A review of the *Chrysanthemoides monilifera* biological control program in Australia: 1987–2005

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Summary

Two subspecies of *Chrysanthemoides monilifera*, namely subsp. *rotundata* (DC.) T.Norl. (bitou bush) and subsp. *monilifera* (L.) T.Norl. (boneseed), have been introduced to Australia from South Africa and are now among our worst environmental weeds. A biological control program was established in 1987 to combat these two invaders. To date, six species of insects have been released on bitou bush, four of which have established. The bitou tip moth (*Comostolopsis germana* Prout) and bitou seed fly (*Mesoclanis polana* Munro) are now widely established in New South Wales and two other agents, the bitou tortoise beetle (*Cassida* sp.) and the bitou leaf roller ('*Tortrix*' sp.) are currently surviving in low numbers in New South Wales but only in the vicinity of their initial release sites. A total of six species have been released for boneseed, but despite repeated and often large releases, none of these agents have established in the field. Predation by indigenous invertebrates is suspected as being a key factor in preventing establishment of the foliage feeding agents in Tasmania, South Australia and Victoria. The leaf buckle mite, *Aceria* sp., is one of several additional agents being investigated for the control of boneseed; it was approved for field release in 2005. Despite the failure of several agents to establish in the field, especially on boneseed, the biological control program has delivered some successes. The pending release of the leaf buckle mite and the targeted selection of future agents specifically for boneseed should help to counteract previous setbacks.

Keywords Bitou bush, *Chrysanthemoides monilifera* subspecies *rotundata*, boneseed, *Chrysanthemoides monilifera* subspecies *monilifera*, biological control, bitou tip moth, bitou seed fly.

Introduction

Biological control is one method of regulating the density and distribution of alien plants and animals. It relies on the use of natural enemies, from the plant or animal's native range, be they parasites, predators, herbivores or pathogens to reduce populations to an acceptable level (Van den Bosch and Messenger 1973). The aim of biological control is not to eradicate species that have become a problem, but rather to halt their unregulated growth. In fact, such control efforts rarely result in complete eradication (Myers *et al.* 2000).

Biological control is a long-term option, with no guarantee of success. For example, a review of biological control programs found that only 55% could be deemed to be at least partly effective, with many biological control programs using multiple agents to achieve success (Sheppard 1992). However, as MacFadyen (2000) argues the probability can be much higher (e.g. 80–90% probability of attaining satisfactory control of the target weed) if biological control programs are properly conducted and resourced. In addition, not all species are suitable targets for biological control, and the initial costs of a biological control project may be very high. However, this control technique is environmentally sound, can be cost effective over the long term, the agents can attack populations that would otherwise be inaccessible, and intervention is not needed once the agents have established (MacFadyen 1998, Briese 2000, MacFadyen 2000). Unfortunately there have been few reviews of specific biological control programs, which outline the agents examined, their successes or failures, and the future directions in a form that is suitable for both a scientific audience as well as those stakeholders that have invested in the program.

This paper presents an overview of the biological control program for *C. monilifera* (bitou bush and boneseed) in Australia over the last 19 years, outlining the history of each agent from initial release to current status, as well as the future directions of the *C. monilifera* program. This review highlights the long-term nature, complexity, failures (e.g. the failure to establish an agent on boneseed) and successes (e.g. the rapid and widespread establishment of the bitou seed fly) of biological control. In addition the commitment that is required to deliver weed control with biological agents. Unfortunately, such reviews are rarely undertaken (Walton 2005), which leaves the public and to a lesser extent funding agencies with limited understanding of the program or its status and future directions. It is thus hoped that this review will lead to greater appreciation of the intricacies of biological control and promote future long-term commitment towards the biological control of weeds in Australia more generally. The cost of the *C. monilifera* program has been estimated at $7.1 million, however, a cost benefit analysis of the program from 1990 to 2030 estimated a cost saving of $53 million, being $7.2 saving in control, $4.4 million in increased amenities, and $41.5 million in increased biodiversity benefit (Page and Lacey 2006).

The target weeds

Bitou bush

Bitou bush (subsp. *rotundata*) is a coastal shrub that in its native range occurs predominantly along the east coast of South Africa. Bitou bush was first recorded in Australia around 1908 near Newcastle, New South Wales. It is thought that this infestation originated from ballast carried from South Africa (see references in Weiss *et al.* 1998). However, the NSW Soil Conservation Service facilitated the invasion of bitou bush with deliberate plantings between 1946 and 1968; bitou bush was planted to stabilize sand dunes following the mining of rutile and zircon (Barr 1965), especially along the northern coastline of New South Wales. The capacity of bitou bush to invade native vegetation was subsequently recognized and deliberate plantings were halted. Unfortunately, control programs did not follow and by 1976 bitou bush was naturalized along much of the New South Wales coast. The potential impact of this introduced plant was not recognized however, until several years later. Between 1981 and 1982 a comprehensive bitou bush survey was undertaken along the entire New South Wales coastline. This survey showed that bitou bush infested 660 km of the coastline and was the dominant plant for approximately 220 km of the area infested (Love 1984). The New South Wales coast was re-surveyed in 2000–01, revealing that the length of coastline infested had increased to 900 km (representing a 36% increase to 80% of the coastline), of which bitou bush was the dominant plant along approximately 400 km (36%) (Thomas and Leys 2002). With a few exceptions, bitou bush occurs continuously from the Shoalhaven River north to the Queensland border with most of the north coast of New South Wales being heavily infested. South of the Shoalhaven River to Batemans Bay, areas free of bitou bush are interspersed with heavy infestations. South of Batemans Bay, bitou bush only occurs in isolated disjunct infestations (Figure 1a). In addition, the survey recorded bitou bush up to 10 km inland, which was further than previous estimates, invading fore dunes through to coastal eucalypt woodlands. Significant infestations also occur on the sand dunes at Menindee Lakes, western New South Wales and on Lord Howe Island where an eradication program is currently being undertaken. Outside of New South Wales bitou bush poses a threat to coastal Victoria (particularly the northern part) and southern Queensland.

