
Coleoptera sp. 14		Coleoptera	UN	UN	U	R
Coleoptera sp. 15		Coleoptera	UN	UN	U	R
Coleoptera sp. 2		Coleoptera	UN	UN	U	R
Coleoptera sp. 3		Coleoptera	UN	UN	U	O
Coleoptera sp. 4		Coleoptera	UN	UN	U	O
Coleoptera sp. 5		Coleoptera	UN	UN	U	R
Coleoptera sp. 6		Coleoptera	UN	UN	U	R
Coleoptera sp. 7		Coleoptera	UN	UN	U	R
Coleoptera sp. 8		Coleoptera	UN	UN	U	R
Coleoptera sp. 9		Coleoptera	UN	UN	U	R
Compsus auricephalus	Curculionidae	Coleoptera	RO	UN	W	A
Coscinoptera poss. sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Coscinoptera mucida	Chrysomelidae	Coleoptera	UN	UN	W	R
Coscinoptera soricina	Chrysomelidae	Coleoptera	UN	UN	L	R
Coscinoptera sp.	Chrysomelidae	Coleoptera	UN	UN	U	O
Cryptocephalus irroratus sp. prob.	Chrysomelidae	Coleoptera	UN	UN	W	A
Cryptocephalus militaris	Chrysomelidae	Coleoptera	UN	UN	U	O
Cryptocephalus sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Cryptocephalus sp. 1	Chrysomelidae	Coleoptera	UN	UN	U	R
Cryptocephalus sp. 2 nr. xanthospilus	Chrysomelidae	Coleoptera	UN	UN	U	A
Cryptocephalus trizonatus	Chrysomelidae	Coleoptera	UN	UN	W	O

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
Cryptorynchinae sp.	Curculionidae	Coleoptera	UN	UN	U	R
Cryptothelea poss. sp.	Psychidae	Lepidoptera	LE	UN	U	R
Cryptothelea gloverii	Psychidae	Lepidoptera	ST, LE	UN	W	A
Curculionidae sp.	Curculionidae	Coleoptera	UN	UN	U	A
Curculionidae sp. 1	Curculionidae	Coleoptera	UN	UN	U	R
Curculionidae sp. 2	Curculionidae	Coleoptera	UN	UN	U	A
Curculionidae sp. 3	Curculionidae	Coleoptera	UN	UN	U	O
Curculionidae sp. 4	Curculionidae	Coleoptera	UN	UN	U	O
Curculionidae sp. 5	Curculionidae	Coleoptera	UN	UN	U	R
Curculionidae sp. 6	Curculionidae	Coleoptera	UN	UN	U	R
Curculionidae sp. 7	Curculionidae	Coleoptera	UN	UN	U	R
Curculionidae sp. 8	Curculionidae	Coleoptera	UN	UN	U	R
Cylindrocopturus elongatus	Curculionidae	Coleoptera	ST	UN	U	R
Cylindrocopturus tetralobatus	Curculionidae	Coleoptera	ST	UN	W	R
Dasymetopa sp.	Ulidiidae	Diptera	UN	UN	U	R
Derbidae sp.	Derbidae	Hemiptera	UN	UN	U	R
Diabrotica balteata	Chrysomelidae	Coleoptera	RO	AL	W	A
Diabrotica litterata	Chrysomelidae	Coleoptera	RO	AL	W	A
Diabrotica sp.	Chrysomelidae	Coleoptera	LE	LE	U	A
Diabrotica? sinuata	Chrysomelidae	Coleoptera	UN	UN	W	O
Dicymolomia julianalis	Pyralidae	Lepidoptera	UN	UN	W	O
Diptera sp. 1		Diptera	UN	UN	U	R
Diptera sp. 2		Diptera	UN	UN	U	O
Dysodia poss. sp.	Thyrididae	Lepidoptera	UN	UN	U	R
Elachistidae sp.	Elachistidae	Lepidoptera	PO	UN	U	O
Elateridae sp. 1	Elateridae	Coleoptera	UN	UN	U	R
Elateridae sp. 2	Elateridae	Coleoptera	UN	UN	U	O
Enchenopa monoceros	Membracidae	Hemiptera	UN	UN	W	A
Enchophyllum n. sp.	Membracidae	Hemiptera	UN	UN	U	R
Epicaerus sp. 1	Curculionidae	Coleoptera	UN	UN	U	O
Epicaerus sp. 2	Curculionidae	Coleoptera	UN	UN	U	R
Epitragus sp. 1	Tenebrionidae	Coleoptera	UN	UN	U	A
Epitragus sp. 2	Tenebrionidae	Coleoptera	UN	UN	U	A
Euacidalia sp.	Geometridae	Lepidoptera	UN	UN	U	O
Eueupithecia cisplatensis	Geometridae	Lepidoptera	LE	UN	U	A
Eulophidae sp. 1	Eulophidae	Hymenoptera	UN	UN	U	A
Eumolpinae sp. 1	Chrysomelidae	Coleoptera	PO	UN	U	O
Euryscopa sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Eurytomidae sp.	Eurytomidae	Hymenoptera	UN	UN	U	A
Euzophera n. sp. near	Pyralidae	Lepidoptera	ST, LE	UN	U	O

