

Agents for biological control of *Mimosa pigra* in Australia: review and future prospects

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Abstract

We review progress in the assessment and release of insects for the biological control of mimosa, *Mimosa pigra* L., including studies on biology, interactions with host plant, host specificity, rearing and release. We list and discuss the approximately 45 agents that have been formally assessed. It is likely that other agents were informally assessed and rejected during native range work. A total of 32 species was exported from the Americas and tested in quarantine facilities. The total number of released agents is currently 13 (excluding *Scamurius* sp. from another project against *Mimosa diplotricha* Sauvalle). Following assessment, many species were rejected either before or after introduction into quarantine. Reasons for rejection include lack of adequate host specificity, difficulties with rearing, difficulties with testing, lack of availability, and because they were not considered to be sufficiently damaging to the target weed. For example, six species of leaf beetles (three root-breeders and three leaf-breeders) were rejected due to inadequate host specificity. Two species of leaf-tying Lepidoptera were rejected because it was too difficult to test their host specificity. The tip weevils, *Temnocerus* spp., were too difficult to rear in the quarantine laboratory. *Morpheis pyracmon* (Cramer) was rated highly in the past but has not been found recently in the field, and so was discarded due to a lack of availability. The gracilariid moth, *Marmara* sp., was removed from the list of priorities because the damage to the stem appeared to be insignificant. Other agents that were not previously on the list of greatest potential agents of Harley *et al.* (1995), such as *Temnocerus* spp. and the geometrids *Macaria pallidata* (Warren) and *Leuciris fimbriaria* Stoll, were added due to observations of their abundance and damage. Seven agents are currently being assessed and we anticipate that several of these will be released if funding continues. The establishment of released agents is recorded here but their impact is discussed elsewhere. Studies on plant-insect interactions have advanced our knowledge of the ecology of weeds and their herbivores. Advances in host-testing methodology have been made that will assist future work on this and other weeds.

Keywords: mimosa, agent selection, host specificity, biological control of weeds, risk assessment.

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Introduction

A biological control project against mimosa, *Mimosa pigra* L., has been active since 1979. From 1979 to 1982, surveys of natural enemies were conducted by CSIRO from a field station in Curitiba, Brazil. The station was re-established in Acapulco, Mexico in 1984 and moved to Veracruz, Mexico in 1987. Since then, the Mexican Field Station has worked continuously on mimosa (Segura and Heard 2004). In addition to Mexico and Brazil, exploratory trips in search of biological control agents were made to Costa Rica, Honduras, Venezuela, Belize and Cuba. The emphasis on exploration ceased around 1993, by which time most natural enemies had been identified. Harley *et al.* (1995) reviewed progress to 1994. After 1994, the study of the natural enemies identified as being potential agents continued. Throughout the project, the order of priority of species for host-specificity testing was continuously reassessed as more was learnt about them.

CSIRO and the Northern Territory Government provided funds for the mimosa project from 1979 to the present, with contributions from the Australian Centre for International Agricultural Research (1984 to 1991), and more recently from the Australian Department of Environment and Heritage (1991 until present). The Mexican Field Station simultaneously conducted work on other target weeds such as *Sida* spp., *Jatropha gossypifolia* L., *Hyptis suaveolens* (L.) Poit., *Argemone* spp. and *Parkinsonia aculeata* L.

In this paper, we review the progress that has been made since 1994 (post the period covered in Harley *et al.* 1995). We will describe the work done on each insect that has been studied. In some cases this work led to the release of the agent. Otherwise, we will discuss the rationale for the decision to reject them from further consideration, or to reduce their priority relative to other species. Species that we believe still have potential are discussed.

The establishment of agents is recorded here but their impact is discussed elsewhere (Paynter 2004). Details on the release and redistribution of agents are reported elsewhere (Hoskins and Rea 1999). For details of work on pathogens, see Evans *et al.* (1995) and Hennecke (2004).

Underpinning this applied work, strategic research was done in the area of insect–plant interactions. The aim was to help us understand the biology and ecology of the insects and to optimise their impact. Research was also done on the methodology of host-specificity testing. This has led to more accurate, quicker and cheaper assessment of agents, enhancing our capacity to do future biological control projects more efficiently (Heard 1995a,b, 1999).

Assessment of the suitability of agents for release in Australia

Here we describe the research undertaken to evaluate insects for their suitability for release in Australia and for optimising their potential as effective agents. The criteria examined include: impact on the target weed, host specificity, ability to test the host specificity, availability in the field, ability to ship live to Australia, and ability to rear in the laboratory. Study of the impact on the target weed allows the prediction of the efficacy of an agent. These studies consist of observations and experiments in the native range and in the quarantine laboratory. The disciplines of biological control and ecology in general have had limited success in predicting which agents will be effective in a new environment. However, some guidelines seem to have some validity and were considered.

Host-specificity testing

An understanding of host specificity allows the prediction of the direct ecological effects of introducing an agent and hence its environmental safety. The host specificity of some agents could not be tested with the resources available. Similarly, some agents could not be reared. These were serious constraints on which insects we introduced.

A variety of techniques was used to determine the host specificity of agents. Tests were done in the field in Mexico and in Australian quarantine. The extent of each varied, e.g. *Malacorhinus irregularis* Jacoby was entirely tested in quarantine. For some species, preliminary testing was done in Mexico, e.g. *Sibinia ochreosa* Casey and *Sibinia peruana* Pierce. In other cases, the results of quarantine testing were validated in the field in Mexico, e.g. the leaf-tying Lepidoptera *Pococera gelidalis* (Walker), *Aristotelia* sp., and *Apotoforma rotundipennis* (Walsingham).

The three types of experimental design were used where appropriate: choice, no-choice and choice-minus-control (Heard and van Klinken 1998). The interpretation of the data and decision-making followed a risk-assessment approach (McFadyen and Heard 1997). Further aspects of host-specificity testing are discussed elsewhere (Heard 1997, Heard and van Klinken 1998, Heard 1999, 2000, 2002).