The impact of bitou bush to native plant communities was recently determined in New South Wales during the development of the Bitou Bush Threat Abatement Plan (see DEC 2006). The Threat Abatement Plan identified 158 plants species, three plant populations and 26 ecological communities at risk from bitou bush invasions, many of which are not formally listed as threatened.

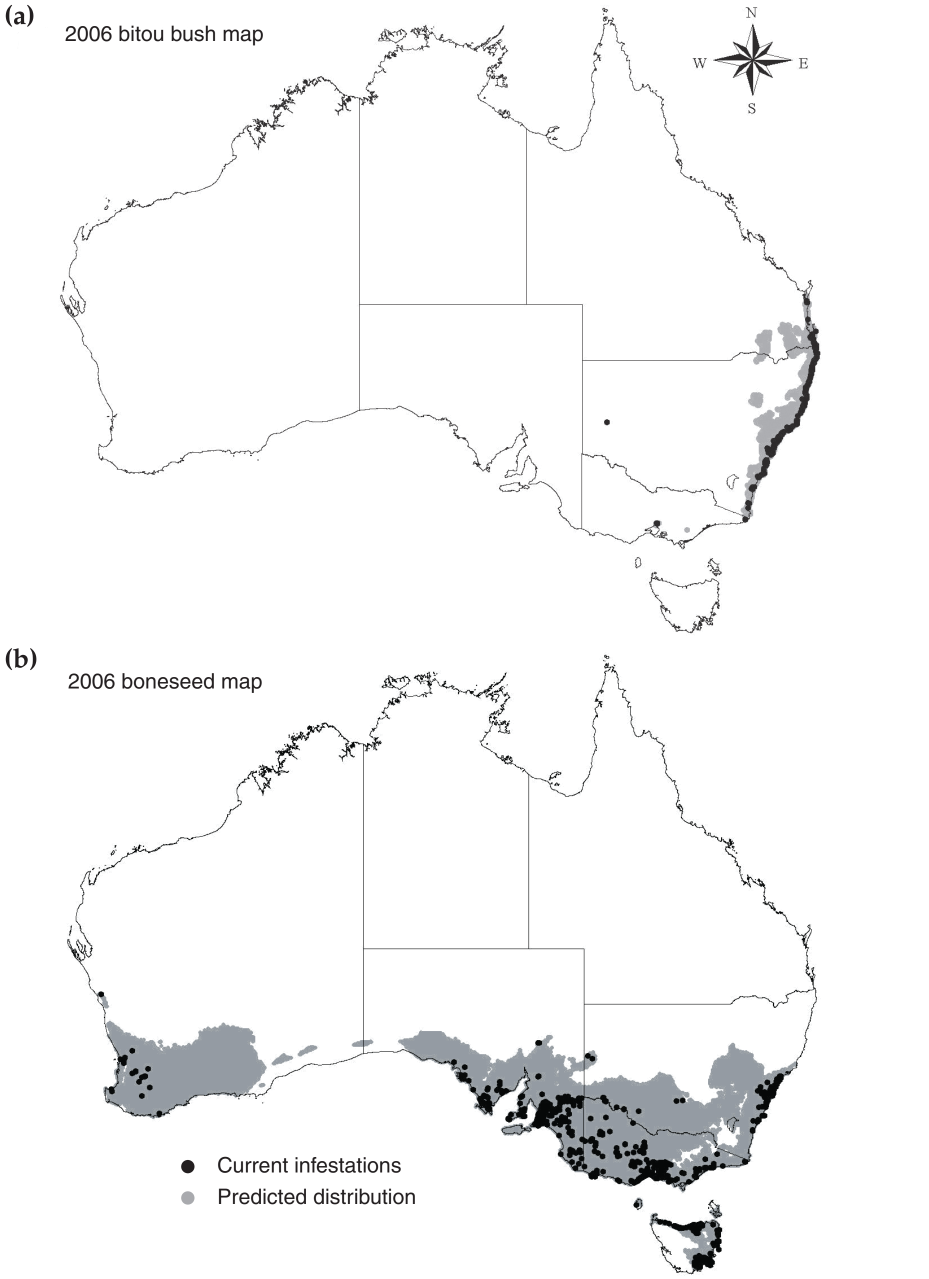

Figure 1. Current and potential distribution in Australia of (a) bitou bush, and (b) boneseed.