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
nigricantella ragonot						
Flatidae sp. 1	Flatidae	Hemiptera	UN	UN	U	A
Fulgoroidea sp. 1		Hemiptera	UN	UN	U	A
Fulgoroidea sp. 2		Hemiptera	UN	UN	U	O
Fulgoroidea sp. 3		Hemiptera	UN	UN	U	R
Galgupha (Microcompsus) sp.	Thyreocoridae	Hemiptera	UN	UN	U	O
Galgupha sp.	Thyreocoridae	Hemiptera	UN	UN	U	R
Gelechiidae sp.	Gelechiidae	Lepidoptera	UN	UN	U	R
Gelechiinae sp.	Gelechiidae	Lepidoptera	PO, FL	UN	U	A
Gelechioidea sp.		Lepidoptera	PO	UN	U	O
Geometridae sp.	Geometridae	Lepidoptera	UN	UN	U	A
Geometridae sp. 1	Geometridae	Lepidoptera	LE	UN	U	R
Geraeus sp.	Curculionidae	Coleoptera	UN	UN	U	R
Glyptoscelis chontalensis	Chrysomelidae	Coleoptera	UN	UN	W	A
Glyptoscelis planigera or v. nr.	Chrysomelidae	Coleoptera	UN	UN	U	R
Glyptoscelis sonorensis	Chrysomelidae	Coleoptera	UN	UN	U	A
Glyptoscelis sp.	Chrysomelidae	Coleoptera	UN	UN	U	O
Guayaquila sp.	Membracidae	Hemiptera	UN	UN	U	O
Hesperiidae sp.	Hesperiidae	Lepidoptera	UN	UN	U	R
Heteropsylla sp.	Psyllidae	Hemiptera	AP	UN	U	A
Homalodisca ichthyocephala	Cicadellidae	Hemiptera	UN	UN	W	O
Homoptera sp.		Hemiptera	UN	UN	U	R
Hypothenemus hampei	Curculionidae	Coleoptera	SE	UN	W	A
Hypothenemus rotundicollis	Curculionidae	Coleoptera	ST	UN	W	A
Hypselonotus sp.	Coreidae	Hemiptera	UN	UN	U	R
Icerya sp.	Margarodidae	Hemiptera	UN	UN	U	A
Iridopsis aglauros?	Geometridae	Lepidoptera	LE	UN	W	A
Iridopsis defectaria	Geometridae	Lepidoptera	LE	UN	W	R
Issidae sp. 1	Issidae	Hemiptera	UN	UN	U	A
Lactica sp.	Chrysomelidae	Coleoptera	UN	UN	U	A
Largus cinctus	Largidae	Hemiptera	UN	UN	W	R
Largus sp.	Largidae	Hemiptera	UN	UN	U	A
Lasiocampidae poss. sp.		Lepidoptera	LE	UN	U	A
Lepidoptera sp.		Lepidoptera	UN	UN	U	A
Lepidoptera sp. 2		Lepidoptera	PO	UN	U	R
Lepidoptera sp. 3		Lepidoptera	PO	UN	U	R
Lepidoptera sp. 4		Lepidoptera	UN	UN	U	R

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
Lepidoptera sp. 5		Lepidoptera	UN	UN	U	R
Lepidoptera sp. 7		Lepidoptera	UN	UN	U	R
Lepidoptera sp. 9		Lepidoptera	UN	UN	U	R
Leptoglossus zonatus	Coreidae	Hemiptera	UN	UN	W	R
Lobopoda sp.	Alleculidae	Coleoptera	UN	UN	U	R
Lophalia prob. cyanicollis	Cerambycidae	Coleoptera	UN	UN	U	R
Lophopoeum carinatulum	Cerambycidae	Coleoptera	SE	UN	W	A
Loxa viridis	Pentatomidae	Hemiptera	UN	UN	W	R
Lyrcus sp.	Pteromalidae	Hymenoptera	UN	UN	U	A
Macaria abydata	Geometridae	Lepidoptera	LE	UN	W	A
Macaria sp.	Geometridae	Lepidoptera	LE	UN	U	O
Madarellus sp.	Curculionidae	Coleoptera	UN	UN	U	A
Megacerus leucospilus	Chrysomelidae	Coleoptera	SE	UN	U	R
Megacerus ricaensis	Chrysomelidae	Coleoptera	UN	UN	U	R
Megalostomis poss. sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Megalostomis (Pygidiocarina) tomentosa tomentosa	Chrysomelidae	Coleoptera	NO	UN	L	O
Megalostylus sp.	Curculionidae	Coleoptera	UN	UN	U	R
Melandryidae sp.	Melandryidae	Coleoptera	UN	UN	W	R
Melipotis acontioides	Noctuidae	Lepidoptera	LE	UN	C	A
Membracidae sp.	Membracidae	Hemiptera	UN	UN	U	A
Membracidae sp. 1	Membracidae	Hemiptera	LE	LE	U	R
Membracidae sp. 2	Membracidae	Hemiptera	LE	LE	U	R
Membracidae sp. 3	Membracidae	Hemiptera	LE	LE	U	R
Membracidae sp. 4	Membracidae	Hemiptera	UN	UN	U	R
Membracidae sp. 5	Membracidae	Hemiptera	UN	UN	U	R
Membracidae sp. 6	Membracidae	Hemiptera	UN	UN	U	R
Metallactus prob. sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Metallactus sp. 1	Chrysomelidae	Coleoptera	UN	UN	U	O
Mimosestes amicus	Chrysomelidae	Coleoptera	SE	UN	L	A
Mimosestes insularis	Chrysomelidae	Coleoptera	SE	UN	L	R
Mimosestes mimosae	Chrysomelidae	Coleoptera	SE	UN	L	A
Mimosestes nubigens	Chrysomelidae	Coleoptera	UN	UN	L	R
Mimosestes sp. 1	Chrysomelidae	Coleoptera	SE	UN	U	A
Mozena sp.	Coreidae	Hemiptera	UN	UN	U	R
Murgantia histrionica	Pentatomidae	Hemiptera	UN	UN	W	O
Mycetaspis personata	Diaspididae	Hemiptera	UN	UN	W	A
Myochrous austrinus	Chrysomelidae	Coleoptera	UN	UN	U	A
Myochrous elachius	Chrysomelidae	Coleoptera	UN	UN	W	A
Myochrous melancholicus	Chrysomelidae	Coleoptera	UN	UN	U	A
Myochrous sp. 1	Chrysomelidae	Coleoptera	UN	UN	U	A

