Development of new biological control agents of parkinsonia *Parkinsonia aculeata*

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Parkinsonia in Australia

- WoNS
- Pastoral and environmental weed
- Biocontrol recommended as an adjunct to current management practices

History of Biocontrol

- USDA did surveys in Argentina in 1970s
- Qld/WA/NT project made collections from 1983 – 86 focussing on USA and north Mexico
  - Chose that region as thought to be centre of origin. Centre of origin is important as it likely to be the region of:
  - Richest fauna of natural enemies
  - Greatest proportion of host specific natural enemies
- Revealed a low diversity of natural enemies for a tropical tree
- Resulted in introduction of three agents between 1989 and 1995 of which only one, *Pentobruchus germaini,* established

History of Biocontrol

- *Pentobruchus germaini*
  - now widely established but effectiveness is limited by low seed predation rates caused by egg parasitism

Recent history

- Hiatus until 2002 when 4 years of survey work recommenced under NHT WONS funding
- Focus was on surveys in Central America which was recently deemed to be the native range, centre of origin
- Few new potential agents revealed

Portfolio of recent related projects

- LWA
  - Development of new biocontrol agents for Parkinsonia, 06-08
- AWRC
  - Field host range of high priority biocontrol agents of Parkinsonia, 08-09
- MLA
  - Development of new biocontrol agents for bellyache bush and Parkinsonia, 07-10

Objectives of current project (06-08)

1. Broaden the surveys for natural enemies
2. identify the total pool of potential biocontrol agents
3. prioritise agents on the basis of likely efficacy and safety
4. conduct preliminary host-specificity testing with the highest ranked potential agents
5. Further prioritisation to indicate species ready for importation into Australian quarantine for comprehensive host-specificity testing

Objectives of current project (continued)

In addition, this project aims to develop, apply and test new approaches to comprehensively surveying natural enemies of target weeds in their native-range and thereby greatly increase the efficiency of that phase in future biological control programs

6. Quantitative analyse of surveys to ensure future survey effort is best directed
7. Application of DNA bar-coding to solve taxonomic impediment

1. Broaden the surveys for natural enemies

Identify the total pool of potential biocontrol agents

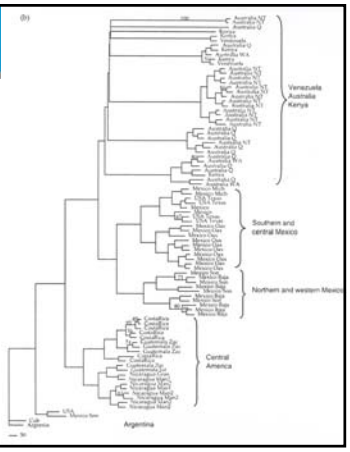
So where is the native range?

Or more importantly, where is the centre of origin?

Centre of origin?

New study on biogeography and systematics (Hawkins al 2007)

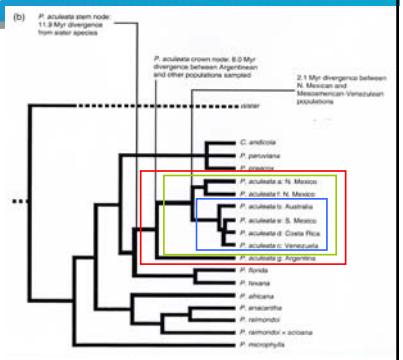
- AFLPs show heavy structuring



Centre of origin?

New study on biogeography and systematics (Hawkins et al 2007)

Gene sequences show deep and old lineages



1. Broaden the surveys for natural enemies

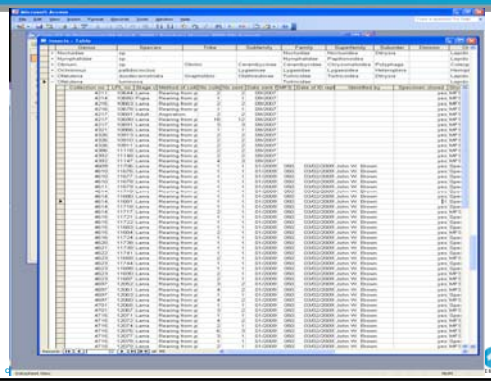
	Locations	Collections
Mexico	75	124
Guatemala	5	8
Nicaragua	10	22
Costa Rica	6	7
Venezuela	49	58
Dominican Republic	**1**	**1**
Ecuador	**2**	**2**
Peru	**3**	**3**
Brazil	**6**	**6**
Total	157	231

- Plus collection records from 17 sites in Argentina and Paraguay obtained

2. Identify the total pool of potential biocontrol agents

- New employee at Mexican field Station has strong taxonomic skills
- Rapidly sorting, identifying to higher levels
- Finding and contacting specialists
- Sending material to specialists
- Receiving material and entering taxonomic information into database.