Boneseed

Boneseed (subsp. *monilifera*) grows further inland than bitou bush (both in its native range and Australia) and in its native range occurs predominately in the western cape of South Africa. The exact date and manner of arrival of boneseed to Australia is unknown. The first known record is from a Sydney garden in 1852. The first known locations elsewhere in Australia include Melbourne 1858, Adelaide 1892, Ulverstone (Tasmania) 1931 and Perth 1948 (Weiss *et al*. 1998). Since its introduction, boneseed has been cultivated widely in most states. The majority of the present day infestations can be attributed to escapes from gardens and nurseries (Adair and Ainsworth 2000). Today, boneseed is widespread in South Australia (e.g. Mt Lofty Ranges), Victoria (e.g. the Mornington Peninsula, the You Yangs, Otways National Park, Dandenong Ranges National Park and scattered in the Wimmera region) and Tasmania (particularly the north, east and south-east coastal regions), with scattered infestations in Western Australia (mainly confined to the south-western region consisting of approximately 20 widely dispersed small patches). In New South Wales, boneseed occurs from the Hunter River southwards, as well as in south-west New South Wales (e.g. Dareton). Additional scattered infestations occur on the Central Coast, and in the Sydney Metropolitan Area. Boneseed has the potential to invade extensive areas, especially in Tasmania (Ireson *et al*. 2002, Figure 1b).

The need for a biological control program

Given that bitou bush and boneseed have invaded quite different regions of Australia, their collective distribution pattern is substantial. For example, the combined distribution of these two subspecies shows that they have invaded over 3% of the Australian continent or 2.5 M ha (Sinden *et al*. 2004).

Outside of Australia, *C. monilifera* is considered a minor environmental weed in New Zealand (boneseed only), France and the Islands of St. Helena (South Atlantic Ocean) and Sicily (Weiss *et al*. 1998). However, within Australia the extent of its impact is only just starting to be realized. For example, an examination of the impacts to biodiversity showed that bitou bush threatens more than 150 plant species in New South Wales alone (Downey 2004, DEC 2006) – an increase of more than 140 species on the previous estimate (see ARMCANZ *et al*. 2000). This increase is due to a better recognition of the impacts, rather than a sudden increase in the problem.

The significance of both bitou bush and boneseed as major weeds was officially recognized in 1999, when *C. monilifera* was listed as a 'Key Threatening Process' under the NSW *Threatened Species Conservation Act 1995* (see NSW SC 1999, Downey and Leys 2004), and in 2000 as a Weed of National Significance (WoNS) (Thorp and Lynch 2000). A national strategy to manage bitou bush and boneseed (ARMCANZ *et al*. 2000), as well as best practice management guides have been developed (Adair and Ainsworth 2000, Vranjic 2000). A Threat Abatement Plan has been prepared to ameliorate the impact of bitou bush and boneseed to native plant communities in New South Wales (see DEC 2006). In addition, within New South Wales, a State (NPWS 2001) and numerous regional bitou bush strategies (e.g. Scanlon 2001) have also been developed. While increased legislation and strategies result in greater emphasis on weed management and outcomes (Downey 2003), they do not necessarily result in better control options, especially for widespread weeds. Thus, an integrated approach to managing weeds is needed (Groves 1989), which must include biological control (Vranjic 2000).

The *Chrysanthemoides monilifera* biological control program in Australia

In 1987, the biological control program for bitou bush and boneseed was approved by the then Standing Committee on Agriculture, following a nomination by the New South Wales National Parks and Wildlife Service (Holtkamp *et al*. 1999). However, the need for a biological control program was expressed several years prior to this (Love and Dyason 1984, Groves 1985).

As *Chrysanthemoides* is a small genus, with no related plants of economic value in Australia, the potential success of any biological control was high, provided appropriate agents could be found. In addition, surveys of the morphological variation revealed little variation in either subspecies (bitou bush or boneseed) in Australia (Simmons and Flint 1985). This suggests that there is likely to be limited genetic variability within the Australian populations, and that Australian populations may be derived from a limited number of introductions (Stahle 1997) with the reduced genetic diversity potentially enhancing susceptibility to biological control agents.

A series of surveys for likely agents in South Africa, conducted between 1987 and 1990, indicated that there were at least 113 species of phytophagous insects and four pathogens associated with the *Chrysanthemoides monilifera* species complex (Adair and Scott 1989, Scott and Adair 1992). Following further evaluations a list of potential agents was produced, consisting of 42 phytophagous insect species (33 of which were undescribed) and four pathogens (Scott and Adair 1992). These potential agents were then prioritized, based on their effect on the host plant, host specificity, climate matching, resistance to predation and pragmatic factors (i.e. ease of collection and rearing in the laboratory). The final outcome was a much-reduced list of 17 phytophagous insects and two pathogens with the greatest potential for the biological control of *C. monilifera* in Australia. This search failed to identify a suitable root-feeding agent (Adair and Edwards 1996). Sixteen of the 19 agents identified by Adair and Edwards (1996) have been given further consideration as potential agents in Australia (see Table 1).