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
Myochrous sp. 2	Chrysomelidae	Coleoptera	UN	UN	U	A
Neocompsa exclamationis	Cerambycidae	Coleoptera	UN	UN	W	O
Neolasioptera n. sp.	Cecidomyiidae	Diptera	ST	UN	P	A
Nezara viridula	Pentatomidae	Hemiptera	UN	UN	W	A
Nezara viridula?	Pentatomidae	Hemiptera	UN	UN	W	O
Nymphalidae sp.	Nymphalidae	Lepidoptera	UN	UN	U	O
Obrium sp.	Cerambycidae	Coleoptera	UN	UN	U	R
Ochrimnus pallidocinctus	Lygaeidae	Hemiptera	UN	UN	U	O
Ofatulena duodecemstriata	Tortricidae	Lepidoptera	ST	UN	L	A
Ofatulena luminosa	Tortricidae	Lepidoptera	ST, PO	UN	P	A
Olethreutinae sp.	Tortricidae	Lepidoptera	UN	UN	U	O
Oncideres bouchardi	Cerambycidae	Coleoptera	ST	UN	U	R
Oncideres sp.	Cerambycidae	Coleoptera	ST	UN	U	R
Oncopeltus cingulifer	Lygaeidae	Hemiptera	UN	UN	W	R
Oncopeltus sexmaculatus	Lygaeidae	Hemiptera	UN	UN	W	R
Orophus sp.	Tettigoniidae	Orthoptera	UN	UN	U	A
Orthoptera sp. 1		Orthoptera	UN	UN	U	O
Orthoptera sp. 2		Orthoptera	UN	UN	U	R
Oryctometopia fossulatella	Pyralidae	Lepidoptera	PO	UN	W	A
Oxymerus aculeatus lebasii	Cerambycidae	Coleoptera	UN	UN	W	O
Pandeleteius nodifer	Curculionidae	Coleoptera	UN	UN	W	A
Pantomorus globulicollis	Curculionidae	Coleoptera	UN	UN	L	A
Paragrilus rugatulus	Buprestidae	Coleoptera	ST	UN	U	R
Paulululsus hispaniolae	Curculionidae	Coleoptera	UN	UN	U	O
Pellaea stictica	Pentatomidae	Hemiptera	UN	PO	W	A
Pentatomidae sp.	Pentatomidae	Hemiptera	UN	UN	U	O
Pentatomidae sp. 1	Pentatomidae	Hemiptera	UN	UN	U	R
Pentatomidae sp. 4	Pentatomidae	Hemiptera	UN	UN	U	R
Pentatomidae sp. 6	Pentatomidae	Hemiptera	UN	UN	U	R
Pentatomidae sp. 7	Pentatomidae	Hemiptera	UN	UN	U	A
Pentatomidae sp. 8	Pentatomidae	Hemiptera	UN	UN	U	R
Pentatomidae sp. 9	Pentatomidae	Hemiptera	UN	UN	U	O
Penthobruchus germaini	Chrysomelidae	Coleoptera	SE	UN	P	A
Phalacridae sp.	Phalacridae	Coleoptera	UN	UN	U	R
Phthia picta	Coreidae	Hemiptera	UN	UN	W	R
Physonota sp.	Chrysomelidae	Coleoptera	UN	UN	U	R
Plagiohammus imperator	Cerambycidae	Coleoptera	UN	UN	U	R
Platynota near subargentea	Tortricidae	Lepidoptera	LE	UN	W	A