The host test list against which agents are tested has evolved through the project. A rationale for the list is given in Forno and Heard (1997). The list is long, about 70 species, because of the size and importance of the legume family. This list is now outdated, if one accepts the notion that economi-

cally important species that are distantly related should not be included (Briese 2003). Information on the acceptability of distantly related species has never contributed to the determination of the host range of any biological control agent for mimosa.

Species tested and recently released in Australia

The agents assessed are described below and listed in Table 1. In this first section, we discuss those agents tested and released since Harley *et al.* (1995). We then discuss those agents that have been tested but not released, followed by a section dealing with native biological-control agents. The final section covers those future potential agents on which preliminary work has been done but where more assessment is required.

Coelocephalapion aculeatum (Fall)

This flower-feeding apionid from Mexico was first released in 1992. Adults feed and oviposit into unopened flower buds. Larvae complete their development on the flowers. Host-specificity studies showed that larvae could complete their development only on mimosa, except for very low levels on *Neptunia dimorphantha* Domin. (Forno *et al.* 1994). This insect provided a case study in plant-insect interaction relevant to biological control (Heard 1995a,b). Although initially reported to have established, it has not been found in surveys for several years (Paynter 2004).

Coelocephalapion pigrae Kissinger

This apionid, from Venezuela and Brazil, was discovered after the release of *C. aculeatum* and was first released in 1994 (Heard and Forno 1996). Larvae develop on inflorescences. Adults feed on these inflorescences and also on leaves. It rapidly colonised all infestations of mimosa (Paynter 2004). The ability of adults of this insect to feed on the leaves of the host may contribute to its success, by allowing it to survive during periods of low flower abundance. In addition, this species prefers perfect flowers containing female parts, rather than male flowers, potentially allowing a greater impact on seed set (Heard, unpublished data).

Chalcodermus serripes Fahraeus

This weevil (Photo 1) is a very common seed predator in the native range (Photo 2), along with *Sibinia fastigiata* Clark. Adults oviposit on green seeds approaching full size. Larvae develop inside the pod, each on a single seed. It was not possible to rear this insect in the quarantine laboratory in Brisbane but specificity testing could be conducted on imported adults. Testing focused on

the oviposition and feeding preferences of adults, which proved to be entirely specific (Heard *et al.* 1999). Insects from three provenances – Mexico, Venezuela and Amazonian Brazil – were tested, allowing importations from these three areas. This provided more genetic diversity and importations at all times of the year.



Photo 1. Adult of *Chalcodermus serripes* on a pod of *Mimosa pigra* in Mexico, note the pods damaged by feeding and oviposition. Photo: Tim Heard, CSIRO.

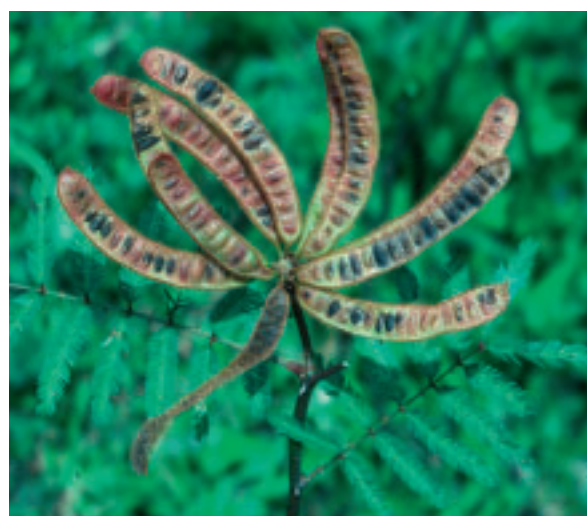


Photo 2. Pods in Mexico damaged by *Chalcodermus serripes* and *Sibinia fastigiata*. Photo: Wendy Forno, CSIRO.

Normally, the Australian Quarantine and Inspection Service (AQIS) requires that imported insects be reared through one generation in quarantine before release. Because this insect could not be reared in quarantine, the import permit included a request to release adults collected in the field in the native range. Following extensive negotiation, permission was obtained to release field-collected adults following an intensive regimen, including the following:

Table 1. The status of natural enemies for biological control of *Mimosa pigra*.