Eleven of these species have undergone host-specificity testing in quarantine in Australia (see Adair and Scott 1989, 1991, 1997, Adair 1997, Adair and Bruzzese 2000), with ten of these species approved for release. Following release, these species were evaluated in the field (see Meggs 1995, Edwards *et al*. 1999, Holtkamp 2002, Ireson *et al*. 2002, Swirepik *et al*. 2004a, 2004b). It is anticipated that the eleventh species will be released in the near future, with several others also being considered for possible approval (Table 1). To date four species have established on bitou bush and are causing damage to their host plant if only on a local scale.

In addition to these classical biological control agents, initial investigations were undertaken to determine if the non-specific pathogen *Sclerotinia sclerotiorum* (Lib.) De By. could be used as a mycoherbicide to control bitou bush (see Cother 1997, 2000). Despite some promising results a mycoherbicide has not been developed.

The bitou bush program

Six of nineteen potential agents have been released on bitou bush, however, only four have successfully established to date (Table 1). The established agents are the bitou tip moth (*Comostolopsis germana*), bitou tortoise beetle (*Cassida* sp.), bitou seed fly (*M. polana*) and bitou leaf roller moth (*Tortrix* sp.).

Bitou tip moth The shoot-tip feeding Geometrid moth (*C. germana*), known commonly as the bitou tip moth, was the first agent to be released on bitou bush in 1989 (Adair *et al*. 1995). Subsequently, more than 200 releases of the bitou tip moth were made between 1990 and 1997. The releases covered 72 sites in New South Wales ranging from the Queensland border to Tathra in the south of the State (Holtkamp 2002). The bitou tip moth is now widely established along most of the New South Wales coast. The exact distribution of the tip moth has not been established, but it is believed to have spread throughout the entire range of bitou bush in Australia, with the exception of a disjunct population in the Menindee lakes area of western New South Wales and possibly northern Victoria. In many areas the bitou tip moth has reached high population levels and is having a significant impact on the flowering and seed production of bitou bush (Holtkamp 2002). Elsewhere population levels are still increasing to their maximum potential carrying capacity, which may exceed 400 larvae m^{-2} (R. Holtkamp unpublished data). Even at levels a quarter of this value (i.e. 100 larvae m^{-2}) seed production can be reduced by 50–80% (Holtkamp 2002). The presence of two Hymenopteran parasitoids, *Diadegma* sp. and *Brachymeria* sp., however, has reduced some tip moth populations. For example, in some areas up to 50% of the bitou tip moth larvae are parasitized (Holtkamp 1993). The reduction in seed production attributed to the bitou tip moth is likely to have a significant effect on the long term management of bitou bush infested sites as soil seed bank accumulation is reduced. The seed bank will degrade to relatively low levels within three years if seed input is halted (Weiss *et al*. 1998), thereby reducing management and control effort required in the long-term. However, such statements are not based rigorous assessments and thus the exact nature of the decline is not known.

Black boneseed beetle Another foliage-feeding agent, the black boneseed beetle, *Chrysolina scotti* Daccordi (formerly *Chrysolina* sp. 1), was the second agent released onto bitou bush. The release of this agent occurred after extensive host testing of the then undescribed beetle (Adair and Scott 1991, 1997). The first release of the beetle occurred at Tathra, New South Wales in 1990 and was subsequently released at four other sites but has failed to establish. One reason for this failure is that observational data indicates that the larvae of the black boneseed beetles are prone to predation by ants and spiders (R. Holtkamp unpublished data); the Australian ant fauna is particularly large and diverse in comparison to other areas of the world (Shattuck 1999).

Painted boneseed beetle The third agent released was the painted boneseed beetle (*Chrysolina* sp. B) at Ulladulla, southern New South Wales in 1995. It was subsequently released at seven other sites but has failed to establish. Ant and/or spider predation may also be responsible for the failure of this agent to establish in the field.

Bitou tortoise beetle The fourth agent released was the bitou tortoise beetle (*Cassida* sp.) at La Perouse (a suburb of Sydney) in 1995. A total of 12 releases were made with locations spread over most of New South Wales (R. Holtkamp unpublished data). Recent surveys conducted during 2004 have shown it to be present at most of these initial release sites. However, the populations are highly localized to these sites and contain low densities (R. Holtkamp unpublished data). Based on these surveys it appears that the impact of the bitou tortoise beetle may be limited in the immediate future. Initial research suggested that the bitou tortoise beetle had a greater potential in Australia (see Kleinjan and Scott 1996) than what has been exhibited to date (P. Edwards personal communication).

Bitou seed fly Studies in South Africa, on the distribution, attack strategy (i.e. seed feeder) and impact of three seed fly species led to a prioritized release of these species in Australia (see Edwards and Brown 1997). These studies also determined the areas in Australia that were likely to be infested by each of these three species. A subsequent study evaluated the host specificity of two of these seed flies (*M. polana* and *M. magnipalpis* Bezzi) as potential biocontrol agents (see Adair and Bruzzese 2000).

The fifth agent released was the bitou seed fly (*M. polana*) in 1996 (Figure 2). This insect was the first of the flower and seed-feeding agents to be released against *C. monilifera* in Australia. It was first released at two sites, Iluka Bluff (mid north coast, New South Wales) and Dunbogan (near Port Macquarie, New South Wales) with an additional nine releases made on the New South Wales north and central coasts between 1996 and 1998.

