

Evaluating *Mimosa pigra* biological control in Australia

Quentin Paynter¹

Abstract

Over the last 19 years, 11 insect and 2 fungal species have been released as biological control agents against mimosa, *Mimosa pigra* L. (Mimosaceae), in Australia. This paper outlines the fate of these agents and describes current efforts to measure their impact on mimosa in the Northern Territory of Australia. Three techniques have been used to attempt to estimate the impact of biological control. Firstly, insecticide was used to exclude biological control agents to compare the growth and performance of sprayed and unsprayed plants. This approach was not successful because systemic insecticides failed to exclude stem-mining moths, particularly during the dry season. The second technique used was to conduct large-scale agent surveys and compare plant fitness in relation to agent abundance. This work is ongoing and only the methods employed are described here as we intend to publish the results elsewhere. Finally, litter trays have been used to compare plant fecundity with past measurements taken before biological control agents were widely established in Australia. Preliminary results presented here indicate seed rain has declined by approximately 70% since the mid 1980s.

Keywords: mimosa, biological control, evaluation, impact, plant fecundity.

Introduction

Although mimosa, *Mimosa pigra* L., can still be highly invasive in the Northern Territory (NT), of Australia there is evidence the rapid expansion of mimosa has been greatly reduced. Lonsdale (1993) showed that populations doubled in size every 14 months in the late 1970s and early 1980s. This is partially due to strategic control of satellite infestations (e.g. Cook *et al.* 1996) and large-scale programs that cleared approximately 10,000 ha of mimosa across a range of sites including Oenpelli, Melaleuca Station and Wagait Aboriginal Reserve on the East Alligator, Adelaide and Finnis River floodplains, respectively. There is also evidence that culling feral water buffalo as part of the Brucellosis

and Tuberculosis Eradication Campaign (BTEC), which reduced overgrazing of floodplain grasses (Braithwaite and Roberts 1995), further reduced mimosa's ability to invade (Lonsdale 1993). Ongoing population dynamics studies indicate that biological control is also having an impact. Seed banks beneath mimosa stands on the Adelaide and Finnis River floodplains have declined significantly (Barratt *et al.* 2004), compared with those measured by Lonsdale *et al.* (1988), and are approaching levels recorded in the native range by Lonsdale and Segura (1987). Plant mortality, attributed to attack by *Neurostrotta gunniella* Busck and *Carmenta mimosa* Eichlin and Passoa, has been recorded (Paynter, unpublished data).

The purpose of monitoring biological control is to measure the degree of establishment of control agents, their rate of spread and their impact on the target weed. This knowledge should assist the development of a theoretical basis for under-

¹ CSIRO Division of Entomology, PMB 44, Winnellie, NT 0822, Australia.

standing why an agent succeeds or fails. Evaluation is essential if we are to improve the efficiency of future biological-control programs against either the same target weed in other localities, or against other weed species where the ecology of the target weed is similar.

This paper reviews the fate of all agents released against mimosa in Australia: those which failed to establish or persist, those which established but failed to have an impact, and those which have established and have a measurable impact on mimosa performance in Australia.

Establishment and spread

Species for which it is too early to tell if establishment has occurred/failed

Three species have only recently been released, the leaf-feeding looper, *Macaria pallidata* (Warren), first released in July 2002 (Table 1), and the two seed-feeding weevils, *Chalcodermus serripes* Fahraeus and *Sibinia fastigiata* Clark. Although the latter were, respectively, first released in 1996 and 1997 (Table 1), difficulties mass-rearing these species prevented large-scale field releases from being made until more recently. In 1999, a few individuals of *C. serripes* were recovered at a release site on the Finnis River, one year after the last release had been made there, indicating local persistence for a year. None have been seen since.

Species which failed to establish or persist

Four species have apparently failed to establish and/or persist: both pathogens, *Phloeospora mimosae-pigrae* Evans and Carion and *Diabole cubensis* (Arthur & J.R. Johnst.); the flower-feeding beetle *Coelocephalopion aculeatum* Fall; and the seed-feeding bruchid *Acanthoscelides quadridentatus* Schaeffer.