Appendix 2. Comprehensive list of natural enemies on parkinsonia						
Genus species	**Family**	**Order**	**Larval feeding site**	**Adult feeding site**	**Host range**	**Abundance**
Platynota rostrana complex	Tortricidae	Lepidoptera	LE	UN	W	A
Platynota stultana	Tortricidae	Lepidoptera	LE	UN	W	A
Platyomus sp.	Curculionidae	Coleoptera	UN	UN	U	O
Pococera gelidalis	Pyralidae	Lepidoptera	LE	UN	L	R
Pococera n. sp. 1	Pyralidae	Lepidoptera	LE	UN	U	A
Pococera n. sp. 2	Pyralidae	Lepidoptera	LE	UN	U	O
Pococera sp.	Pyralidae	Lepidoptera	LE	UN	U	A
Prodiplosis sp.	Cecidomyiidae	Diptera	FL	UN	W	A
Proscopiidae sp.	Proscopiidae	Orthoptera	UN	UN	U	O
Pseudobaris sp.?	Curculionidae	Coleoptera	ST	UN	U	R
Pseudococcidae sp.	Pseudococcidae	Hemiptera	UN	UN	U	A
Psyllidae sp.	Psyllidae	Hemiptera	LE	LE	U	A
Psyrassa basicornis	Cerambycidae	Coleoptera	ST	UN	U	A
Psyrassa castanea	Cerambycidae	Coleoptera	ST	UN	W	O
Pteromalidae sp. 1	Pteromalidae	Hymenoptera	UN	UN	U	A
Pyralidae sp. 2	Pyralidae	Lepidoptera	UN	UN	U	R
Rhinacloa cardini	Miridae	Hemiptera	LE	LE	L	A
Rhinacloa sp. 1	Miridae	Hemiptera	LE	LE	U	A
Rhyssomatus sp.	Curculionidae	Coleoptera	UN	UN	U	R
Rudenia leguminana	Tortricidae	Lepidoptera	ST, FL, PO	UN	L	A
Septoria sp. nov.	Mycosphaerellaceae	Capnodiales	NO	NO	P	O
Sphaenothecus facetus	Cerambycidae	Coleoptera	UN	UN	W	O
Sphaenothecus maccartyi	Cerambycidae	Coleoptera	UN	UN	W	A
Sphaenothecus trilineatus	Cerambycidae	Coleoptera	UN	UN	U	A
Sphyrocoris obliquus	Scutelleridae	Hemiptera	UN	UN	W	R
Stator limbatus	Chrysomelidae	Coleoptera	UN	UN	L	R
Stator sordidus	Chrysomelidae	Coleoptera	NO	NO	L	R
Stator testudinarius	Chrysomelidae	Coleoptera	SE	UN	L	A
Steatococcus sp.	Margarodidae	Hemiptera	UN	UN	U	A
Synchlora frondaria	Geometridae	Lepidoptera	PO, FL	UN	W	A
Thasus gigas sp. prob.	Coreidae	Hemiptera	UN	UN	U	A
Tingidae sp.	Tingidae	Hemiptera	LE	LE	U	R
Tolype nanus	Lasiocampidae	Lepidoptera	LE	UN	W	A
Tolype prob. sp.	Lasiocampidae	Lepidoptera	UN	UN	U	R
Torymidae sp.	Torymidae	Hymenoptera	UN	UN	U	A
Trachyderes sp.	Cerambycidae	Coleoptera	UN	UN	U	A
Typophorus nigritus	Chrysomelidae	Coleoptera	UN	UN	W	R
Urodera (Boreurodera) crucifera crucifera	Chrysomelidae	Coleoptera	UN	UN	L	A
Vanduzeea segmentata	Membracidae	Hemiptera	UN	UN	W	O
Xyonysius sp.	Lygaeidae	Hemiptera	UN	UN	U	R

9.3 Appendix 3. Recommendations for Specimen Storage and Field Sampling for DNA Work

Killing Methods

There is limited information available in the literature on the effect of killing methods on DNA quality, and this is probably less important than the long-term storage methods, as long as specimens are transferred to the storage medium soon after killing. The studies available give conflicting results. Dean & Ballard (2001), using *Drosophila simulans* found no difference when specimens were killed by 7-9 minutes in cyanide or ethyl acetate, freezing or immersion in 70% ethanol at room temperature. On the other hand, Dillon et al (1996) found that hymenoptera that were killed with ethyl-acetate and air-dried had lower yields of DNA and couldn't be amplified in PCR reactions. Quicke et al (1999) suggest that ethyl acetate may be a problem because specimens are often maintained damp for some time before they are mounted. It is important that enzymatic breakdown by endonucleases is prevented, particularly if specimens remain damp, and it is unknown if ethyl acetate does this.

Preservation Methods

While published literature on different preservation methods is limited, there is certainly more information than on killing methods. Researchers have tested DNA quality of specimens dried in silica gel (Post *et al.*, 1993, Simuliidae; Mandrioli *et al.*, 2006, Lepidoptera), direct drying, critical point drying and chemical drying (Austin & Dillon, 1997, Hymenoptera; Quicke *et al.*, 1999, Hymenoptera), freezing in an ultra cold freezer or liquid nitrogen (Post *et al.*, 1993; Dillon *et al.*, 1996; Quicke *et al.*, 1999, Hymenoptera) in various preservation fluids – acetone (Mandrioli *et al.*, 2006), 2-propanol (Post *et al.*, 1993; Mandrioli *et al.*, 2006), Carnoy's (Post *et al.*, 1993, Simuliidae; Mandrioli *et al.*, 2006), ethanol (70-100%), (Post *et al.*, 1993; Dillon *et al.*, 1996, Hymenoptera; Quicke *et al.*, 1999; Vink *et al.*, 2005, arachnids; Mandrioli *et al.*, 2006, Lepidoptera), RNAlater (Vink *et al.*, 2005), propylene glycol (Vink *et al.*, 2005), 1:1 acetic acid: TE buffer, 4% formaldehyde (Gurdebeke & Maelfait, 2002), formal saline (Post *et al.*, 1993) and methanol (Post *et al.*, 1993), and pinned in the presence or absence of naphthalene (Dean & Ballard, 2001, *Drosophila simulans*) for periods of 1 month to 2 years. Vink et al (2005) also tested different fluids at different storage temperatures (40˚C, 19-24˚C, 2-4˚C, -20˚C and -80˚C).

