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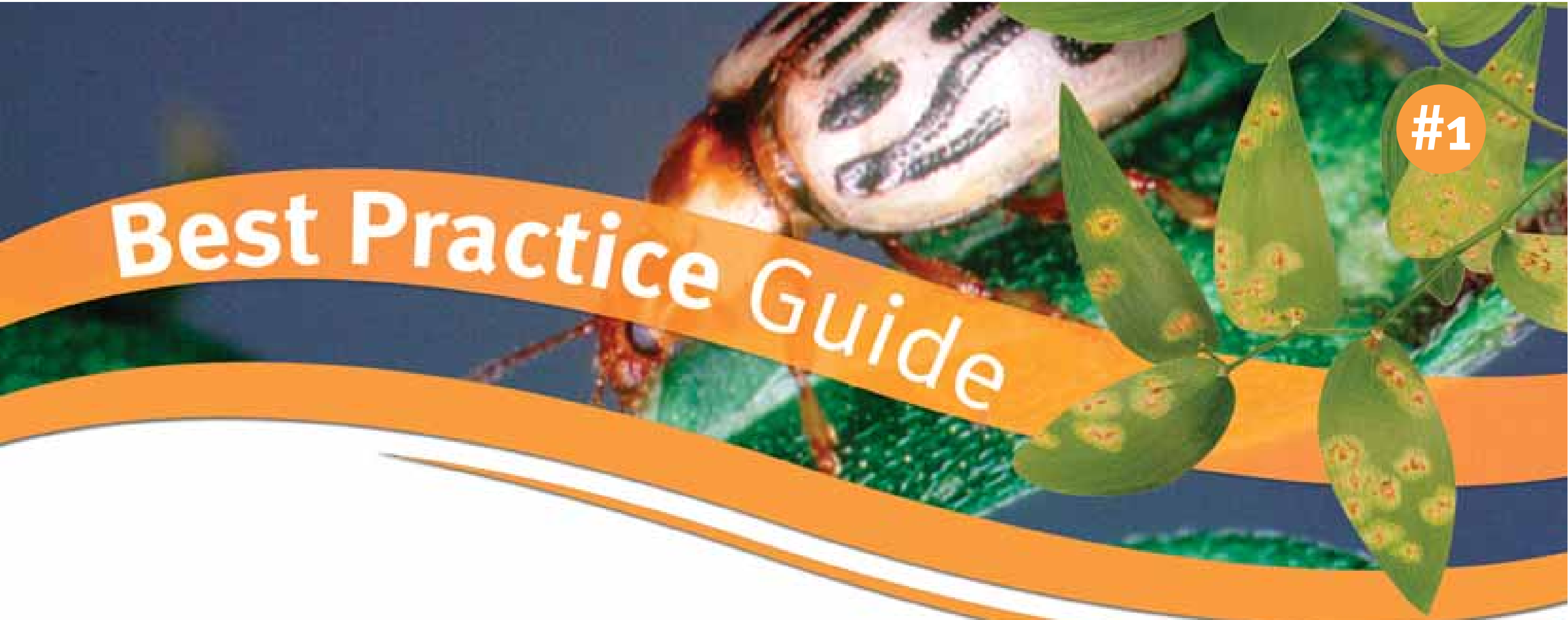

Impact evaluation of weed biological control agents

What is impact evaluation?

Impact evaluation of biological control agents is performed either before or after their release in the introduced range of the target weed.

Pre-release impact evaluation is carried out in laboratories and glasshouses or in the field in the weed's native range to predict impact of candidate agents on individual plants or populations and to assist in selection of the most promising agents.

Post-release impact evaluation measures the effectiveness of agents in reducing target weed populations in the introduced range and quantifies the benefits for associated plant communities, ecosystems, the economy and society in general.

In some instances, the direct effects of released agents may be monitored on non-target plant species identified as possible hosts during host-specificity tests prior to release: but this is not the focus of this guide.

Post-release impact evaluation is crucial to understanding the overall success rate of a biological control program. Here the effects of grazing, herbicides and biological control agents (*Longitarsus echii* and *Mogulones larvatus*) are being monitored on Paterson's curse.
Photo: CSIRO

Why perform post-release impact evaluation?