Within two years of its release (i.e. August 1998), the bitou seed fly (*M. polana*) could be found from Fraser Island in Queensland to Tathra in southern New South Wales – a total distribution of over 1200 km of the coastline (Edwards *et al*. 1999). The initial rapid establishment and expansion of the bitou seed fly populations brought with it great hope for the impact of the seed fly on bitou bush seed banks and population dynamics. A study on the impact of the seed fly on bitou bush seed production was carried out at five sites on the New South Wales coast from Iluka in the north to Moruya in the south during 2001-2. The seed fly reduced seed production by an average of 27% (ranging from 23 and 31%, Stuart *et al*. 2002). Noble and Weiss (1989) predicted that for bitou bush to be controlled by a pre-dispersal seed predator, a reduction in annual seed rain in excess of 95% would be required. Based on this prediction, the modest reduction measured in seed production attributed to the seed fly is unlikely to reduce the rate of invasion and or recolonization of bitou bush. However, such seed reductions may result in a decrease in the number new populations.

Using a climate-driven process-based population model, Stuart (2002) simulated the likely impact the seed fly would have on bitou bush seed banks and recruitment when integrated with herbicide. The model suggested that if observed reductions in seeding of between 23 and 31% were allowed to accumulate over a 10 year period and this was then followed by a herbicide application to remove the mature bitou bush canopy, there would be no significant difference in seedling emergence rates when compared to no seed fly attack (177 versus 182 seedlings m^{-2}). The same simulations were run to include seed rain reductions of 55 and 99.9% over a 10 year period; even after a reduction of 99.9% over 10 consecutive years and the inclusion of herbicide application the model suggested that there would still be recruitment of 8.8 seedlings m^{-2}. If no follow-up treatment and/or native revegetation were to take place; seedlings at this low density would still be able to quickly form a dominant canopy. Especially as bitou bush plants have been observed to produce seed in their first year on the New South Wales north coast.

Bitou leaf roller moth The sixth and latest agent released was the bitou leaf roller moth (*Tortrix* sp.). The larvae of the moth roll the leaves together using a 'web', forming shelters within which they feed. Initially, the moth was regarded as the most damaging biological control agent available for release against *Chrysanthemoides* in Australia (P. Edwards unpublished data, Scott and Adair 1990), however, such hopes have not been realized. The moth was first released onto bitou bush in Australia in March 2001. Since then 136 releases of the leaf roller moth have been made at 45 sites from the Queensland border to Moruya in southern New South Wales (Swirepik *et al.* 2004b). The leaf roller moth has established at six of the 45 release sites. At a further 10 sites the leaf roller has been recovered, but it is not yet considered to be established at these sites, because no follow-up observations have been made for at least one year. The leaf roller moth has failed to establish at 29 of the 45 sites.

A study of larval mortality in caged and uncaged populations of the leaf roller moth was conducted by Swirepik *et al.* (2004a) in New South Wales. The authors attributed a high initial decline in larval numbers in all treatments to competition between neonate larvae for feeding sites. However, they also found that predators such as ants and spiders caused post-release mortality of up to 98% within 10 weeks of release.

Swirepik *et al.* (2004b) suggest an abiotic factor such as site may also influence release success. Analysis of plant growth rate, rainfall and evaporation data during two summer release periods found that even after rainfall had been taken into account there was a site or habitat effect on bitou bush growth rate and survival of the leaf roller moth. Available nitrogen in the canopy may also be having an influence over establishment success in the leaf roller moth (A. Swirepik unpublished data); this phenomenon is the subject of ongoing research.

During 2004 an in-depth study was conducted on post-release predation and the effect of site on the establishment of the leaf roller moth (Strakosch 2004). The study involved releasing the same number of leaf roller moth eggs at five headland and five dune sites with a replicated 'plus or minus' predator exclusion treatment at each site. The study also looked at the total number of invertebrate species using the bitou bush canopy, with a restricted carbon dioxide fogging technique. The invertebrate survey found that there were no differences in species richness between headland or dune sites but there was a significant difference in the abundance of predatory arthropods between dune and headland sites. The increased levels of predation recorded at the dune sites can be attributed to the significantly higher numbers of predators present on the dune sites compared to the headland sites. These results have shifted the focus of the ongoing leaf roller moth release program, in which release sites on headlands are preferred to dune sites. When bitou bush infested headlands cannot be found, concentrated releases at dune sites with a high bitou bush canopy growth rate are used.

The boneseed program

Six agents have been released on boneseed in Australia; black boneseed beetle (*Chrysolina scotti*), bitou tip moth (*Comostolopsis germana*), blotched boneseed beetle (*Chrysolina picturata* Clark), painted boneseed beetle (*Chrysolina* sp. B), lacy-winged seed fly (*Mesoclanis magnipalpis*) and boneseed leaf roller moth (*Tortrix* sp.). In addition the leaf buckle mite (*Aceria* sp.) has recently been approved for release under the *Quarantine Act 1908* and *Environment Protection and Biodiversity Conservation Act 1999* (Table 1).

Figure 2. Bitou seedfly (Photo courtesy of CSIRO).

Black boneseed beetle The black boneseed beetle, the first agent to be released against boneseed was released at Studley Park in Melbourne during October 1989. The beetle was subsequently released at a further 18 sites in Victoria, mostly on the Mornington Peninsula and the You Yang Ranges in 1989. Six releases were made at just one site (Morialta National Park) in South Australia in 1989, while in Tasmania, the black boneseed beetle was released at a total of 10 sites in the same year. The beetle failed to establish at all sites.