Potential reasons for failure include:

- *Differences in plant phenology between the NT and the native range of the biological control agent.* For example, *C. aculeatum* is an obligate flower-feeder. In the NT, flowering and seed production almost ceases during the dry season (Lonsdale 1988), a factor that may also have hindered establishment of the seed-feeder, *A. quadridentatus*. It is noteworthy that adults of the flower-feeder *Coelocephalopion pigrae* Kissinger, which is very similar to *C. aculeatum* but did establish in the NT, can feed on leaves and therefore survive periods when flowers are not present on the plants.
- *Climatic differences between the NT and the native range of the biological control agent.* For example, germination of the rust fungus *D. cubensis* requires temperatures less than 25°C and a dew period of greater than 10 h (Seier and Evans, 1996), a combination of conditions that is rare in the NT.

Table 1. Status of biological control agents released against mimosa in Australia.

Weed and agent	Plant part attacked	First release	Status
Insects			
Coleoptera (beetles)			
Bruchidae (seed beetles)			
<i>Acanthoscelides quadridentatus</i>	Mature seed	Apr 1983	Did not persist
<i>Acanthoscelides puniceus</i>	Mature seed	Apr 1983	Established
Chrysomelidae (leaf beetles)			
<i>Chlamisus mimosae</i>	Leaves and stems	Nov 1985	Established
<i>Malacorhinus irregularis</i>	Leaves and roots	Oct 2000	Established
Curculionidae (weevils)			
<i>Coelocephalopion aculeatum</i>	Flower-buds	Jan 1992	Did not establish/persist
<i>Coelocephalopion pigrae</i>	Leaves and flower-buds	May 1994	Established
<i>Chalcodermus serripes</i>	Mature green seed	Apr 1996	Establishment not confirmed
<i>Sibinia fastigiata</i>	Young green seed	Dec 1997	Establishment not confirmed
Lepidoptera (moths)			
Gracillariidae			
<i>Neurostrota gunniella</i>	Tunnels in pinnae and small stems	Feb 1989	Established
Sesiidae			
<i>Carmenta mimosa</i>	Tunnels in large stems	Jul 1989	Established
Geometridae (loopers)			
<i>Macaria pallidata</i>	Leaves	Jul 2002	Establishment not confirmed
Fungi			
<i>Diabole cubensis</i>	Leaves	Jun 1996	Did not establish/persist
<i>Phloeospora (Mycosphaerella) mimosae-pigrae</i>	Leaves, stems and pods	Jan 1995	Did not establish/persist

- *Competition with other biological control agents.* For example, *P. mimosa-pigrae* has a long incubation period. The stem-mining moth, *N. gunniella* is ubiquitous in NT mimosa infestations (Smith and Wilson 1995) and leaves infested with both *P. mimosa-pigrae* and *N. gunniella* were shed before the pathogen produced spores, but not when only *P. mimosa-pigrae* was present (Paynter and Hennecke 2001). The seed-feeder *A. quadridentatus* may also have been excluded by competition with the closely related bruchid seed-feeder *Acanthoscelides puniceus* Johnson.

Species for which establishment has been confirmed

Six biological control agents are currently confirmed to have established and persisted in Australia: the twig and stem-mining moths *N. gunniella* and *C. mimosa*; the seed-feeder *A. puniceus*; the flower-feeder *C. pigrae*; and the leaf-feeding beetles *Chlamisus mimosae* Karren and *Malacorhinus irregularis* Jacoby. The impact of a number of these species has been investigated:

- *N. gunniella* herbivory reduced seed production by up to 60% and radial canopy growth by 14% over one growing season (Lonsdale and Farrell 1998) and a single generation of larvae reduced seedling growth by 30% (Paynter and Hennecke 2001). However, *N. gunniella* herbivory was considered insufficient to control mimosa on its own (Lonsdale and Farrell 1998).
- Although both species of *Acanthoscelides* released (*A. puniceus* and *A. quadridentatus*) initially established, only *A. puniceus* has been recorded in recent collections. Herbivory by these agents was considered a failure, destroying only 0.8% of mimosa seed (Wilson and Flanagan 1991), although recent surveys indicate the proportion of seeds attacked by *A. puniceus*, may now be greater, up to ca. 20% (Q. Paynter, unpublished data).
- In addition, the leaf-feeding beetle *Chlamisus mimosae* Karren established only in the Finniss River catchment, where it is insufficiently common to have an impact on mimosa. Cultures of this insect suffered from predation by mites and hymenopterous parasites (Wilson *et al.* 1992) and there is anecdotal evidence that the poor performance of this agent in the field is also due to larval predation by green ants (T. Heard, pers. comm. 2002).
- Steinbauer (1998) showed that mimosa plants, infested with *C. mimosa*, showed a reduced relative growth rate compared with uninfested plants, especially when grown in the shade.

However, these data are laboratory based and do not provide a full picture of the impact.

Recent priorities have therefore been to determine the impact of *C. mimosa* and *C. pigrae*, as well as the combined impact of all agents. Work to determine the rate of spread of *M. irregularis*, which was confirmed to have established at one site in July 2002, has also commenced.

Work on evaluating *C. pigrae* has been regarded as of lower priority than *C. mimosa* because this agent rapidly spread throughout mimosa's range following release in the NT. *Carmentis mimosa* is spreading more slowly, increasing its range at ca. 2 km per year (Ostermeyer 2000) and it has been important to determine its impact to decide the merits of allocating resources towards additional releases.

Three approaches to measuring impact have been used, namely: (a) using insecticides to exclude biological control agents, (b) comparing plant performance at sites where agents are present with plant performance at sites where biological control agents are absent, and (c) comparing past (pre-introduction of biological control agents) demographic data with present demographic data.

Measuring impact

Experiment I. Insecticide exclusion study

Introduction

Farrell *et al.* (1992) reviewed the benefits of various techniques to demonstrate impact of biological-control agents and concluded that manipulative experiments, such as the use of insecticide exclusion, are preferable to experiments that correlate insect abundance with plant performance. We therefore conducted an insecticide exclusion trial at Beatrice Lagoon, near Humpty Doo, NT, Australia (see Lonsdale 1988 for site description). The experiment was designed to assess the impact of all biological control agents on growth, fecundity and survival of mimosa plants using a systemic insecticide.

Design

In the first week of June 2000, individual mimosa seedlings (approx. 20 cm tall) were planted, 4.5 m apart, in a grid (20 rows by 10 columns) of 200 cultivated plots. Prior to planting, all plots were cleared of vegetation (cultivated area 1 × 1 m; cleared area 5 × 5 m around each seedling) to exclude intra- and interspecific competition. Insecticide and control treatments were randomly allocated (100 each).

Plants allocated to the insecticide treatment were initially sprayed at monthly intervals with the systemic insecticide Confidor® (Bayer Australia Ltd, Pymble, NSW; one sachet in five litres), which had been demonstrated to effectively control *N. gunniella* infestations in glass-house conditions (B. Hennecke, pers. comm.). Control plants were sprayed with an equivalent volume of water. At the January 2001 census, it was found that insecticide did not eliminate *C. mimosa* attack, so the spraying rate was increased to twice a month and the dose rate was doubled to two sachets in five litres.

Data collection

Plant height measurements and *C. mimosa* counts were performed monthly. *Neurostrotta gunniella* counts were also performed monthly, initially by counting frass holes on stems, and later, when the plants had grown larger, by randomly selecting 50-cm-long green stem tips from 30 sprayed and 30 unsprayed plants, as described by Smith and Wilson (1995).

Initially, monthly seed production was measured by harvesting the pods from all plants. Then, when seed production increased, by harvesting 30 sprayed and 30 control plants, selected at random.