While confirming establishment, prevalence and abundance of released agents over space and time is a crucial milestone for any biological control program, ultimate success can only be demonstrated by undertaking post-release impact evaluation. Claiming success of a biological control program without quantitative assessment is questionable because other factors such as variation in climate, land use and / or management practices may have contributed to a weed population's decline.

Measuring impacts of agents on target weed populations can provide the necessary information to fine-tune a biological control program's strategy and improve efficiency and effectiveness of future programs for other weeds. For example, the data gathered may inform practitioners that other agents affecting different life stages of the target weed will need to be introduced to exert additional stresses on weed populations or that change in other management practices may increase agent impact. Rigorous assessment of programs also provides data to demonstrate the effectiveness of biological control and hence justify continued investment in research and implementation.

Who should undertake post-release impact evaluation?

The research group that initiates the biological control program often takes responsibility for post-release impact evaluation of agents, but rarely gets the continuation of funds necessary to undertake long-term evaluation. Sufficient resources and funding should be negotiated and put aside at the start of programs to support at least a minimal level of impact evaluation activities soon after an agent is released. Additional funding should be accessible later to support long-term monitoring. Multi-disciplinary teams comprising ecologists, entomologists / plant pathologists, economists and modellers are most effective in devising appropriate monitoring programs to gather the necessary data for subsequent analyses. Post-graduate student projects are also a possible avenue for more theoretical intensive studies to be undertaken, such as developing an understanding of the indirect impacts of agents on the ecosystem. Community groups and state and local government officers may also become involved, particularly when monitoring protocols and sampling regimes are simple and not too time-consuming. A program can greatly benefit from such involvement because it increases the number of sites monitored at regional scales.

Prerequisite benchmarks for evaluation

Performance targets defined at the beginning of biological control programs provide quantitative benchmarks against which effectiveness can be later evaluated. These targets estimate the required decline in weed density (or other population level parameters) over a defined timeframe to reduce impact of weed populations below an economic or ecological threshold. For example, a 25% reduction in weed cover within 10 years may be considered as necessary to obtain a satisfactory increase of desirable vegetation at infested sites. More generally, determining the relationship between a quantitative measure of the weed population (including per capita assessment) and the economic or ecological impacts may be more helpful because it allows an observed level of decline to be equated to a degree of impact and hence benefits. Devising such relationships however, requires knowledge of the economic and / or ecological impacts of the weed and what level of control is necessary to ameliorate these impacts. Partnerships between economists, plant ecologists and plant population modellers are recommended to document impacts of the target weed in order to devise realistic performance targets for a program. Such *a priori* explicit criteria against which agents or programs can later be judged are still rarely made, to the detriment of biological control as a discipline.

Impact evaluation of the bridal creeper rust fungus (*Puccinia myrsiphylli*) and responses of associated vegetation.
Photo: Peter Turner

Economic impact evaluation

Cost-benefit analyses are fundamental to evaluating the economic impacts of biological control programs quantitatively. The output of such analyses, expressed as a benefit-cost ratio or as a net present value, is a measure of the return on investment from the program. A benefit-cost ratio is derived by dividing the value of the losses avoided by affected industries or other stakeholders (in other words what economic damage would have been caused by the weed in the absence of the project, including costs for control), by the research and development costs of the biological control program. A net present value is an indicator of how much cash value a project adds to the value of the industry concerned. Both costs and benefits are discounted at a specified rate to account for differences in the time when they were incurred; costs are up-front while benefits increase steadily as agents gradually impact on the weed.

Cost-benefit analyses are more robust when carried out after there has been ample time for the field impact of agents to be realised and sufficient long-term ecological impact data to be assembled. They are also more convincing when based on accurate data on research and development costs of programs, economic and social costs of the target weed and its rate of spread and distribution, before and after releases of agents.

Unpriced values for benefits such as the preservation of native biodiversity and scenic amenity, improved recreational access to land and water, reduction of fire hazard, decreased exposure of environment to herbicides, and costs such as 'collateral damage' on non-target plants (if applicable), are not included in the analysis unless a definitive dollar value has been estimated. Nevertheless, unpriced environmental and social benefits and costs should be captured and described to provide a broader perspective to the economic analysis.