Agent	Plant part attacked	Status
Coleoptera (beetles)		
Bruchidae (seed beetles)		
<i>Acanthoscelides puniceus</i> Johnson	Mature hard seeds	Released Apr 1983.
<i>Acanthoscelides quadridentatus</i> (Schaeffer)	Mature hard seeds	Released Apr 1983.
Chrysomelidae (leaf beetles)		
<i>Chlamisus mimosae</i> Karren	Leaves, stems	Released Nov 85.
<i>Cryptocephalus</i> nr <i>miserabilis</i>	Leaves	Studied 81, rejected – not specific.
<i>Lexiphanes</i> nr <i>guerini</i>	Young leaves	Studied 85/86, rejected – not specific.
<i>Diplacaspis</i> nr <i>prosternalis</i>	Stem and leaves	Studied 85/86, rejected – not specific.
<i>Syphrea bibiana</i> (Bechyne)	Leaves, roots	Studied 84, 97, 01. Currently being reared and studied in quarantine.
<i>Syphrea</i> sp. (<i>Syphrea flavipes</i> group near <i>S. cardiaca</i>)	Leaves, roots	Studied 95–97, rejected – not specific.
<i>Genaphthona</i> nr. <i>transversicollis</i>	Leaves, roots	Studied 95–97, rejected – not specific.
<i>Paria</i> sp.	Leaves, roots	Studied 95–97, rejected – not specific.
<i>Malacorhinus irregularis</i> Jacoby	Leaves, roots	Released Oct 00.
Curculionidae (weevils)		
<i>Coelocephalapion aculeatum</i> (Fall)	Flower-buds	Released Jan 92.
<i>Coelocephalapion pigrae</i> Kissinger	Leaves and flower-buds	Released May 94.
<i>Coelocephalapion spretissimum</i> (Sharp)	Flower-buds	Accidentally introduced in quarantine as contaminant with <i>C. aculeatum</i> .
<i>Chalcodermus serripes</i> Fahraeus	Mature green seed	Released May 96.
<i>Chalcodermus persimilis</i> O'Brien	Mature green seed	Studied 94/95, rejected – not specific.
<i>Sibinia fastigiata</i> Clark	Young green seed	Released Dec 97.
<i>Sibinia seminicola</i> Clark	Young green seed	Studied in Mexico, 93–94. Rejected, normal host is <i>Mimosa asperata</i> (but see text on Brazilian provenance).
<i>Sibinia ochreosa</i> Casey	Flowers	Studied in Mexico, 97–99. Host testing 15% complete.
<i>Sibinia peruana</i> Pierce	Flowers	Studied in Mexico, 97–99. Host testing 15% complete.
Rynchitidae		
<i>Temnocerus debilis</i> (Sharp)	Young leaves and tips	In Brisbane quarantine, 98–02. Developing rearing and host-testing methodology.
<i>Temnocerus</i> sp. (Venezuela)	Young leaves and tips	To be imported.
Cerambycidae (longicorns)		
<i>Platymopsis humeralis</i> White	Girdles and breeds in stems	Redistributed 97–99, damaging native insect.
Lepidoptera (moths)		
Gracillariidae		
<i>Neurostrota gunniella</i> (Busck)	Tunnels in pinnae and small stems	Released Feb 89.
<i>Marmara</i> sp.	Tunnels under surface of stems	Studies in Mexico, c. 95. Rejected – not sufficiently damaging.
Sesiidae		
<i>Carmenta mimosa</i> Eichlin & Passoa	Tunnels in large stems	Released Jan 89.
Gelechiidae		
nr <i>Aroga</i> sp. = <i>Aristotelia</i> cf <i>howardi</i> = <i>Gelechia benitella</i> = new genus, new sp.	Leaves and stems	Studied 84–85, rejected – not specific.
<i>Aristotelia</i> sp. near <i>dasyopoda</i>	Leaves	Studied in Mexico and Australia, 97–01. Rejected – unable to determine specificity.
Pyralidae		
<i>Pococera gelidalis</i> (Walker)	Leaves	Studied in Mexico and Australia, 97–01, rejected – not specific.
Tortricidae		
<i>Apotoforma rotundipennis</i> (Walsingham)	Leaves	Studied in Mexico and Australia, 97–01. Rejected – unable to determine specificity.

Table 1. (cont' d) The status of natural enemies for biological control of *Mimosa pigra*.

Agent	Plant part attacked	Status
Cosmopterigidae <i>Ithome</i> sp.	Pods	Studied in Mexico, 97–00. Rejected– rare and not available when pods occur in Australia.
Cossidae <i>Morpheis pyracmon</i> (Cramer)	Stems	Mexico and Brazil, 95– 96. Rejected – insect not found.
Geometridae (loopers) <i>Macaria pallidata</i> (Warren) <i>Leuciris fimbriaria</i> (Stoll)	Leaves Leaves	Released Sep 02. Studied in Mexico, 98–02. Imported to Australia, 02. Colony in quarantine, developing host-testing methodology.
Hemiptera (bugs)		
Pseudococcidae <i>Spilococcus prosopidis</i> (Cockerell) Numerous other species	Young leaves Young leaves	Studied in Mexico, 95–96. Rejected – not found. Studied in Mexico, 95–96, rejected – not specific.
Cicadellidae Several species	Leaves	Studied in Mexico, 96–98. Rejected – not specific, difficult to rear, larvae not found.
Coreidae <i>Scamurius</i> sp. <i>Mictis profana</i> (F.)	Leaf tips Leaf tips	Released 88. Released – did not establish (See text, normal host is <i>M. diplotricha</i>). Damaging native insect.
Hymenoptera (wasps)		
Eurytomidae <i>Risbecoma pigrae</i> Rasplus	Seeds	Attempted to collect in Mexico, 00–02. Needs further assessment.
Fungi		
<i>Phloeospora mimosae-pigrae</i> Evans & Carrion <i>Diabole cubensis</i> L. <i>Microstroma ruizii-belinii</i> Evans & Carrion <i>Mycosphaerella mimosicola</i> Henn. <i>Botrydiplodia theobroma</i> Pat.	Leaves, stems and pods Leaves Stems	Released Jan 95. Released Jun 96. Studied in Mexico. Rejected – not damaging in native range. Studied in Mexico. Rejected – only on <i>M. asperata</i> . Studied in Australia. Cosmopolitan fungus with broad host range, causes die-back.

1. all insects will be held in quarantine for at least two weeks for inspection and treatment
2. the identity of each individual will be confirmed by two scientists familiar with the species, this to be done by microscopic examination while the insects are anaesthetised
3. each individual will be inspected for the presence of mites or other phoretic organisms
4. each individual will be rinsed in hypochlorite solution to surface-sterilise
5. each individual will be inspected for external signs of disease or parasites
6. a sample (5%) of all insects will be squashed and inspected by an insect pathologist for disease.

Approximately 30 importations, mostly from Mexico and Brazil, but including one from Venezuela, commenced in 1996 and continued until 2000. This resulted in the release of approximately 4,600 field-collected adults. Three shipments found to be contaminated with a microsporidium were destroyed. In 2000, staff from the Northern Terri-

tory Weeds Branch developed a method for mass rearing this species. Hence, the justification and need for release of field-collected adults ceased to exist. Laboratory rearing resulted in the release of a further 4,700 adults until the effort ceased in 2002. Approximately 9,300 adults have been released at seven sites (N. Graham, pers. 2003). Establishment has not yet been confirmed (Paynter, 2004).