Most studies found 100% ethanol [although Vink et al (2005) found 70% to be as good as 100%] or cold storage (-20˚C, -80˚C or liquid nitrogen) to be the best methods of preservation. For example, Dillon et al (1996) found that specimens stored in 100% ethanol were as good as fresh samples, or those stored at -80°C. They also mention that wasps seem to preserve DNA better than other insects. Table 3 in Quicke et al 1999 (attached), gives a good summary of the literature at that point. They recommend cold storage or critical point drying or chemical drying using HMDS after initial brief preservation in ethanol (70%) for specimens that will be used for both DNA and morphology. Austin & Dillon (1997) also found all methods of chemical drying tested to be suitable for DNA. They mention that 96% ethanol at room temperature or colder will preserve DNA, but is not good for morphology. However, since then several studies have tested other preservation fluids not mentioned in this study, and some of these other fluids were found to be better.

Mandrioli et al (2006) found acetone to be the best fluid for specimen storage. After 2 years these specimens were as good as the specimens stored frozen at extremely low temperatures (liquid nitrogen or ultra cold freezer), and much better then specimens stored in ethanol. They suggest that this is because acetone penetrates the tissues more quickly. Acetone is readily available in most countries as nail polish remover. There is no mention of the suitability of acetone for morphological specimens.

Vink et al (2005) found that RNAlater (a commercially available preparation) and propylene glycol were significantly better than various concentrations of ethanol after 6 weeks storage, particularly at temperatures greater than 4˚C (they note that DNA starts to degrade at room temperature in ethanol after 5 days). These fluids also have the advantage that they can be carried on aircraft and sent by mail. However, Williams (2007) found that RNAlater was unsuitable for long term storage of molluscs for DNA. Vink et al (2005) also mention that these fluids are not recommended for specimens for morphological studies, as they may cause soft tissue shrinkage. Their final recommendation is to put the legs in RNAlater or propylene glycol, and the rest of the specimen in 70% ethanol.

Gurdebeke & Maelfait (2002) found a solution of 1:1 acetic acid: TE buffer to be unsuitable for DNA preservation. They also tested 4% formaldehyde and found moderate preservation (similar to 70% ethanol), for DNA quantity. However when the DNA quality was tested with RAPD (a PCR-based method), it failed.

Post et al (1993) had poor or negligible yields of DNA from samples stored in Carnoy's or formal saline. Mandrioli et al (2006) had similar results with Carnoy's. Post et al (1993) also found ethanol to be better than methanol or propanol, for samples stored up to 1 year. Although propanol gave a high yield, the DNA was highly degraded.

Dessaeur et al (1990) have suggested that continuing activity of nucleases in alcohol preserved specimens can make them poor sources of DNA, and these might be inhibited by the addition of EDTA.

Vink et al (2005) also compared storage temperatures with the various fluids, and found that at 40˚C, after 6 weeks, all samples were degraded to the point that nuclear DNA could not be amplified (but mitochondrial DNA was still OK). Storage temperatures at the Mexican Field Station would not be this high, but may be higher than the "room temperature" tested in this study (19-24˚C). They found 4˚C to not be significantly different to 19-24˚C for this short period of time, but -20˚C and -80˚C were significantly better than other temperatures (but not significantly different to each other).

Transportation

Most specimen preservation fluids must be removed before specimens can be transported, for safety reasons. Williams (2007) simulated different methods of transportation by lowering alcohol concentration of specimens for different time periods. The final recommendations were that specimens should be soaked as long as possible before removing the alcohol (at least 3 days) to ensure penetration into tissues and the time in lower concentration should be minimised. There are commercially available solutions for specimen storage that can be sent by mail (RNAlater, DMSO), but they were found to be unsuitable for long-term storage.

Summary and recommendations

Summary table:

Preservation method	Suitability for DNA	Reference	Suitability for morphology	Reference
Dried in silica gel	Poor	(Mandrioli *et al.*, 2006)	Good	
Critical point drying and chemical drying (standard methods for Hymenoptera, not typically used for other groups)	Good	(Austin & Dillon, 1997; Quicke *et al.*, 1999)	Good	(Quicke *et al.*, 1999)
Ultra cold freezer or liquid nitrogen	Good	(Post *et al.*, 1993; Dillon *et al.*, 1996; Quicke *et al.*, 1999; Mandrioli *et al.*, 2006)		
Acetone	Good	(Mandrioli *et al.*, 2006)		
Propanol or isopropanol	Moderate	(Post *et al.*, 1993; Mandrioli *et al.*, 2006)		
Carnoy's	Poor	(Gurdebeke & Maelfait, 2002; Mandrioli *et al.*, 2006)		
95-100% ethanol	Good-moderate	(Post *et al.*, 1993; Dillon *et al.*, 1996; Austin & Dillon, 1997; Quicke *et al.*, 1999; Vink *et al.*, 2005; Mandrioli *et al.*, 2006)	Poor	(Austin & Dillon, 1997)
<95% ethanol	Moderate	(Gurdebeke & Maelfait, 2002; Vink *et al.*, 2005)		
RNAlater	Conflicting recommendations	(Vink *et al.*, 2005; Williams, 2007)	Poor	(Vink *et al.*, 2005)
Propylene glycol	Good	(Vink *et al.*, 2005)	Poor	(Vink *et al.*, 2005)
1:1 acetic acid: TE buffer	Poor	(Gurdebeke & Maelfait, 2002)		
4% formaldehyde	Poor	(Gurdebeke & Maelfait, 2002)		
Formal saline	Poor	(Gurdebeke & Maelfait, 2002)		
Methanol	Moderate	(Post *et al.*, 1993)		

Based on the literature, I would recommend collecting into 100% ethanol, and replacing this with fresh 100% ethanol within a day or two of collecting, if they were only to be used for DNA studies. For anything that can be stored in 70% ethanol (e.g. Coleoptera), 100% ethanol will

preserve the specimen adequately for morphology, provided that the specimen is relaxed by rehydration before pinning (S. Cameron, pers. comm.). Putting multiple whole specimens in a single vial would require no changes to the current LPL numbering system. For specimens such as Lepidoptera, I would recommend separating one leg from each specimen as a DNA sample and placing it in one of these fluids, and putting the remainder in 70% ethanol or other preferred preservation for morphology. This is a lot of extra work, so it may be feasible only for selected species that will be the subject of molecular work in the near future. This would also be tricky with the current databasing system, because an LPL number represents multiple specimens, so a leg would not be matched to the individual specimen it came from, only the batch of specimens from the same locality, date & collection method. This may be a problem where there is genetic variation within a population.