Analysis

Mean plant height, growth, seed production, and the abundance of agents on sprayed and unsprayed plants were compared using *t*-tests. Data were normalised by Log (n+1) transformation, where appropriate.

Results

The height of plants and biomass (estimated using a correlation between stem diameter and dry weight ($\log_e \text{ weight} = -2.92 + 2.783(\log_e \text{ diameter})$; $r = 0.971$; $n = 358$; $P < 0.001$; T. Schatz and W. Müller, unpublished data) were not significantly affected by insecticide treatment (Figures 1 and 2). The plants first set seed in February 2001 and the cumulative seed production per plant (February–April 2001) was not significantly affected by insecticide treatment (Figure 3).

Damage by *N. gunniella* was initially significantly reduced, but not eliminated, by the insecticide treatments. However, efficacy of the insecticide treatment declined as the dry season commenced, despite an increase in frequency and dose of insecticide treatment. The degree of reduction varied from ca. 75% ($P < 0.001$) in the October 2000 census to only 30% ($P < 0.05$) in the April 2001 census and by a similar amount in the May census, although this difference was not significant (Figure 4). Furthermore, apart from the February 2001 census ($P < 0.05$) there was no significant effect of insecticide treatment on *C. mimosa* abundance

(Figure 5). A smaller scale dry-season trial was performed, using another systemic insecticide Rogor® (Richgro Garden Products, Canning Vale, WA), which also failed to suppress *C. mimosa* and *N. gunniella* (Q. Paynter, unpublished data). The experiment was then abandoned in June 2001, after it had been running for a year.

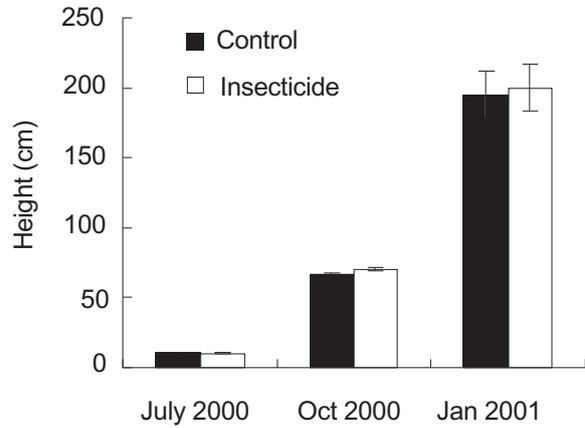


Figure 1. Height of plants (cm).

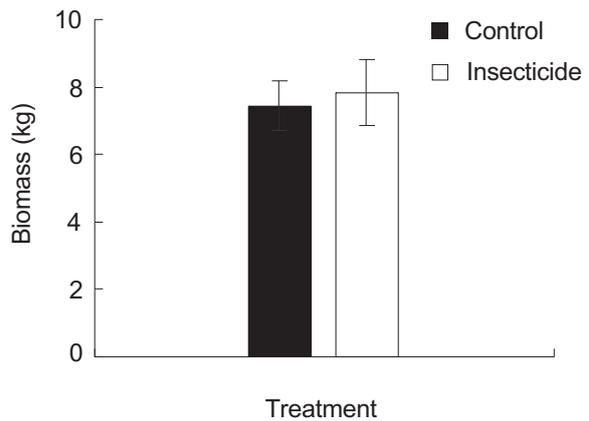


Figure 2. Estimated mean biomass (April 2001 census).

Discussion

This experiment failed because the systemic insecticide used did not exclude *C. mimosa*. This was not entirely unexpected; indeed, Farrell *et al.* (1992) noted difficulties excluding *N. gunniella* using insecticides, and recent advice for controlling *Carmentis chrysophanes* Meyrick, a pest of lychees and persimmons, is to remove and burn infested branches <<http://www.dpi.qld.gov.au/horticulture/5459.html>>.