Bitou tip moth Attempts were made to establish the bitou tip moth onto boneseed at a total of 37 sites across south-eastern Australia (11 in Victoria, seven in Tasmania and 19 in South Australia). The tip moth persisted at one site in Victoria at the Keith Turnbull Research Institute (KTRI) near Melbourne, where the rearing of the tip moth took place in field plots, but apart from this site the bitou tip moth failed to establish on boneseed elsewhere in Australia. The reasons for the failure of the tip moth to establish on boneseed have not been identified although it may be due to one or a combination of factors including poor climate match of the agent (Adair and Edwards 1996), or the impact of natural enemies (i.e. predation and parasitism).

Blotched boneseed beetle The third agent released against boneseed was the blotched boneseed beetle. It was released in 1992 at seven sites across Victoria and two sites in South Australia but never released in Tasmania. The blotched boneseed beetle failed to establish at all sites.

Painted boneseed beetle The painted boneseed beetle was released in 1994 at four sites in Victoria and two sites in Tasmania. As with the other *Chrysolina* species released previously, the painted boneseed beetle failed to establish. Such failures in the field cannot be predicted despite thorough host specificity testing prior to release (e.g. Adair and Scott 1991, 1997).

Lacy-winged seed fly The fifth agent released against boneseed was the lacy-winged seed fly. The seed fly was approved for release in 1997 although rearing of this insect through the mandatory one generation in quarantine proved almost impossible. This was because the quarantine conditions did not promote boneseed or bitou bush fruit production, hence there were no developing seeds for the seed fly larvae to feed on (Adair 1997). In recognition of this difficulty, the Australian Quarantine and Inspection Service (AQIS) issued a special 'direct release' permit enabling the 'one generation' condition to be waived. Lacy-winged seed flies were then collected from South Africa as larvae within *Chrysanthemoides* fruit and upon emergence, individual adult flies were released from quarantine after being carefully checked for pathogens and parasites. In 1998, five releases of the lacy-winged seed fly were made in Victoria at Davey's Bay, the You Yangs and within a field plot at the KTRI. Two releases were made in South Australia

Table 1. Agents investigated for the biological control of *Chrysanthemoides monilifera* in Australia and their status (based on the potential agents identified by Adair and Edwards 1996).

No.	Biological control agent	Agent released for bitou bush	Agent released for boneseed	Year first released in Australia	Established on bitou bush	Established on boneseed
1.	Bitou tip moth (*Comostolopsis germana*)	yes	yes	1989	yes	no
2.	Black boneseed leaf beetle (*Chrysolina scotti*)	yes	yes	1989	no	no
3.	Blotched boneseed leaf beetle (*Chrysolina picturata*)	no	yes	1992		no
4.	Painted boneseed beetle (*Chrysolina* sp. B)	yes	yes	1994	no	no
5.	Bitou tortoise beetle (*Cassida* sp.)	yes	no	1995	yes	
6.	Bitou leaf beetle (*Ageniosa electoralis* Vogel)			rejected after evaluation – it attacked non-target plants		
7.	Bitou seed fly (*Mesoclanis polana*)	yes	no	1996	yes	
8.	Lacy-winged seed fly (*Mesoclanis magnipalpis*)	no	yes	1998		no
9.	Boneseed leaf roller moth (*Tortrix* sp.)	yes	yes	2000	no	recovered from one release site only
10.	Bitou leaf roller moth (*Tortrix* sp.)	yes	no	2001	yes	
11.	Leaf buckle mite (*Aceria* sp.)		pending	approval for release granted in March 2005		
12.	Lesser seed fly (*Mesoclanis dubia* Bezzi)			not tested		
13.	Boneseed rust fungus (*Endophyllum osteospermi* (Doidge) comb. nov.)			host-specificity testing still pending		
14.	Tip wilt beetle (*Obereopsis pseudocapensis*)			initial testing only (see Neser and Morris 1985)		
15.	Unidentified moth (*Gelechiidae*)			untested		
16.	Unidentified gall-forming cecidomyiid (*Cecidomyiidae*)			untested		
17.	Soft rot fungus (*Sclerotinia sclerotiorum*)			endemic fungus considered for, but not used as a potential mycoherbicide		

at Morialta and Belair National Parks. In 1999, a further three releases were made in Victoria at the You Yangs and KTRI and one release in South Australia at Morialta National Park. The last release of the lacy-winged seed fly occurred in August 2000 at KTRI.

Despite repeated releases at the Victorian sites, the lacy-winged seed fly failed to establish. It is likely that the flowering periods of boneseed are too short for the seed fly to survive from one year to the next. A current strategy to establish the lacy-winged seed fly in Australia is to target releases onto bitou bush in southern NSW. Once established on bitou bush, attempts will be made to harvest seed flies and release them into boneseed infestations. Consignments of seed fly-infested fruit were shipped to Australia in mid to late 2005 to initiate such releases.