2. Identify the total pool of potential biocontrol agents



3. Prioritise agents on the basis of likely efficacy and safety

- Consider factors such as:
  - Host specificity
    - Known specificity of relatives
    - Preliminary results from native range
  - Abundance
  - Distribution
  - Damage to plant
    - Severity of damage
    - Placement of damage
  - Ability to test host specificity

3. Prioritise agents on the basis of likely efficacy and safety

Preliminary list of the top potential biocontrol agents of Parkinsonia

	Species	Notes
1	Nr *Rudenia leguminana*	A defoliating / flower bud feeding caterpillar
2	*Ofatulena luminosa*	Stem borer
3	*Calosima* spp.	Tip moths
4	*Eureupithecia cisplatensis*	Defoliating looper caterpillar
5	*Glyptoscelis sonorensis*	Defoliating leaf beetle
6	Cerambycidae spp.	Pod borer
7	Cerambycidae spp.	Stem borers
8	Eulophidae spp.	Flower feeding wasp
9	*Tetrastichus* sp.	Flower feeding wasp
10	*Septoria* sp.	Leaf and stem fungal cankers

4. Conduct preliminary host-specificity testing with the highest ranked potential agents

- Rearing, biology and preliminary specificity testing taking place
  - the garden and field plot of the Mexican Field Station
  - USDA station in Argentina
- Open field tests at file site at Ebano in Mexico

Open field tests at Ebano, Mexico

- Ebano: Large field site in river delta, relatively close to MFS
- Extensive populations of parkinsonia found here together with at least 22 other related legume species
- Ideal for survey of natural host use of various species by insects collected on parkinsonia
- Aim to complete survey over all the phenological stages so leaf, stem, flower and fruit insects can be studied
- Also allows field studies on biology of high priority agents
- Case study: *Ofatulena luminosa*

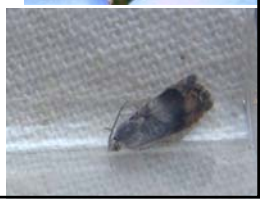
Open field tests at Ebano, Mexico

Open field tests at Ebano, Mexico

Bagged tips and stems for rearing of insects to adult after damage observed on plant

Ofatulena luminosa

Case study: *Ofatulena luminosa*
- Stem boring larva
- Only emerged from Parkinsonia, no other legumes



Host specificity testing based on the USDA lab Buenos Aires, Argentina

Parkinsonia looper: *Eueupithecia cisplatensis*

Host specificity testing based on the USDA lab Buenos Aires, Argentina

Parkinsonia looper: *Eueupithecia cisplatensis*

Host specificity testing based on the USDA lab Buenos Aires, Argentina

Tested plants	N° of replicates	Feeding damage	% adult emergence
Fabaceae			
Caesalpinoideae			
Bahuinia candicans	6	0	0
Caesalpinia gillesii	6	0	0
Gleditsia amorphoides	7	0	0
Parkinsonia aculeata	**5**	**Heavy**	**44**
Peltophorum dubium	6	0	0
Senna corimbosa	6	0	0
Senna spectabilis	6	0	0
Mimosoideae			
Acacia caven	8	0	0
Acacia visco	5	0	0
Prosopis alba	5	0	0

Host specificity testing based at the USDA lab Buenos Aires, Argentina

Parkinsonia looper: *Eueupithecia cisplatensis*

Host specificity testing based at the USDA lab Buenos Aires, Argentina

Parkinsonia looper: *Eueupithecia cisplatensis*

Objectives of current project (continued)

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6. Quantitative analyse of surveys to ensure future survey effort is best directed

•Some relevant questions:

- Is divergence in insect assemblages on Parkinsonia more strongly influenced by climatic factors (current ecology) or biogeography (evolutionary history)?
- Where might further sampling reveal more insect species?

Biogeography of the Neotropics

- Complex geological history of Neotropics
  - Connections between North and South America
  - Formation of mountain ranges e.g. Andes
- Disjunct distribution of suitable habitat types – current and historical
- Occurs in various biogeographic provinces
  - 26 of 70 biogeographic provinces

Regions and transition zones (Morrone et al 2006)

Subregions

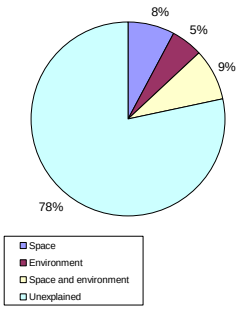
Dominions

Provinces

Methods

- Generalised Dissimilarity Modelling (GDM)
  - Correlates dissimilarities in species assemblages with ecological and geographic distance, and other predictors
  - Response variable:
    - Dissimilarity in species assemblage
  - Predictor variables:
    - Spatial:
      - Geographic distance
      - Biogeography from 0 (same province) to 4 (different region)
    - Climate
      - BIO1 = Annual Mean Temperature
      - BIO2 = Mean Diurnal Range
      - BIO4 = Temperature Seasonality
      - BIO12 = Annual Precipitation
- ANOSIM dissimilarities
  - between samples, grouped by biogeographic provinces
- Species accumulation curves –
  - testing survey completion for each significantly different group of provinces