Insecticide efficacy was highest during the wet season (in February 2001, *N. gunniella* and *C. mimosa* damage was significantly reduced by 75% and 42%, respectively) and lowest in the dry season (in May 2001, insecticide had no significant

effect on *N. gunniella* or *C. mimosa*). Miller and Siriworakul (1992) suggested that increased evaporation of foliar sprays at low humidities, or perhaps poor absorption due to the closure of stomata during periods of lower humidity, could explain low herbicide efficacy in the dry season. Low soil moisture content can also give a lower receptive leaf area, due to wilting and closure of leaves and reduced rate of transport and deposition of herbicide at sites of physiological action (Miller and Siriworakul 1992); perhaps the same may be true for the uptake of a systemic insecticide.

Even if we had used an insecticide treatment that effectively excluded biological control agents, insecticide exclusion experiments have another disadvantage – they may also kill pollinators, so that the impact of biological control agents on plant fecundity cannot be measured (Lonsdale and Farrell 1998). For this reason we also tried other approaches to collecting impact data.

Experiment 2. Comparing plant fitness in relation to agent abundance: large-scale agent surveys

Introduction

Whilst some species, such as *N. gunniella* (Wilson and Flanagan 1990), spread rapidly through mimosa stands in the NT, *C. mimosa* is spreading more slowly, increasing its range at a rate of *ca.* 2 km per year (Ostermeyer 2000). This has enabled sites to be identified where *C. mimosa* is present or absent, so that experiments can be set up with inter-dispersed control and impact sites (see Lonsdale and Farrell 1998). Furthermore, concurrent surveys can also be performed for ubiquitous biological control agents, so that the abundance of all agents present can be correlated to plant performance (see Lonsdale and Farrell 1998)

Materials and methods

Initially, 10 sites on the Finnis River catchment (5 with *C. mimosa*; 5 without) were chosen in 1999 and, in 2000, a further 8 sites (4 with *C. mimosa*; 4 without) were selected on the Adelaide River catchment. Five litter trays were set up, to monitor the seed rain, within the stands at each site, and 25 plants were tagged per site to monitor their survival over time. Litter samples have been collected annually, and insect surveys have also been performed at the same time as follows.

- *Neurostrota gunniella*. Ten 50-cm stems are collected from each site and the number of frass holes counted, as described by Smith and Wilson (1995).
- *Coelocephalapion pigrae* is sampled by beating the tips of 10 stems (approx. 20 cm long), selected at random along the stand edge,

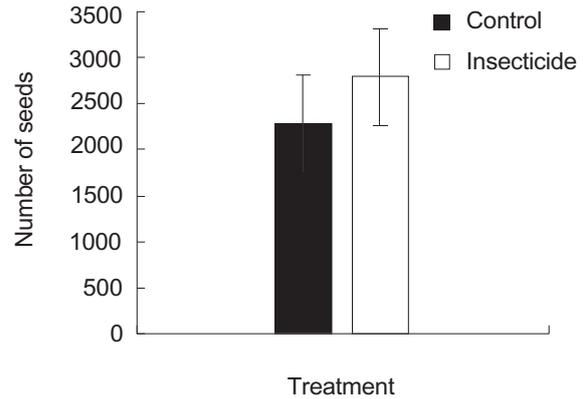


Figure 3. Mean seed production per plant (February–April 2001).

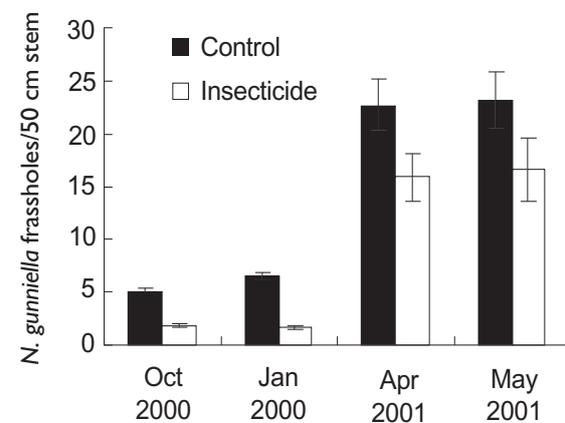


Figure 4. *Neurostrota gunniella* abundance.