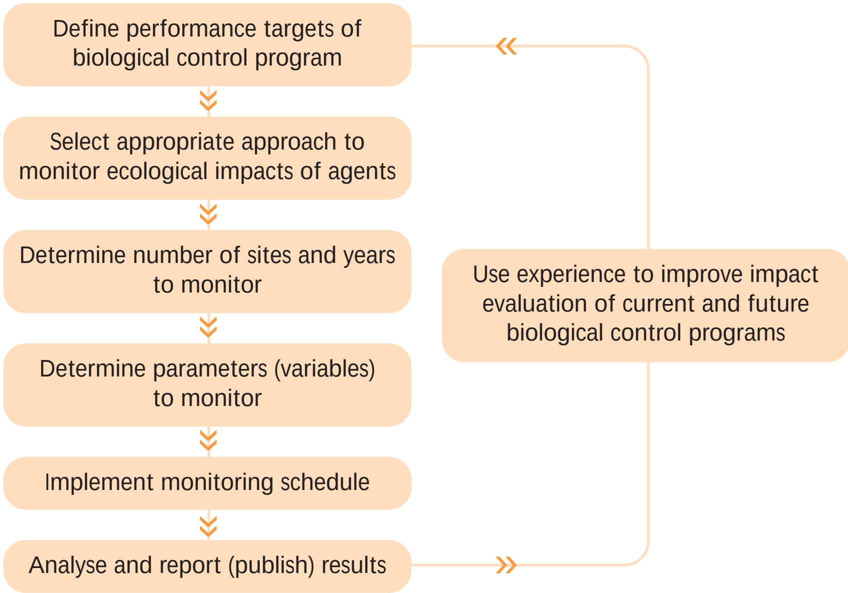

Figure 1: Key steps for developing a monitoring program to evaluate post-release ecological impacts of biological control agents.
Adapted from Roni et al 2005

Ecological impact evaluation

Several different approaches, varying in their level of complexity and amount of resources required for implementation, have been used to evaluate short- and long-term ecological impacts of biological control agents. Some approaches are more effective or suitable than others under certain circumstances (eg terrestrial versus aquatic systems), and each has pros and cons (Table 1). To be most effective, post-release ecological impact evaluation should be planned well before any agents are released to ensure that crucial pre-release measurements are gathered. This includes determining the design, scale and duration of the monitoring program and the types and timing of data collection (Fig. 1).

Approaches

Before- and after-release assessments

Photopoints: The simplest and cheapest approach to evaluate impact of biological control agents is to establish fixed reference points at weed-invaded sites before or soon after the release of agents and to take a series of photographs from those at regular intervals after release (see the parthenium case study). This approach is suitable when impact of the agent(s) is visually conspicuous and a dramatic decline in weed density and cover is observed. It can be difficult however, to measure modest changes in weed density from such a photo series. Where appropriate, photographs of vertical views of fixed areas (eg quadrats) are more suitable for measuring slight changes in density because they can be analysed with high precision using image analysis software. Decrease in weed density may not be easily detected in photographs if other vegetation is replacing the controlled weed and therefore additional information on the type of plants present at photopoints has to be collated. Photographs also do not provide evidence that the decline in weed populations is due to the agent(s), unless it is coupled with an assessment of agent damage and density.

Comparing historical and contemporary data: Published and unpublished historical information, such as field ecological data and aerial or satellite photos of specific weed-infested sites, collected regardless of the initiation of a biological control program, can provide baseline data to compare with contemporary data taken at the same sites. Aerial and satellite imagery can be particularly powerful by providing a long-data series prior, during and after release of biological control agents over a large, spatial scale. The use of historical data for comparison is most useful when the agent is widespread and if data of equal quality exist for several sites.

Table 1: Summary of advantages and disadvantages of various approaches to evaluate post-release ecological impacts of biological control agents.