Percentage variation explained by each group of predictors



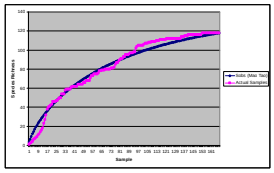
- Difficult to separate effects of space and climate (environment).
- Typical result, both are strongly correlated
- Other factors that may also be having an impact:
  - Temporal factors, e.g. season, plant reproductive stage
  - Phylogeographically distinct plant lineages, extra level of complexity caused by a combination of spatial and environmental variables.
  - Incomplete knowledge of insect assemblage at every site

ANOSIM results

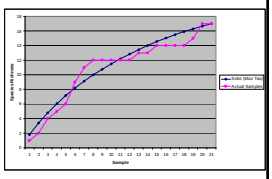
- Significant differences (P<0.05) at the level of province, dominion, region and subregion
- Post-hoc pairwise comparisons between provinces:
  - Group A:
  - Group B:
  - Group C:
  - Group D:
  - Ungrouped (not significantly different to any other sites) – all are smaller samples:
    - Arid Ecuador
    - Caatinga in NE Brazil
    - Western Panamanian Isthmus
- Reasonably congruent with phylogeographic lineages of the host plant (Hawkins et al 2007)
- Also reasonably congruent with biogeographic dominions of Morrone et al (2006).

Species accumulation curves

Group C: Mexican Gulf and Mexican Pacific Coast



Group B: Chaco and Pampa Provinces



Survey completion in sample groups

- Surveying is approaching completion for group C, i.e. Mexican Gulf, Mexican Pacific Coast and Tamaulipas provinces
  - Region close to CSIRO Field Station
- More surveying required for group B, i.e. Chaco and Pampa provinces
  - Field work recently commenced and ongoing in Argentina
- Poorly sampled provinces with insufficient information for analysis – also probably require more sampling:
  - Arid Ecuador
  - Caatinga
  - Western Panamanian Isthmus

Implications for biocontrol

- Insect distributions affected by climatic and spatial variables, therefore need for native range surveying to include diverse range of sampling sites, both climatically and spatially.
- Potentially many unsampled insect species on *P. aculeata* in Argentina – ongoing sampling.
- Only 21% of variation explained by climate and space. Need to investigate variation due to:
  - Sampling effort
  - Temporal variation, e.g. seasons, plant reproductive stage
  - Plant phylogeography

7. Application of DNA bar-coding to solve taxonomic impediment

- DNA barcoding approach sequences a standard gene (COI)
- Encountered limitation due to poor quality DNA (fragmentation)
- Recommendations on how to preserve specimens in future
- Attempted mini-barcodes with some success
- Demonstrated that routine bar-coding of all specimens is not viable. Instead use it to answer specific questions.
- Chose case studies: *Calosima* spp., *Mimosestes amicus, Rudenia leguminana*

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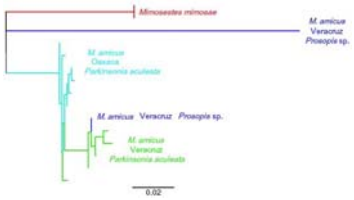
- *Calosima* spp. (stem-boring Lepidoptera)
- Bar coding proved useful to confirm the morphological evidence that two species were present.

7. Application of DNA bar-coding to solve taxonomic impediment

- *Mimosestes amicus* (seed-feeding bruchid beetle)
- Used mini-barcodes due to poor quality DNA
- Polyphagous on numerous legume species
- Are host races present?



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

7. Application of DNA bar-coding to solve taxonomic impediment

- *Rudenia leguminana* (tip moth)
- Damaging and common tip borer and leaf feeder
- Widespread on many legume species
- Wide geographic range suggests species complex
- Also open field tests showed use of Parkinsonia exclusively
- Bar coding suggested the presence of more than one species
- Further sampling for DNA in combination with morphological analyses and host specificity testing required.

Knowledge assets produced

- Prioritised list of natural enemies with information on their predicted impact on the target
- List of plant species for the specificity testing
- Technical improvements in biodiversity surveying

K&A Activities

- Presentations to Australian Parkinsonia Workshops
- Presentations to Australian Weed conferences
- Publications in scientific literature

Current work

- Open field testing of high priority agents of Parkinsonia agents in Mexico and Argentina (Australian Weeds Research Centre)
- Focus on prioritised list of enemies with information on their pros and cons for use as biocontrol agents
- Modify methodologies based on lessons learnt in last two years
- Write up of the barcoding and biogeographic aspects of the work
- Import into Australian quarantine top priority agents of Parkinsonia to commence detailed assessment (depends on funding)

The future (continued)

Years beyond 09/10 (pending funding)

- Continue to import and assess agents according to their priority
- Continue native range work on higher priority agents

•A call for funding for research projects from the Federal government under the sequel to AWRC is expected in the next couple of months. We will apply for funds to import species for detailed assessment in Australian quarantine.

•In the meantime, species for import into Australia have been selected (*Eueupithecia cisplatensis* and *Ofatulena luminosa*) and applications to import them are being prepared.

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Thank you

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