Boneseed leaf roller moth The boneseed leaf roller moth was first released in April 2000 at the You Yangs in Victoria. It has since been released at a total of 30 sites in Victoria, three sites in the Mount Lofty Ranges of South Australia and 34 sites in Tasmania. In Victoria, releases of the moth have occurred across a wide range of climates and habitats, from the Mallee in far north-western Victoria, the temperate forests of the Dandenong Ranges and the coastal areas of the Mornington Peninsula and south-western Victoria to improve the chance of establishment. The boneseed leaf roller moth failed to establish, despite an intensive release strategy.

Boneseed leaf buckle mite In March 2005, approval was granted to release the boneseed leaf buckle mite in Australia. This tiny eriophyid mite feeds on developing *C. monilifera* leaves, inducing the formation of hairy, white to brown erinea or leaf galls which cause distorted leaf growth (Adair 1997). Currently, strains of the leaf buckle mite demonstrating high virulence and impact on boneseed populations in South Africa are being collected for importation into Australia. Following approval from quarantine, trial releases are expected to commence in 2006 in Victoria, Tasmania and South Australia. While the strains to be imported will be specifically for boneseed, it is possible that bitou bush adapted strains could be investigated for introduction into Australia in the future.

Factors affecting the establishment of biological control agents on boneseed

A range of environmental factors may have contributed to the failure of boneseed agent establishment in Australia, however, evidence suggests that biotic resistance (or predation) by indigenous invertebrates may have been a key factor, with *Chrysolina* species (i.e. the blotched and black boneseed beetles), the bitou tip moth and the boneseed leaf roller moth being particularly vulnerable as they all spend large parts of their life cycle unprotected on the foliage of boneseed. In Victoria, Meggs (1995) showed that ants and spiders played a role in preventing the black boneseed beetle from establishing and discussed the possibility of a facultative mutualism between the ants and the honeydew-secreting nigra scale (*Parasaissetia nigra* Nietner – Homoptera: Coccidae). This scale is also common on boneseed in Tasmania, together with several ant species and a range of other generalist predators including spiders and mites (Ireson *et al*. 2002). The blotched leaf beetle and the painted boneseed beetle both oviposit in the soil and Adair and Edwards (1996) suspected that poor chemical defence-mechanisms in the eggs of the *Chrysolina* species associated with *Chrysanthemoides* made them susceptible to predation. In Tasmania, a natural enemy exclusion experiment (Ireson *et al*. 2002) suggested that predators were a key factor in preventing the establishment of leaf roller moth, with about 70% of unprotected egg batches of this species being damaged compared to only 4% of protected batches.

The lack of success in establishing biological control agents on boneseed indicates that future selections should avoid species that may be vulnerable to predation. A species of leaf buckle mite is about to be released, which is expected to be better equipped for avoiding predation. This is because adult feeding habits produce structures on the leaves which enable the mites to protect themselves from larger predators and extreme weather conditions. Some small predators are not inhibited by such structures, however. In addition, climate matching should be used to help determine future agents (see Clark 2001).

Factors affecting the success of seed seeders

One factor that underpins the long-term population dynamics of any plant is the probability of each seed becoming a flowering or reproductive individual. Unfortunately, this probability is only known for a few species (e.g. scotch broom (*Cytisus scoparius* (L.) Link: see Downey and Smith 2000). Using the probability value for scotch broom, Downey (unpublished data) suggests that a reduction in seed production of 53% should be sufficient to reduce the probability to <1, despite claims that a seed reduction of 70–99% is needed (Parker 2000). Thus as this probability is unknown for bitou bush (or boneseed) the actual impact of the seed fly on long-term bitou bush dynamics could vary to that predicted by Stuart (2002). Although widely established, based on Stuart's (2002) predictions the seed fly offers little hope for the successful biological control of bitou bush on its own and underlines why the strategy for biological control involves a guild of agents including those that attack the canopy directly such as the bitou tip moth and bitou leaf roller moth.

The role of biological control in the integrated management of *C. monilifera*

The interaction between biological control and other control techniques is important to prevent adverse impacts on the biological control agents, either directly (mortality) or indirectly (loss of food source or host). An overview of the possible interaction between herbicides and biological control agents on bitou bush was presented by Ainsworth (1997), Ainsworth and Holtkamp (1999), and Adair and Holtkamp (1999).

The listing of bitou bush and boneseed in NSW as a Key Threatening Process under the *Threatened Species Conservation Act 1995* (TSC Act), initiated the development of the first threat abatement plan (TAP) for a weed in Australia (DEC 2006). The Bitou TAP is the most comprehensive on-ground management plan for reducing impacts on biodiversity from an environmental weed, however, it does not specifically include actions relating to biological control. The initiation of any new agent is a longer term prospect than the life of the TAP (i.e. five years). Therefore, embarking on such a program would not meet the objective of the TAP which is to reduce, abate or ameliorate the threat (see the TSC Act), during the life of the TAP. Thus the initiation of any new program will require substantial long term commitment given no new bitou bush agents are being investigated (see future direction section below). Despite no direct actions in the TAP relating to biological control the occurrence or release of biological control agents at priority sites, should be used to help deliver broader control.

In NSW, the Department of Environment and Conservation and the National Bitou Bush and Boneseed WoNS Program in collaboration with CSIRO Entomology and the NSW Department of Primary Industries held two workshops aimed at providing land managers with the skills to identify the biological agents for bitou bush in the field and redistribute such agents to new locations in their area. It is hoped that these skills, especially the latter will enable land managers to continue the biological control program for bitou bush after the current funding has ceased.