Sibinia fastigiata

This weevil (Photo 3) is a very common seed predator (Photo 2) in the native range of Mexico, Central and South America. Adults oviposit on small green seeds. Larvae develop inside the pod, each on a single seed. The first two larval instars feed on the perimeter of the seed allowing it to continue growing. The final instar then consumes the entire seed. It was not possible to rear *S. fastigiata* in the quarantine laboratory in Brisbane but specificity testing was conducted on imported long-lived adults. Testing focused on the oviposition preferences of adults, which proved to be

entirely specific (Heard *et al.* 1997). Adults feed non-destructively on pollen and possibly nectar from open flowers, so it was not necessary to test the host specificity of adult feeding. Insects from Mexico and Amazonian Brazil were tested, allowing importations from these two provenances. This provided more genetic diversity and allowed collections for importations at all times of the year.



Photo 3. An adult of *Sibinia fastigiata* on a young pod of *Mimosa pigra*. Photo: Tim Heard, CSIRO.

As already noted, AQIS normally requires that imported insects be reared through one generation in quarantine before release. Because this insect could not be reared in quarantine, the import permit included a request to release adults collected in the field in the native range. Following extensive negotiation, including a review of disinfection chemicals (Wittenberg and Heard 1997), permission was obtained to release field-collected adults following an intensive regimen, including the following:

1. all insects will be held in quarantine for at least two weeks for observation and treatment
2. the identity of each individual will be confirmed by two scientists familiar with the species, this to be done by microscopic examination of the anaesthetised insects
3. each individual will be inspected for the presence of mites or other phoretic organisms
4. clean mechanically with 1% Trimove (quaternary ammonium compound cleaning agent) using a 30-second soak followed by a 10-second spray action over a sieve to clean crevices
5. rinse in sterile water and dry with forced air
6. immerse for 1 minute in fresh undiluted alcoholic povidone-iodine 10% (1% available iodine as an aqueous iodophor preparation).
7. air dry
8. store in heat-sterilised or chemically disinfected environment.
9. each individual will be inspected for external signs of disease or parasites
10. a sample (5%) of all insects will be squashed and inspected by an insect pathologist for disease
11. a sample (5%) of all insects will be sacrificed for examination of concealed surfaces by dissection.

The chemical treatments for surface cleansing proved to be very damaging to the adults, decreasing their longevity and fecundity (Heard 2001). Further negotiations with AQIS resulted in the replacement of this overly stringent regimen with the same regime used for *C. serripes* (see earlier).

Approximately 25 importations, from both Mexico and Brazil, have been received since 1997, and they continue to the present. This has resulted in the release of approximately 2,450 field-collected adults. Six shipments were found to be contaminated with a microsporidium and were destroyed. Only shipments since 2001 have been processed through quarantine using the new, gentler treatment. It is too early to know if this species is established (Paynter 2004). We recommend that these shipments continue in order to give this insect the best chance of establishment.

Malacorhinus irregularis

Malacorhinus irregularis (Photo 4) is a chrysomelid beetle known only from Mexico. The adults feed on leaves of the host and the larvae develop on seedlings and, to a lesser extent, on other plant parts such as imbibed seeds and roots. The larvae complete development from egg to adult in about 36 days and adults can live for up to 6 months (Heard *et al.* 2000). This insect is not common in Mexico; searches were made between 1995 and 1998 before a sufficient number of adults to start a culture was found. The paucity there was attributed to low abundance of mimosa seedlings. It was predicted that, with the availability of large numbers of seedlings, the insect could become common and damage this critical stage in the plant's lifecycle.

Host-specificity tests were conducted to determine the suitability of seedlings and leaves for larval development, and suitability of leaves for adult feeding. No larval survival occurred on any plant species other than mimosa. The extent of adult feeding on the test plants was negligible, being less than 1% of that which occurred on mimosa (Heard *et al.* 2000). The first release occurred in 2000 and mass-rearing and releasing has been conducted continuously since then. This species has already established in one river system (Paynter 2004).



Photo 4. Gregarious adults of *Malacorhinus irregu-laris* in the field in Australia. Photo: Quentin Paynter, CSIRO.

Macaria pallidata

Harley *et al.* (1995) listed *Macaria pallidata* (Photo 5) as being restricted to *Mimosa* spp. but having no potential “because ectophagous larvae of Lepidoptera are often subjected to high levels of parasitism and predation”. However, this is a common and damaging species in Mexico where the ant fauna on mimosa is also abundant, presumably so are parasitoids. Hence, we raised it up the list of priority agents to be assessed. Four shipments of larvae from Veracruz, Mexico, were received into Brisbane quarantine in 1998 and 2000 and used to start a colony for study.



Photo 5. An adult of *Macaria pallidata* on a leaf of *Mimosa pigra*. Photo: Tim Heard, CSIRO.

Adults of this moth are short-lived, nocturnal and feed on nectar sources. Females deposit eggs directly on leaves and stems. Larvae begin feeding by removing the top surface of the leaf. They feed, exposed without protection, on young and mature foliage completely stripping plants when larval densities are high. During the day, they rest on the tips of leaves in positions that imitate stems. They move very little from their original position until forced to look for more food after almost

completely consuming pinnules. Larvae drop on a silken thread when disturbed. The larvae develop through five instars. Pupation is in the soil or among damaged plant tissue. Time from egg to adult under laboratory conditions at 25–27°C is 25 days. It is a multivoltine species with several generations per year. In addition to Mexico, this species appears to be extremely widespread across tropical America as material lodged in the Museum of Natural History, UK, is from the Amazon basin, Nicaragua, Colombia, Paraguay and the Guianas region of Venezuela (Heard *et al.* 2001).

The host specificity of this species was tested using laboratory larval development tests on 70 test plant species. Development to adult occurred on six species other than mimosa. However, the survival rates were so low that these plants could not sustain a population of this insect species. The maximum survival rate on a non-target species was 1.1% compared with 64% on mimosa. When the mean lifetime fecundity is considered, a survival rate of 1.1% is the minimum required for population maintenance in the absence of other mortality factors. Open field trials in Mexico, although not comprehensive, support the conclusion that this insect is specific to mimosa. Approval was obtained to release this insect and first releases were made in 2002 (Heard *et al.* 2001). It is too early to know whether this agent has established (Paynter 2004).