Approach		Description	Advantages	Disadvantages
Before- and after- release assessments	Photopoints	Comparison of photographs taken at fixed reference points at weed-invaded sites	Simple and cheap Suitable when dramatic decline in weed populations is observed	Cannot definitively attribute weed decline to agent Difficult to measure modest changes in weed density May be misleading if other vegetation replaces controlled weed
	Comparing historical and contemporary data	Comparison of historical data on weed with similar contemporary measurements	Relatively cheap to implement because it takes advantage of previous work Suitable if agent is already widespread and pre-release data exist for several sites	Cannot definitively attribute weed decline to agent May be difficult to locate quality pre-release records
	Stakeholder surveys	Comparative surveys of land managers' perceptions of prevalence of the target weed	Relatively cheap to implement Useful for large-scale evaluation	Cannot definitively attribute weed decline to agent Provides only qualitative (and possibly biased) assessments of program outcomes
	Comparing quantitative data	Comparison of pre- and post-release measurements on weed and agent(s) taken across multiple sites and years	Persuasive if a long-term, sustained reduction in the weed correlates with presence of agent Can improve study by including control plots (with no agent) at monitored sites Suitable to measure changes in associated ecosystem	Requires more resources than above methods. Difficult to separate effects of agent from other confounding factors (unless control plots included) Needs to be carried out over long periods if high variability in weed parameters is observed between years
Comparing sites or plots with and without agents		Comparison of sites or plots where agents are present with others where they are not (no pre-release data collected)	Suitable if agent has already been released, but is not widely established Suitable to measure changes in associated ecosystem if agent-free sites are retained over long periods	Difficult to separate effects of agent from other confounding factors that affect weed growth at different sites / plots Needs to be carried out across several similar sites and over multiple years Not suitable if agent disperses rapidly.
Correlative studies		Correlation between agent density or damage severity and weed performance	Suitable if agent has already been released and is widely established Suitable for assessment at individual plant level	Difficult to separate effects of agent from other confounding factors that affect weed growth at different sites Needs to be carried out across several similar sites and over multiple years
Agent exclusion experiments		Comparison of plots where agent is excluded, using cages or pesticide applications, with others where they are present	Allows assessment of relationship between agent densities or damage severity and weed performance, without external confounding factors Unintended procedural effects can be managed by measuring effects of the exclusion treatments without agent present and use this to interpret experimental results	Resource- and time-consuming to establish and maintain Generally carried out on a small scale and over the short term Exclusion treatments can alter the system investigated (eg insecticide can exclude pollinators and affect weed reproduction) Difficult for medium to large woody weeds
Demographic modelling		Models to demonstrate mechanistic connection between agent impact and weed densities	Useful to exclude other explanations of population decline of the weed over time Useful to predict future dynamics of weed populations under pressure from agent and other control tactics Useful when no or limited pre-release data are available Useful to assist planning of timeframe of monitoring activities	Requires specialised expertise to develop models Data needed to parameterise models may not be available Inevitable trade-off between simplicity of model and how well it fits the 'real' data

Photopoints at an infestation of bridal creeper at Gairdner River in Western Australia. Top photograph was taken in September 2005 less than 2 months after the rust fungus (*Puccinia myrsiphylli*) was released, while the photograph on the bottom was taken in September 2006, 15 months after the release.
Photos: Peter Turner

As for photos taken at fixed reference points, this approach cannot attribute, with certainty, the decline in weed populations to the agent(s).

Stakeholder surveys: Perceptions of land managers on the prevalence of a target weed and the economic and / or social problems that it causes can be surveyed before the initiation of a biological control program. They can then be compared with answers obtained in subsequent surveys performed years after the release of agent(s). Such surveys are a relatively inexpensive way to obtain a qualitative assessment of the outcomes of a program, but may be deceptive because stakeholders' perceptions can be influenced by whether or not they are supporters of biological control or perceptions may change independent of the biological control program itself. Therefore surveys should be coupled with more detailed site-specific quantitative studies to provide hard data to support findings.

Comparing quantitative data: Quantitative data collected on selected populations of the target weed, associated plant communities and ecosystem processes in the immediate years before the release of biological control agent(s) can be compared with similar data gathered after their release (see the mimosa and bridal creeper case studies). Such studies have to be carried out at multiple sites across the weed distribution and over several years to capture the often high temporal variations in population levels and to ascertain that observed changes are primarily due to the agent(s). The inclusion of control plots at monitored sites, where agents are not released or are excluded (see below), can also provide additional information to identify possible confounding effects. These control plots however, may be short-lived if the agents rapidly colonise them, or exclusions cannot be adequately maintained over long periods.