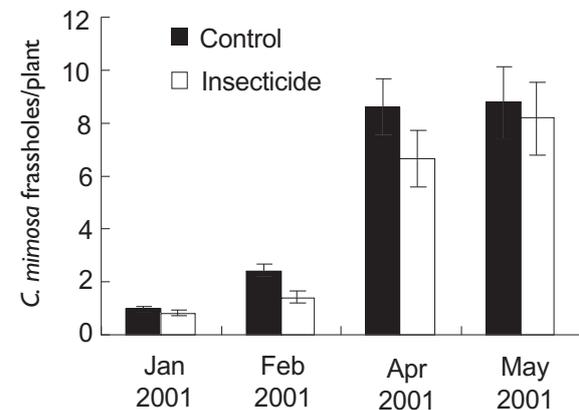


Figure 5. *Carmentia mimosa* abundance.

against the edge of a 21 cm diameter funnel, attached to a *ca.* 7 cm diameter by 7.5 cm deep plastic jar containing 70% alcohol to collect and preserve insects dislodged from the beaten vegetation. The samples were then sorted and insects counted back at the laboratory.

- *Carmentia mimosa* was sampled by randomly selecting 10 plants/site, measuring the diameter of the trunk (so the dry weight of mimosa

could be estimated – see Experiment 1, above) and counting the number of frass holes on each plant, so that the number of *C. mimosa* frass holes/kg of mimosa could be estimated.

- *Acanthoscelides puniceus* attack has also been quantified by counting the proportion of seed collected in the litter samples that contains exit holes made by the adult beetles.

In 2002, additional data were collected: 15 soil cores were taken from each site, and random quadrats (0.5 × 0.5 m) were thrown and the percentage cover of mimosa, competing vegetation and the number of mimosa seedlings and mature stems were recorded.

Preliminary results and discussion

It is too early to report the results of this experiment in detail, but it should provide replicated, quantitative data on the impact of *C. mimosa*. Preliminary results indicate *C. mimosa* is having a major impact on mimosa in the NT, with high densities reducing seed production by ca. 90%. Seedling regeneration has been reduced by an even greater amount because percentage cover of competing vegetation, which can reduce seedling establishment (Lonsdale and Farrell 1998), is significantly greater under plants attacked by *C. mimosa*, presumably because heavily attacked plants cast less shade.

Experiment 3. Comparing plant fitness with the past: seed rain

Introduction

Whilst using insecticide exclusion proved problematical, correlating insect abundance with plant performance may also prove inadequate. For example, estimates of agent abundance may not be representative for agents that have a very rapid generation time, such as the flower-feeder *C. pigrae*, unless sites are sampled at frequent intervals. This is often not possible due to poor wet-season access to field sites. Another problem with such correlations is that insects may not select host plants at random; for example, a biological control agent that preferentially oviposits on large plants (e.g. *Trichosirocallus briesei* Alonso-Zarazaga and Sanchez-Ruiz on *Onopordum* (Briese *et al.* 2002)) will result in a positive correlation between insect abundance and plant performance.

Another option for estimating the impact of biological control has been to compare the contemporary performance of mimosa plants with the performance of plants recorded before the release of biological-control agents (Lonsdale 1988). This may prove to be a particularly useful method for estimating the impact of *C. pigrae*, which spread rapidly following initial release, so

that no sites now exist where *C. pigrae* is absent. For this species, neither experiments with inter-dispersed control and impact sites (see Lonsdale and Farrell 1998) nor insecticide nor cage exclusion experiments can be performed. The use of insecticides or exclusion cages is problematic because they also kill or exclude pollinators, confounding results (Lonsdale and Farrell 1998). Laboratory manipulation studies are hampered by a ca. 97% natural abortion rate of flower heads (Lonsdale 1988).