***Scamurius* sp.**

Queensland Government workers introduced *Scamurius* sp. (Coreidae) into Australia from Brazil for the biological control of *Mimosa diplotricha* Sauvalle (= *Mimosa invisa*). Host-specificity tests in the laboratory showed that mimosa is also acceptable for adult feeding and suitable for larval development, although native range observations indicated that it will not transfer to mimosa (Wild, 1986). In 1988, Northern Territory Government workers released this tip-feeding bug in mimosa infestations in the hope that it may prove to be an effective agent. No evidence of establishment was ever found (C. Wilson, pers. comm. 2003). This insect is not a recent introduction but is mentioned here because it does not appear on earlier lists of released agents compiled by Forno (1992) and Harley *et al.* (1995). Because this insect was not selected and tested specifically for mimosa, it is not included in any of the lists of mimosa agents, including Table 1.

Species recently assessed but not released

***Sibinia seminicola* Clark**

This species appears on the list of insects with greatest potential (Harley *et al.* 1995). However, it may not be compatible with mimosa. To confirm

field host preferences, we collected large numbers of mature pods of mimosa, and the closely related *Mimosa asperata* L., from many plants growing at several sites in Mexico in 1993, and held them for adult emergence. The results showed that the normal host for *S. seminicola* in Mexico is *M. asperata* (Table 2). The earlier belief that *S. seminicola* was a predator of mimosa may have originated from the previous lumping of mimosa and *M. asperata* together under the one species, *M. pigra* (Turner 1959). On the basis of this information, we dropped *S. seminicola* from the list, as it was unlikely that an insect with such a low preference for, or suitability to, mimosa will be an effective agent. However, *S. seminicola* is often collected on mimosa in Brazil where *M. asperata* does not occur. For example, in 13 shipments of *Sibinia* spp. from Brazil between 1996 and 2001, four shipments included *S. seminicola* at a mean level of 12%. This raises the question as to whether biotypes of *S. seminicola* from Brazil may be adapted to mimosa and be worthy of further assessment.

Chalcodermus persimilis O'Brien

This species was accidentally imported into Australia from Mexico as *C. serripes*. Differences in morphology and behaviour were observed. This led to its description as a new species (Heard *et al.* 1998). Oviposition preferences, adult feeding preferences and larval development of *C. persimilis* were determined for a selected group of legume plant species. Adults fed and oviposited on several species of Mimosaceae. Larvae developed into normal adults on several of these species. The host range of this species was therefore too broad to be considered as a biological control agent of mimosa (Heard *et al.* 1998).

Ithome sp.

This small moth appears to feed exclusively on the pods of mimosa though it may also eat leaves, flowers and stems. Little is known about its biology. Larvae tunnel in pods, eating the seeds and pod wall. They often tie two pods together with silk. The taxonomy is not clear: more than one species may occur and even other genera such

as *Obithome* may be present in collections. Specimens of *Ithome* are common in the collections, 34 individuals from Veracruz were reared in 1994 from field-collected pods of both *M. asperata* and mimosa. It is also known from Cuba.

This species is currently not highly rated as a prospective agent because it is difficult to rear, adults are very small, delicate, and difficult to handle, and it is not very common or damaging in the field. Also, it is not available in the field when pods are present in Brisbane (March–May). More work is needed, e.g. to find the preferred feeding site of larvae. It is possibly not compatible with other seed-feeding insects because *Ithome* damage the pod wall, seeds or perhaps the other species of larvae directly.

Marmara sp.

This moth, known from Mexico, Brazil and Venezuela, was considered a high priority in Harley *et al.* (1995), as the larvae feed extensively under the epidermis of the stem. In subsequent preliminary studies, the direct damage of *Marmara* was reassessed and considered to be insignificant, so this species was dropped from the list of priority species.

Morpheis pyracmon (Camer)

This moth was considered a potential agent by Harley *et al.* (1995). However, no specimens were present in any collection. Furthermore, specific attempts to find this insect in Brazil in 1995 and 1996 failed to locate any specimens.

Spilococcus prosopidis (Cockerell) (or near)

This mealybug was considered a potential agent by Harley *et al.* (1995). However, attempts to find this insect in Tabasco state, Mexico, in 1995, failed to locate a single specimen. According to the literature, *S. prosopidis* is known from USA, on *Prosopis velutina* Wootton and *Poradendron* sp. Mexico is south of its known range (Ben Dov 1994). Numerous other species of pseudococcids were found and identified by G. Watson, Natural History Museum, UK. The species not recorded in Harley *et al.* (1995) are: *Dysmicoccus grassii* (Leon-

Table 2. Emergence of adults of the pod breeding *Sibinia* spp. from two species of *Mimosa* in Mexico.

	<i>Mimosa pigra</i>	<i>Mimosa asperata</i>
No. of collections	11	9
No. of collection sites	4	2
Total number of pods collected	1,256	1,480
No. of adults of <i>Sibinia fastigiata</i> emerged	214	6
No. of adults of <i>Sibinia seminicola</i> emerged	1	507

ardi), *Nipaecoccus neogaesus* Williams & Granara de Willink, *Paracoccus lycopersici* Ezzat & McConnell, *Pseudococcus cryptus/calceolariae/comstocki* complex. None of these are host specific even to legumes, much less mimosa, and hence all members of this group were dropped from further consideration.

Cicadellidae

Harley *et al.* (1995) list about 20 species of leafhoppers collected from mimosa. Some of these were known to have a wide host range and so were rated as having no potential. The remainder were listed as having an unknown host range and so were rated as having unknown potential. Due to the abundance and high level of damage caused by these insects, we decided to reassess them.