Comparing sites or plots with and without agents

In situations where an agent has already been released but has not widely established or spread, it is possible to compare weed population parameters at areas where the agent is present with others where it is not (see the mimosa and parthenium case studies). Results of such comparison studies however, may be influenced by differences in site or plot characteristics such as unsuitable abiotic or biotic conditions that prevent agents establishing and / or affect plant growth (eg competition with other vegetation). These studies should be conducted across several similar sites and over multiple years to account for these possible confounding effects. It may not be possible however, to perform comparative studies for many years if an agent population builds-up rapidly and individuals disperse to control sites.

Correlative studies

Correlative studies are effective to relate agent densities and rate of attack to changes in growth and reproduction parameters of individual weeds. They can demonstrate the overall impact on the weed population and associated vegetation if permanent sites are monitored over several years. They can also be used to clarify contributions from multiple agents. Changes in plant performance recorded in correlative studies may not be due solely to the effect of the agents but also to other external factors, such as moisture availability and microhabitat characteristics. The density and levels of attack of an agent may also be altered by the nutritional quality, size and vigour of the target weed. To overcome some of these problems, correlative studies should be carried out over several sites and years and ideally concurrently with exclusion experiments (see below), to irrevocably demonstrate cause and effect and account for possible confounding factors.

Agent exclusion experiments

Exclusion experiments using cages or achieved through regular applications of insecticides or fungicides are performed to relate agent densities or severity of damage directly to plant performance, independent of confounding external factors (see the parthenium and bridal creeper case studies). These types of experiments can also be used to isolate the effect of single agents (eg pathogen versus arthropod). They are generally more suitable for annual weeds, whose population density levels often fluctuate widely between years, than for medium to large woody weeds (see the mimosa case study). They may sometimes involve the transfer of potted plants of the target weed or transplantation of standardised plants to the field to enable detailed examination of responses to agents under natural conditions, while controlling for differences in soil and plant size, age and genotype. The main advantage of such a pot or planting method is the ability to retrieve entire individual plants at the end of the experiment, making it easier to examine agent impact on below-ground biomass (see the bridal creeper case study).

Procedural difficulties however, are often associated with these types of experiments. For example, physical barriers, such as nylon mesh used in cages, can reduce light levels and evapo-transpiration rates, which in turn may alter plant performance. Cage and insecticide exclusion experiments can also affect plant performance by excluding pollinators leading to reduced seed production in plants within treated plots and producing unrealistic results (see the mimosa case study). The exclusion treatment may also influence the system by affecting populations of endemic pathogens, predators and parasitoids. It is recommended to use a pesticide with a restricted mode of action that is efficient against the specific agent to overcome non-target impacts on associated microbial and insect communities. The pesticide used to exclude the target agent may have unwanted fertiliser or phytotoxic effects that affect plant growth. Such unintended procedural effects do not present an insurmountable problem if they can be quantified. Experiments that compare the performance of plants without the agents in caged or chemically-treated plots versus uncaged or untreated plots, can be used to estimate the effect of applying the exclusion treatment, which can be taken into account when interpreting results of exclusion experiments.

Exclusion experiments are very time-consuming and costly to carry out and therefore are generally conducted on a small scale for only a short period of time. These experiments may also be unsuitable to measure changes in associated vegetation due to their inherent short-term nature.

Demographic modelling

Models are useful to demonstrate a mechanistic connection between agent impact and reduced weed densities in order to exclude other explanations of population decline of the weed over time. With data on weed growth rates and fecundity gathered before and shortly after agent release, a population model can be developed to predict the future dynamics of weed populations, including rate of spread, under pressure from the agent (see the mimosa case study). Comparison of model predictions with long-term post-release data on plant demography will evaluate whether the agents are affecting the population dynamics of the weed and to what extent agent damage is having an impact on population densities.

Modelling based on results from exclusion experiments or correlative studies can also facilitate evaluation of biological control programs for which no or limited prior data are available, even years after they were initiated. Such an approach provides some form of quantitative evidence to support claims that a program has been successful or not.