Future direction of the *Chrysanthemoides monilifera* biological control program

As outlined above, the national *Chrysanthemoides* biological control program in Australia has been in operation for 19 years, with 17 potential agents identified, and 10 agents released. This represents a serious commitment by a wide range of stakeholders to the long-term control of *Chrysanthemoides* in Australia.

Short-term priorities for the bitou bush component

Given the recent research into the establishment of the bitou leaf roller moth, future release priorities for this moth should include strategic releases on bitou bush infested headlands. In addition the current monitoring of the establishment and impact of predators should continue.

A short-term future priority is to continue research into the impact of parasites on the bitou leaf roller moth, as well as to determine the ideal conditions (i.e. climate and habitat) for establishment in the field.

Another short-term priority is to assess the suitability of the bitou leaf roller moth as a potential candidate for use in the CRC for Australian Weed Management's (Weeds CRC) 'weed warriors' program (see Kwong 2002, Weeds CRC 2003). Such a program could promote knowledge of the bitou bush biological control program and aid in future releases of the bitou leaf roller moth.

Information is still needed on the establishment and impact of the bitou tortoise beetle, thus continuation of the current monitoring program is also a short-term priority.

Lastly, the development of strategies to integrate biological control with other management techniques is needed to deliver the sustained long-term control of bitou bush in Australia.

Long-term priorities for the bitou bush component

At this stage, no new specialist agents have been identified for bitou bush, although the possibility of releasing bitou bush-adapted strains of the leaf buckle mite (*Aceria* sp.) should be considered. A leaf buckle mite is known to attack bitou bush in South Africa, causing leaf distortions. This mite could have potential as an additional biological control agent for bitou bush, although it is not known if it is the same species as the leaf buckle mite currently under investigation for boneseed. Thus, an assessment of the effectiveness of boneseed strain should be undertaken on bitou bush.

Given that both the leaf buckle mites (bitou bush and boneseed) were originally thought to be either *Aceria neseri* Meyer or closely related species it is important that a taxonomic revision of *A. neseri* and its relatives be undertaken. Such a study should also compare genetic variation between populations of bitou leaf buckle mites from several boneseed and bitou bush locations in South Africa, the results of which will aid in the potential release of this agent for bitou bush.

The tip wilt beetle (*O. pseudocapensis*) was initially tested for host specificity in 1985 (see Neser and Morris 1985), but testing was never completed. The preliminary host testing showed this agent to be a specialist on *C. monilifera*. Thus, this insect has potential as a new biological control agent for bitou bush. Additional research is warranted to complete the testing and apply for importation and release if testing is successful.

The identification of additional agents would require follow-up examinations in the native range, which are unlikely without significant additional resources and thus such an examination is not proposed at this stage.

Short-term priorities for the boneseed component

The immediate priority is the release of the boneseed leaf buckle mite in Victoria, South Australia and Tasmania. Following importation of the mite from South Africa, mass rearing techniques will be developed to ensure large numbers of mites can be produced. Initially, a small number of closely monitored trial sites will be established on boneseed in each state. Techniques will also be developed to see if community groups can assist in the redistribution process by translocating mite-infested boneseed foliage.

Another short-term priority is to attempt to establish the lacy-winged seed fly in Australia, by first releasing it onto bitou bush. If successful, the release strategy will involve inundation with large quantities of seed flies into boneseed infestations around Australia.

Medium-term priorities for the boneseed component

The boneseed rust (*Endophyllum osteospermi* syn: *Aceidium osteospermi* Doidge) shows considerable promise as a biological control agent for boneseed and potentially bitou bush (see Wood 2002, Wood *et al.* 2004, Wood and Crous 2005). Host testing is partially completed, although the host testing results are yet to be fully analysed. The systemic nature of the rust is favourable for biological control purposes as once the fungus is established within the host, the infection is retained until the death of the plant (Morin 1997); there are however a number of technical and procedural hurdles that will need to be addressed prior to release of this rust in Australia. The first of these is the preparation, submission and approval process for the host specificity report. Should a release permit be granted then the significant technical hurdle of producing an F1 generation of the rust under quarantine conditions will require funding for one to two years as the rust can take this long to develop F1 spores (Wood 2004). It maybe be possible to speed this process up by applying for a direct release permit; obviously this option relies heavily upon the regulators decision, while there will still be technical hurdles to be addressed.

Long-term priorities for the boneseed component

The tip wilt pyralid moth (family: Gelechiidae, undescribed species) has a larval stage that feeds within the plant tissue (Adair and Edwards 1996). It has considerable potential as a biological control agent for boneseed, as it is less vulnerable to predation than the previously introduced foliage feeding agents. Initial host range testing is required to determine the suitability of this agent.

Lastly, the stem-galling cecidomyiid (family: Cecidomyiidae, undescribed species) is abundant in South Africa. The damage that this cecidomyiid causes to boneseed is yet to be quantified (Adair and Edwards 1996), however, its feeding habits may make it less vulnerable to predation, so it may also be considered for future investigation, but this insect has lower priority at this time.

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