There is not enough information to prioritise one of the 3 fluids mentioned above as better for DNA. It may be worth trialling some long series in different fluids for comparison. It is probably preferable to put DNA specimens into propylene glycol, as this can be legally sent by mail or carried in luggage, and one of the above studies shows it to be better than ethanol. Specimens can then be transferred to other fluids in the laboratory.

More consistent across multiple studies is the importance of storage temperature. While typical “room temperature” in a cool climate (19-24˚C) is moderately suitable for DNA storage, in a tropical climate temperatures may be higher than this. Ideally samples should be frozen. It may be worthwhile to purchase extra freezers either for the Mexican Field Station or Long Pocket. This also depends on the reliability of the power supply in Mexico.

References

Austin A & Dillon N. 1997. Extraction and PCR of DNA from parasitoid wasps that have been chemically dried. Australian Journal of Entomology 36, 241-244.

Dillon N, Austin A, et al. 1996. Comparison of preservation techniques for DNA extraction from hymenopterous insects. Insect Molecular Biology 5(1), 21-24.

Gurdebeke S & Maelfait JP. 2002. Pitfall trapping in population genetics studies: Finding the right "solution". Journal of Arachnology 30(2), 255-261.

Mandrioli M, Borsatti F, et al. 2006. Factors affecting DNA preservation from museum-collected lepidopteran specimens. Entomologia Experimentalis et Applicata 120, 239–244.

Post RJ, Flook PK, et al. 1993. Methods for the Preservation of Insects for DNA studies. Biochemical Systematics and Ecology 21(1), 85-92.

Quicke DLJ, Belshaw R, et al. 1999. Preservation of hymenopteran specimens for subsequent molecular and morphological study. Zoologica Scripta 28(1-2), 261-267.

Vink CJ, Thomas SM, et al. 2005. The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics 19(2), 99-104.

Williams ST. 2007. Safe and legal shipment of tissue samples: does it affect DNA quality? Journal of Molluscan Studies 73, 416–418.

9.4 Appendix 4. The value of DNA barcoding in characterizing surveyed natural enemies

In this project, we have worked to overcome the limitations of poor DNA quality due to age and preservation of specimens, for selected groups of biocontrol agents. Note that this differs from the original milestone, where all natural enemies were intended to be barcoded.

PCR-based methods (such as DNA sequencing) only require small amounts of DNA, but fragmentation can be an issue when specimens are older, or poorly preserved. However, methods are available for sequencing short segments of DNA (mini-barcode, Hajibabaei *et al.*, 2006; universal mini-barcode, Meusnier *et al.*, 2008) with almost the same level of accuracy in species identification. A 100 base pair (bp) fragment of DNA sequence will discriminate species with 90% accuracy, compared to 97% with 650bp (Meusnier *et al.*, 2008). Where possible we have obtained full-length barcodes, but where necessary we have also included mini-barcodes.

Barcoding of the Bruchidae from *Parkinsonia aculeata*
Bruchids have frequently been used a biocontrol agents for Leguminosae weeds, including *Parkinsonia aculeata*. Bruchids and their host-plants have also been used for basic research on the evolution of host races, host shifts, and phylogeographic variation at the intraspecific level.

In addition to the host-specific bruchid species that have already been released as biocontrol agents, there may be cryptic species or host-races amongst the more widespread and generalist species – particularly *Mimosestes amicus*, which is commonly collected on Parkinsonia, and causes significant damage to seeds (Heard, 2006). DNA methods may also be useful in assisting with the taxonomy of bruchid species that remain to be identified. In this study, we tested the ability of both shorter and longer fragments of the mitochondrial gene cytochrome c subunit 1 (COI) to give sufficient information to identify species of bruchids, and recognise intraspecific variants (e.g. host-races) or cryptic species.

We used the primers of Morse & Farrell (2005) and Simon *et al.* (1994) to sequence approximately 600 or 700 bp of COI. Internal primers were also designed to give 200 or 300 bp DNA sequences.

DNA sequences were obtained for *Mimosestes amicus* collected from *P. aculeata* and *Prosopis* sp. in Mexico (Oaxaca and Veracruz) and for *M. mimosae* collected from *P. aculeata* in Venezuela. Reliable sequences (i.e. with both the forward and reverse strands sequenced) were obtained for 15 specimens. For most of these specimens a fragment of approximately 600 or 700 bp was obtained. For the 2 specimens from Venezuela that were slightly older (collected in 2004, compared to 2006-2007) and 3 of the specimens from Mexico, a fragment of approximately 300 bp was obtained.