New identifications by Paul Freytag in 1997 of species from Mexico and Venezuela showed that several species are present, including *Kunzella pseudomarginella* (Cardwell), *Zygina* sp. *ceonothana* group, *Kunzeana flavella* Ruppel & DeLong, *Kunzeana vomerella* Ruppel & DeLong, and *Kunzeana* spp. The first two species are not listed in Harley *et al.* (1995) and the *Kunzeana* are only listed as *Kunzeana* spp. The most abundant and widespread species is *K. pseudomarginella* occurring in Mexico and Venezuela. More species identifications would be needed to find out seasonal and geographic patterns of abundance.

Species from this family are abundant: approximately 30 adults per hour per person can be collected at most sites in Mexico. The feeding damage was positively identified and described: it occurs on the tops of mature leaves and consists of white, star-shaped patches, 0.5 mm in diameter, radiating from a central insertion point. Several problems limited research on this taxon. First, only adults were found on mimosa in the field, never nymphs or eggs. Uncertainty as to where nymphs occur led to doubts about host specificity. Possibly, mimosa is not a breeding host, but only suitable for adult feeding. Second, it is difficult to transport adults alive from the field to the laboratory. The greatest success was attained with carrying a caged plant into the field and releasing collected adults directly into it. Even two hours in a cage between the field and the lab resulted in high levels of mortality. It is not likely that they would survive a trip from Central America to Australia. Third, preliminary tests showed them to lack host specificity. Finally, there were problems with taxonomy. Hence, we decided to discontinue working with this group. Some information is summarised below in case work restarts in the future. Future studies would need to include collections from other plant species in the field to determine field host range and gather

information about the sites and host species for nymphal development.

Trials on the mortality (Figure 1) and feeding damage (Figure 2) of cohorts of adults on a range of species showed the lack of specificity of these insects. These are very preliminary data; the trial was not replicated and used a sample that was probably mixed identity. The trial used adult Cicadellidae collected in the field from Playa de Vacas, Mexico, and placed on tips of several plant species. After two days they were changed to new tips and the old ones were checked for feeding damage. Live insects were counted. This was repeated after another two days. The species of insects were not determined. Survival was similar on all plant species except *Mimosa quadrivalvis* L. var. *distachya* (DC.) Barneby, upon which they died rapidly. Feeding was greatest on mimosa species with all other species except *S. distachya* accepted for feeding.

***Syphrea* sp., *Syphrea bibiana* (Bechyné), *Genaphthona* sp., *Paria* sp.**

These four leaf beetles are united by their life history of larval development on roots. Laboratory breeding and testing of the specificity of these species is made difficult by this fact. To assist with the process of prioritising this group, we designed a method to determine the host specificity of adult feeding (Heard and van Klinken 2004). On the basis of this method, only *S. bibiana*, was selected for further assessment of the larval feeding host range (see more on this species below). The remaining species appear to lack sufficiently narrow host specificity of adult feeding for further consideration.

Pococera gelidalis*, *Apotoforma rotundipennis*, *Aristotelia* sp. near *dasyppoda

These three species of moths feed on leaves and tie leaves together to form a protective shelter, causing heavy damage in the native range. All species were imported into Australian quarantine for evaluation of their safety for release. Steps in their natural host-selection behaviour were not expressed in cages, resulting in indiscriminate oviposition on many plant species. As oviposition preferences could not be tested in these circumstances, we tested the innate developmental host range of larvae. Although mimosa was the superior host for all three species, development to adult occurred on several other hosts. We then conducted open field tests in semi-natural conditions in Mexico. We grew four individuals of 26 test plants species, of Australian and Mexican origin, in a field plot. We successively released cohorts of laboratory-reared adults of the three

species. All resulting leaf ties were bagged to capture emerging adults. The numbers of adults reared on the 48 mimosa plants was disappointingly low; 11, 17 and 103 for *A. rotundipennis*, *Aristotelia* sp. and *P. gelidalis*, respectively. The numbers released were 437, 295 and 150, respectively. No adults of *Aristotelia* sp. were reared from the test plants but two adults of *A. rotundipennis* were reared from the closely related *M. asperata*. However, not much value was placed on these results as the poor return of reared adults to released adults suggests that the oviposition pressure on plant species was too low. One useful result was that adults of *P. gelidalis* were reared from one test plant species, *Desmanthus virgatus* (L.) Willd., confirming that it is not sufficiently specific to release in Australia. However, the other two species remain as possible, but difficult to test, candidates (Heard *et al.* 2004).

Coelocephalopion spretissimum (Sharp)

This apionid, from Mexico has been accidentally introduced in quarantine as a contaminant in shipments of other *Coelocephalopion* species. Although Harley *et al.* (1995) listed this species as having potential, it was considered at the time to be a lower priority than the other *Coelocephalopion* species, probably because it was less abundant. It was assessed further.

Native or cosmopolitan species that occur in Australia

Platyomopsis humeralis White

Over 100 native insect species have been recorded on mimosa (Wilson *et al.* 1990, Flanagan *et al.* 1990). The native cerambycid, *P. humeralis*, is probably the most abundant, occasionally causing conspicuous damage by girdling of stems. Efforts

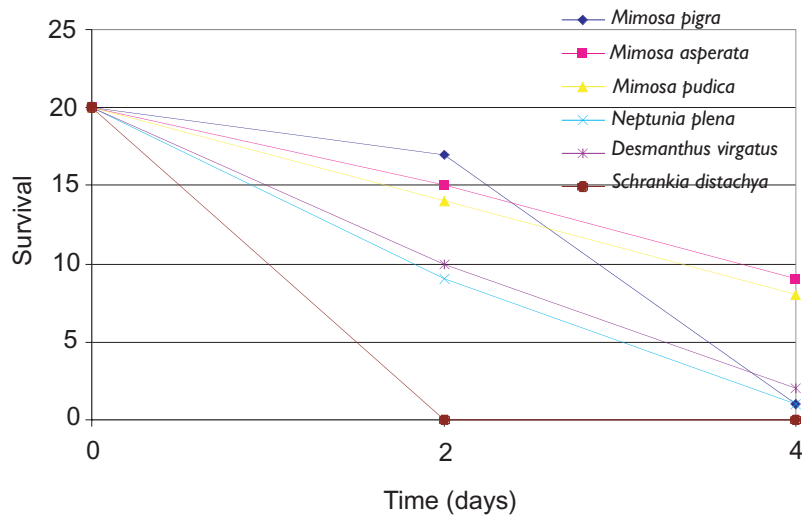


Figure 1. Survival of adult field-collected Cicadellidae when held on various plant species.