Lonsdale (1988) collected regular samples from litter trays that were set up at a biological-control agent release site at Beatrice Lagoon, on the Adelaide River floodplain, between 1984–1986. At that time, the only biological control agents present on the Adelaide River system were the two bruchid seed-feeders (Table 1), which together reduced seed production by less than 1% (Wilson and Flanagan 1991). Using the data he collected Lonsdale was able to estimate seed production, flower abortion rates and pod production. By making similar observations, we measured contemporary seed production, flower abortion rates and pod production and compared them with past measurements (Table 2).

Table 2. Comparison of the annual flower production, the mean number of pods per inflorescence, the mean number of seeds per pod and the annual seed production of mimosa at Beatrice Lagoon pre-biological control in the mid-1980s (Lonsdale 1988) and in 2001.

	1984–1986	2001
Inflorescences/m ²	1,792	2,523
Pods/inflorescence	7.1	4.4
Seeds/pod	21.0	18.5
Percentage successful inflorescences	3.3	1.4
Seeds/m ²	9,103	2,868

Materials and methods

In January 2001, 15 litter trays were set up at Beatrice Lagoon, where Lonsdale (1988) performed his study. Five trays were set up along the edge of the stand, five were set up 15 m in from the edge and five 25 m in from the edge along transects cut into the stand. The litter trays were emptied at monthly intervals, and their contents dried and separated into fractions as described by Lonsdale (1988). At the same time each month, five infructescences per plant (at a stage when the pods were well-developed, but still green, so that individual seed cases were not dehisced) were collected from five plants from the edge, and from 15 m and 25 m in from the edge. The numbers of

Pods per infructescence and number of seeds per pod were counted, enabling the proportion of flowers that abort to be calculated (Lonsdale 1988).

Insect surveys were also carried out, to assess attack at various distances from the edge of the stand to test whether the findings of Smith and Wilson (1995) are true for other agents and to relate potential differences in insect attack to seed production.

For *N. gunniella*, 10 × 50-cm stems were collected from each row. *Coelocephalapion pigrae* was sampled by beating the tips of 10 stems (approx. 20 cm long), selected at random along the stand edge and the 15 m and 25 m transects, against the edge of a 21 cm diameter funnel. The funnel was attached to a ca. 7 cm diameter by 7.5 cm deep plastic jar containing 70% alcohol to collect and preserve insects dislodged from the beaten vegetation. The samples were then sorted, and insects counted, back at the laboratory.

Carmentia mimosa was sampled by randomly selecting 10 stems/row, measuring the diameter of each stem sampled (so the dry weight of mimosa could be estimated – see Experiment 1, above) and counting the number of frass holes in each stem, so that the number of *C. mimosa* frass holes/kg of mimosa could be estimated.

Preliminary results and discussion

The preliminary results are given in Table 2. The results, which are from just one season, and therefore need to be treated with caution, indicate there has been a large reduction in seed production at Beatrice Lagoon, from ca. 9,100 seeds/m² to just ca. 2,900 seeds/m² (i.e. a ca. 70% reduction). This reduction is not due to a decrease in flowering; indeed, the number of inflorescences recorded was actually higher than the mid 1980s. However, both the number of pods/inflorescence and the proportion of successful inflorescences showed a major decline. The results might indicate that the flower-feeder *C. pigrae* is having a large impact; however, *C. mimosa* is also abundant at Beatrice Lagoon and while experiments to monitor both species are ongoing we cannot yet confirm whether the reduction in seed production can be explained by the abundance of *C. pigrae* or is due to the indirect impact of *C. mimosa* herbivory or both

Conclusion

Although much of this work is ongoing, it seems clear that biological control is now having a large impact on mimosa performance. The experiments described above should quantify this impact, demonstrating the benefits of the biological-

control program, and enabling evaluation of the performance of individual agents. The latter should have implications for host prioritisation in future biological-control programs.

Acknowledgements

This project is supported by the Natural Heritage Trust.

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