Phylogenetic analysis of DNA data was carried out using the Neighbour Joining method giving a phylogenetic tree (Figure 1). Distinct monophyletic groups were obtained for *M. mimosae* and *M. amicus*, with a divergence of approximately 8% (with 19 diagnostic differences in approximately 300bp), which is typical for congeneric species. Within the *M. amicus* lineages, two distinct groups are obtained, with a divergence of slightly less than 2% (with 6 diagnostic differences in approximately 600bp, including 4 in the shorter 300 bp region), with a paraphyletic group containing specimens from Oaxaca and a derived monophyletic group containing specimens from Veracruz. It appears from this data that the *M. amicus* specimens from *Prosopis* sp. do not form a distinct host-race to those from *P. aculeata*. One specimen from *Prosopis* sp. groups within the Veracruz clade from *P. aculeata*, while the other is distinct from all other sequences.

Divergence levels between *M. mimosae* and *M. amicus* suggest that the shorter 300bp DNA fragment is sufficient for differentiating congeneric species of bruchids.

There is notable phylogeographic structure within *M. amicus*, with a divergence of approximately 2% between Veracruz and Oaxaca. Phylogeography of *P. aculeata* shows these regions to have closely related lineages of the host-plant. It is possible that the insect species may show stronger levels of phylogeographic structure, as they may be influenced by different ecological factors to the host-plant. The data obtained to date doesn't show strong evidence for host-races in *M. amicus*, at least not between *P. aculeata* and *Prosopis* sp. but more sampling is required to confirm this.

The status of the highly genetically divergent lineage on *Prosopis* sp. is unknown. It does not appear to be a pseudogene (a non-functional nuclear copy of a mitochondrial gene) as most mutations appear to be at 3rd codon positions. Morphologically it is identical to the other individual collected from *Prosopis* sp.

The age of insect specimens was found to be important for extracting quality DNA. Specimens collected in 2004 could only be sequenced for 300 bp. For several older specimens tested, no DNA data was obtained. Some recommendations for collecting insects for DNA search are available in the literature (e.g. Mandrioli *et al.*, 2006). On the other hand, 300 bp seems to be sufficient for distinguishing species, at least those studied here, and differentiating between biogeographic lineages.

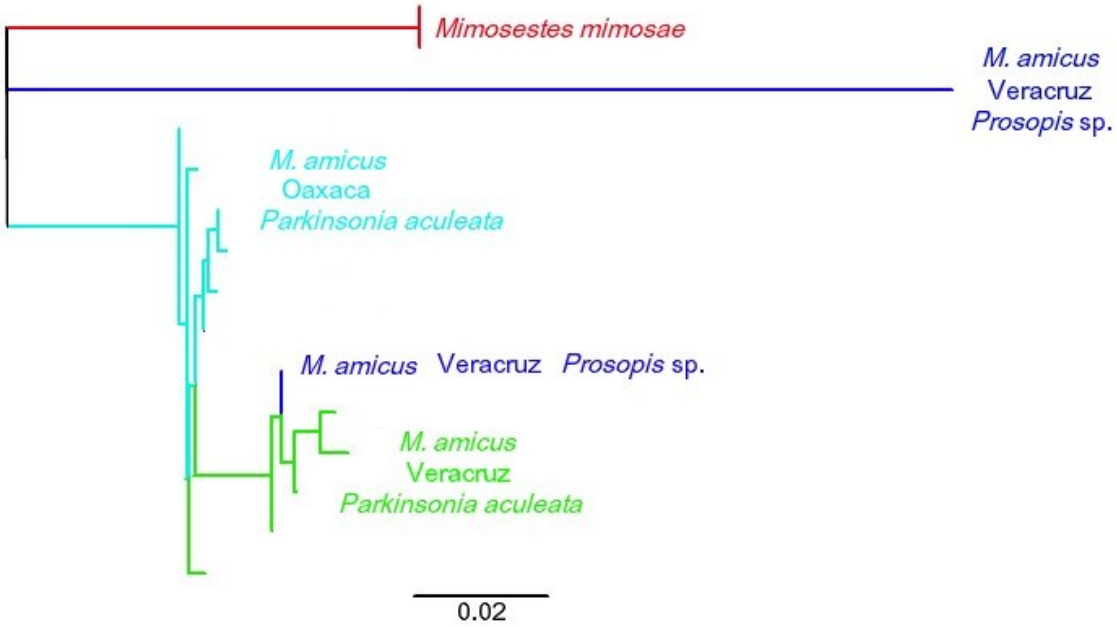

Figure 1: Phylogenetic tree of COI sequences from *Mimosestes* species collected on *Parkinsonia aculeata* and other plant species.

DNA barcoding of *Calosima* n. sp. from *Parkinsonia aculeata*

The caterpillar *Calosima* n. sp. (Coleophoridae: Blastobasinae) has potential as a biocontrol agent but may not be sufficiently abundant to establish cultures. There are two undescribed species present in this genus, collected from *Parkinsonia aculeata*, that are currently being described by David Adamski at the USDA Systematic Entomology Laboratory. As part of this

description, DNA barcodes will be included, and these are also being used to examine variation at the intraspecific level.