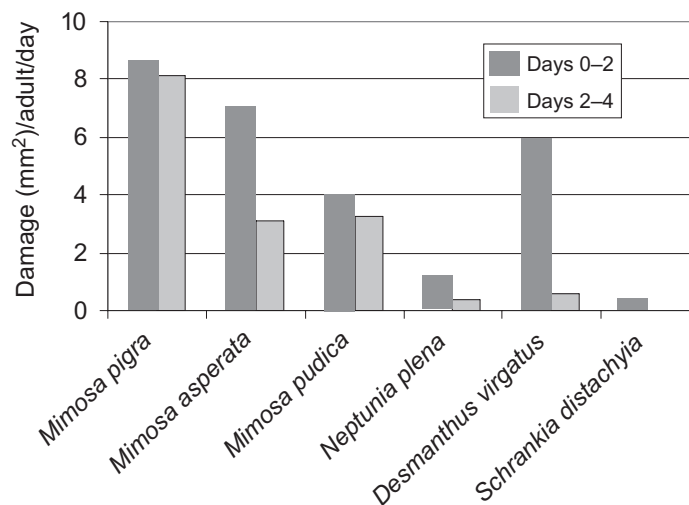


Figure 2. Standardised surface area feeding damage by Cicadellidae on six plant species.

to redistribute this insect are reported by Edwards *et al.* (2000).

***Mictis profana* (F.)**

An insect native to Australia, the crusader bug *M. profana*, can breed on mimosa, where it causes stem tip dieback and is considered to make a positive, if small contribution to biological control (Flanagan 1994).

***Botrydiplodia theobromae* Pat.**

This widespread, tropical, plant-pathogenic fungus was found to cause dieback of mimosa in Australia in the dry season, especially on plants stressed by drought and attacked by *N. gunniella* (Wilson and Pitkethley 1992). The current status of this pathogen is not known and it is not being monitored.

Hit parade of future agents

The following insects are currently under investigation as they have been rated the more promising future agents.

Leuciris fimbriaria

Harley *et al.* (1995) listed *Leuciris fimbriaria* (Photo 6) as having no potential "because ectophagous larvae of Lepidoptera are often subjected to high levels of parasitism and predation". However, this is a common and damaging species in Mexico where the ant fauna on mimosa is also abundant and presumably so are parasitoids. Hence, we raised it up the list of priority agents to be assessed.



Photo 6. Adult of *Leuciris fimbriaria*. Photo: Areli Mira, CSIRO.

This geometrid was reared first at the CSIRO Mexican Field Station, then shipments of larvae were sent to Brisbane for rearing in quarantine. Rearing commenced in Brisbane in 2002. A methodology for host-specificity testing has been

developed and we expect that testing will be completed in early 2004.

***Risbecoma pigrae* Rasplus**

This seed-feeding wasp is common on mimosa in Africa. Damage to seeds is considerable, even to the point where it is considered that attack by this insect is a possible reason why mimosa is not an aggressive invader in that continent (S. Nesar, pers. comm. 2001). It was first described from the Ivory Coast of Africa (Rasplus 1988), and S. Nesar has collected it from north-eastern Namibia, north-eastern South Africa and Uganda. The origin of this species is not known but because this type of seed-feeding chalcid is usually host specific, it has potential for biological control purposes in Australia, irrespective of whether it originated in Africa or America.

Collections of pods have been made in Mexico and the emerging species collected. Of these, a total of 229 Hymenoptera specimens from the states of Veracruz and Guerrero has been sent to Otilie Nesar, Biosystematics Division, Plant Protection Research Institute, Pretoria, South Africa, for identification. None were *R. pigrae*. Further collections are being made in other countries in Central America. Living specimens could be supplied by PPRI, South Africa, if a decision was made to import into quarantine.

Syphrea bibiana

On the basis of the host specificity of adult feeding (Heard and van Klinken 2004), *S. bibiana* (Photo 7) was selected for further assessment of the larval feeding host range. This leaf beetle, known from Mexico, Honduras, Costa Rica and Venezuela, has been imported into quarantine many times over many years, but has proved a difficult subject for assessment. Larvae develop on roots and roots are being used for rearing. However, high mortality generally results. We are trying to develop a more efficient method of rearing. Once this is achieved, a ready supply of insects will become available for further studies and the same method used for adult feeding can be applied to the test plant species in order to test the larval developmental host specificity.

***Temnocerus* (= *Pselaphorhynchites*) spp.**

Harley *et al.* (1995) listed only one species, *Pselaphorhynchites macrophthalmus* (Schaeffer), and stated that it had no potential. However, *Temnocerus* (Photo 8) are common and damaging species in Mexico and other countries. Hence, we raised them up the list of priority agents to be assessed. These small, black, weevil-like rhynchitids breed in the leaf tips. Adults oviposit into

tips and feed on them, and the larvae develop inside tips.

A taxonomic specialist, Robert Hamilton, identified a small series of specimens as *Temnocerus debilis* (Sharp) from Mexico and Honduras and an undescribed species from Venezuela. No *P. macrophthalmus* were identified. We currently have large numbers of specimens awaiting identification.



Photo 7. Adult of *Syphrea bibiana* on a leaf of *Mimosa pigra*. Photo: Areli Mira, CSIRO.



Photo 8. Adult of *Temnocerus debilis* on leaf tip. Photo: Areli Mira, CSIRO.

Limited success with rearing has been achieved, and only a few adults have been reared in Mexico. Similar results were achieved in an Australian quarantine facility using Mexican material. However, preliminary oviposition and adult feeding tests using field-collected adults have shown a high level of specificity. In the near future we hope to import Venezuelan individuals for assessment.

Sibinia ochreosa* and *Sibinia peruana

Huge populations of these small flower-feeding weevils (Photo 9) are present in the native range. Both species co-exist in the field, apparently occupying the same micro-niche. Trials on oviposition preference, longevity and fecundity were conducted at the Mexican Field Station.

For a trial on longevity and fecundity of a mixed species cohort of field-collected adults, adults were placed in bags on flowering mimosa plants. After several days they were changed to new plants and the numbers of live insects were counted. The buds on the plants were dissected for eggs. One replicate was done at a density of 50 adults and another at 10 adults. Adults are long-lived, showing >70% survival at 53 days (Figure 3). Adults laid *ca.* 0.2 eggs/day (*ca.* 0.4 egg/day/female). The age class of inflorescences preferred by these species for oviposition was determined. Young inflorescences at stages 2-3 were preferred for oviposition (Figure 4). This is important information for choosing the plant material for host-specificity tests.



Photo 9. Adults of *Sibinia ochreosa* or *S. peruana* on a flower of *Mimosa pigra*. Photo: Tim Heard, CSIRO.

Host-specificity tests on this insect were conducted in Mexico in 1998 and 1999 on the plant species available. Testing consisted of placing 20 adults into bags on the tips of flowering plants, leaving them to oviposit for two days and then dissecting the flower buds and recording eggs laid. Testing was completed with three replicates of the following twelve species: *Mimosa pudica* L., *Mimosa asperata* L., *Neptunia plena* (L.) Benth., *Leucaena leucocephala* (L.) De Wit, *Desmanthus virgatus* (L.) Willd., *Chaemachrista* sp., *Senna occidentale* (L.) Irwin & Barneby, *Senna obtusifolia* (L.) Irwin & Barneby, *Senna ornitophoides* Lam., *Senna* sp., *Aeschynomene villosa* Poiret and *Caesalpinia pulcherrima* (L.) Sw. None of these species were acceptable for oviposition with the exception of the closely related *M. asperata* that received almost as many eggs as the target weed, mimosa. Both *Sibinia* species were present in the tests. The ratio of *S. peruana* to *S. ochreosa* was 4:1. Testing could

begin in Australia when there is coincidence of flowers in Australia and insects in the field from Brazil, between January and May. These insects cannot be bred in the laboratory and insects are available in the field in Mexico only in the wet season from June to November, hence the need to collect from Brazil.

Considerable effort is required to sort adults into species before tests are conducted. Separation of the females into the two species is relatively easy (Clarke 1984), but separating the sexes for one species, *S. peruana*, could not be done. Sexing of *S. ochreosa* is relatively easy. Separating the males of the two species is also difficult and so the separation was into females of *S. ochreosa* and the rest (males of *S. ochreosa* and both sexes of *S. peruana*). The tests examined acceptability for oviposition only and hence this separation is valid. The only shortcoming is that for tests on *S. peruana*, we do not know how many females are present. This is also not a big problem as the number of eggs counted at the end of the trial is an indication of validity of the trial.

These species are easy to collect and transport, and host testing is technically feasible. Challenges include breeding in quarantine and identification. They are difficult to breed in the laboratory, as they need growing flower buds (flowers of mimosa do

not develop well under conditions of artificial lighting). It is not possible to separate these two species in the field. Females of preserved specimens can be identified to species. This is also possible with live specimens although an anaesthetising device such as a cold table is needed. The question of the effectiveness of a flower-feeding insect on a plant that produces a super-abundance of flowers needs to be addressed.

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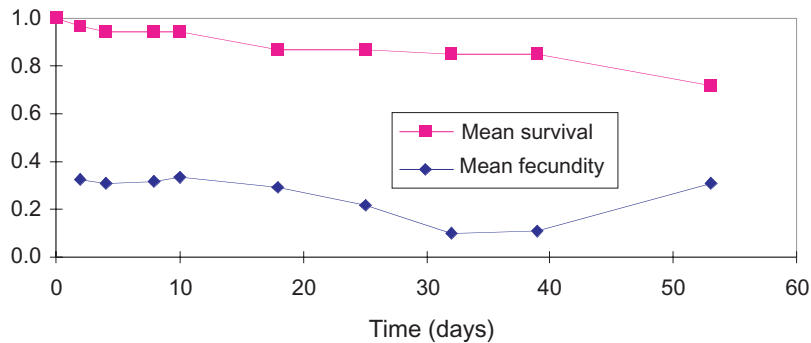


Figure 3. Survival and fecundity of field-collected adults of the flower-feeding *Sibirnia* spp.

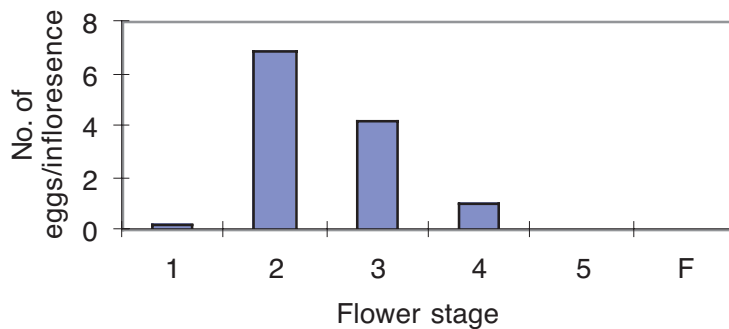


Figure 4. Flower stage preferences of *Sibirnia ochreosa* and *S. peruana* for oviposition (1-5 are increasing developmental stages of flowers with F referring to an open flower. For full description of flower stages, see Heard (1995b).

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