Specimens were collected between 2002 and 2006, and were expected to vary in DNA quality with age. We extracted DNA from individual legs using the Qiagen DNeasy kit, with a final elution in 50µL of buffer, rather than eluting twice with 100µL, to increase the final concentration of DNA. Where possible, the standard DNA barcoding fragment of COI was amplified using the primers of Folmer et al (1994). These primers have been modified to include degenerate sites, increasing the range of taxa across which they will work. The PCR reactions contained 1x 5 Prime HotMaster Mix (which includes DNA polymerase, buffers, dNTPs, and does not require optimization for magnesium concentration), 0.4µM each primer, and up to 13µL DNA template. The PCR cycles were an initial denaturation at 95˚C for 2 minutes, followed by 50 cycles of 95˚C for 30 seconds, 48˚C for 30 seconds, 65˚C (the optimum temperature for activity of the HotMaster enzyme) for 1 minute, followed by a final extension at 65˚C for 5 minutes. For specimens with DNA too degraded to obtain the standard DNA barcoding fragment, the minibarcode was amplified using the universal minibarcode primers of Meusnier et al (2008). The minibarcode PCR reaction contained 1x 5 Prime HotMaster Mix, 0.4µM each primer, and up to 13µL DNA template and the PCR cycles were an initial denaturation at 95˚C for 2 minutes, followed by 5 cycles of 95˚C for 30 seconds, 46˚C for 30 seconds, 65˚C for 1 minute, followed by 35 cycles of 95˚C for 30 seconds, 53˚C for 30 seconds, 65˚C for 1 minute, followed by a final extension at 65˚C for 5 minutes. These PCR products were then sequenced by Macrogen Inc. (Korea).

Minibarcode DNA sequences were obtained for 9 specimens of *Calosima*. These sequences were edited and aligned and a phylogenetic tree was assembled in Geneous version 4.0 (Fig. 2). There are two main clades, both including a mixture of specimens from Mexico and Nicaragua. Within the two main clades, the phylogeographic structure is more consistent with geography. The net sequence divergence between the two clades is approximately 4%, which is at the lower end of typical levels of divergence between congeneric species. There is also a high level of divergence within the two clades, which may reflect isolation by distance or further unrecognised species. The specimens are currently being examined by David Adamski to see whether the phylogeographic groupings correspond to morphological divergences.

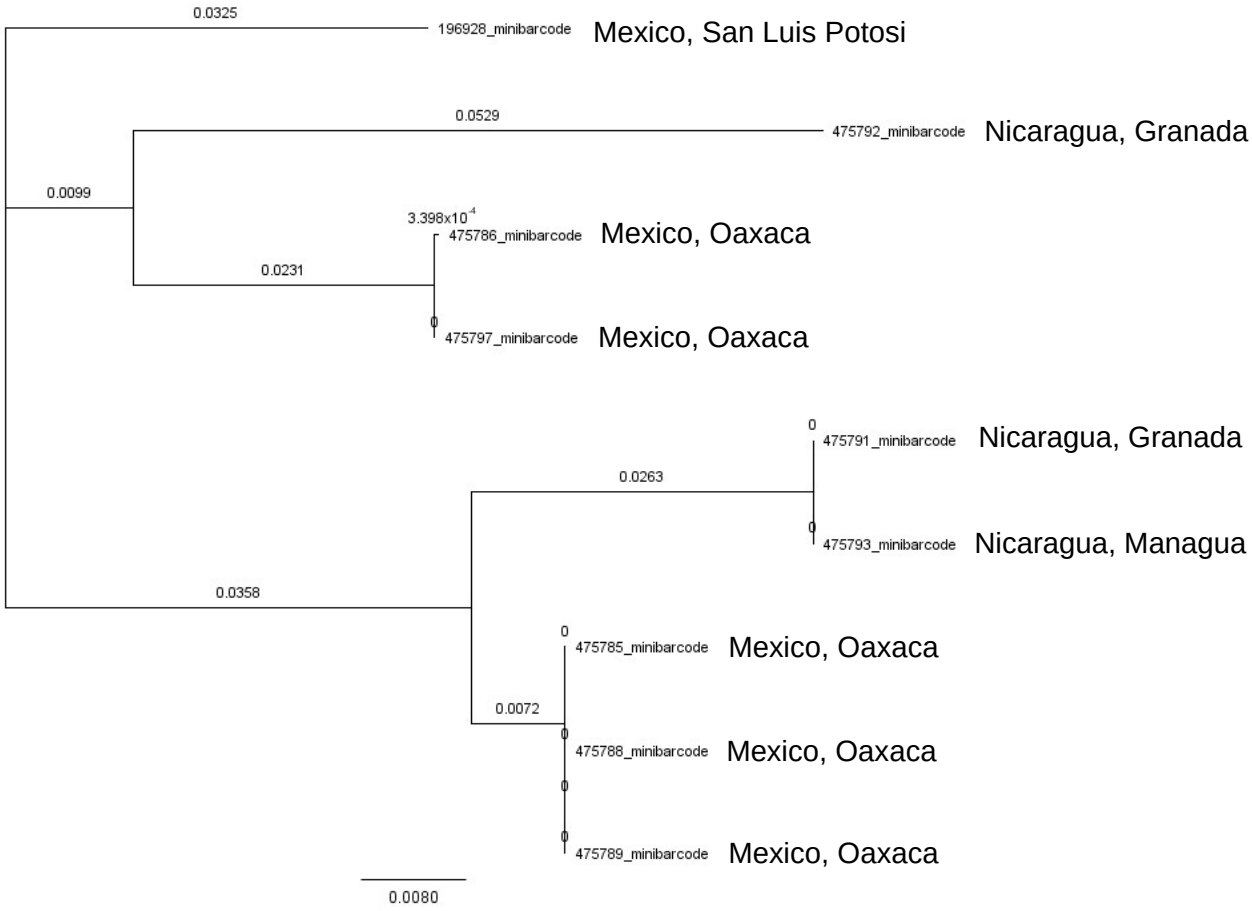

Figure 2: Phylogenetic tree of *Calosima* n. sp. populations across Central America.