Quantitative data collection

The types of quantitative data and time of collection depend on the performance targets set for the biological control program, the approach selected for impact evaluation and the key life history stages of the target weeds and agents (see the parthenium and bridal creeper case studies). For example, it is necessary to measure changes in seed production to assess impacts of a seed-feeding agent: although it is also important to verify if this parameter alters the size of the seedbank and recruitment of seedlings. Timing of data collection may also be influenced by logistic considerations, such as limited access to sites during floods in wet seasons (see the mimosa case study).

Ideally, agent ecological impacts should be measured at the plant, population

Table 2: Possible measurements and most suitable approaches to evaluate post-release ecological impact of biological control agents at different levels.

Level	What to measure	How to measure
Plant	– growth (number, size and biomass of above- and below-ground parts) – reproduction (number and biomass of flowers, fruits and seeds) – survival – agent density and damage levels	– before- and after-release assessments – agent exclusion experiments – correlative studies
Population	– photo references – weed density or cover – age structure – seedling recruitment and survival – viable seedbank density – weed stand size and spread – agent density and damage levels	– before- and after-release assessments (including stakeholder surveys and comparison with historical data) – agent exclusion experiments – comparing sites / plots with and without agents
Ecosystem	– species richness and abundance of desirable plant species – biomass productivity (eg pasture carrying capacity, crop yield) – species richness and abundance of undesirable plant species (weeds) – species richness and abundance of beneficial arthropods and microbes – ecosystem characteristics (eg soil nutrient levels, plant community structure, water quality) and processes (eg rate of nutrient cycling and decomposition)	During: – before- and after-release assessments – agent exclusion experiments (but ensure procedures do not confound any ecosystem level comparisons) – comparision of sites / plots with and without agents

and ecosystem levels (Table 2), but availability of resources may considerably limit the extent of data collection. Practitioners are encouraged to measure agent abundance and responses of individual plants, and wherever possible to also monitor changes in weed populations, by measuring parameters such as seed production, viable seedbank, seedling recruitment, plant density, cover and stand size. Monitoring of associated plant communities (species richness and abundance) and other ecosystem characteristics (eg rate of litter decomposition, soil nutrient levels, water quality in aquatic systems), is also recommended to assess whether the ultimate goals of the program have been achieved.

Resource constraints may also restrict the number of sites that can be included in the impact evaluation phase. Establishment of relationships between key plant performance parameters and the impact that agents have on these relationships at a few representative sites can help streamline the number and type of measurements that need to be taken, and enable subsequent evaluation to take place across many more sites. For example, inflorescence length and tap root diameter of Paterson's curse (*Echium plantagineum*) were found to give a good estimate of seed production, which is labour intensive and time consuming to measure.

Recording a range of abiotic (eg rainfall, temperature and soil nutrients) and biotic parameters (eg presence / abundance of predators, parasitoids or competitors of the agents) may be useful for later interpreting and explaining variability in results. Monitoring several representative sites across the weed distribution to examine the effect of the agents across different climatic regions and habitats may also assist to identify limitations on agent impact (see the bridal creeper case study).

Long-term monitoring

The duration of long-term impact monitoring of agents depends on how fast populations build up to damaging levels, how long weed populations and the associated ecosystem take to respond and the overall variability of the system. Continued, regular monitoring (in some cases for up to 30 years) is often necessary to account for confounding factors such as spatial and temporal climatic variability. For example, populations of a weed (such as an annual) and agents that fluctuate widely between years need to be monitored more regularly to capture this variability. Terminating impact monitoring before agents have had a chance to achieve their full potential provides misleading results and understates the success of biological control programs.

Lack of resources is often a key factor that prevents annual monitoring from being carried out for long periods of time. In such instances, sites can be re-assessed at longer intervals (ideally every 2–3 years and up to 10 year intervals) to capture long-term impacts of agents by comparing with data collected before or soon after releases.

Demographic modelling can also be used to estimate how long an agent may take to affect key growth parameters of a weed, and assist in planning the timeframe of long-term monitoring activities.

Other considerations

Integrated weed management

In cases where post-release impact evaluation of biological control agents indicates that they have only been partially successful at reducing populations of the target weed, their integration with other management regimes may increase overall levels of control. Empirical testing of biological control agents with other control strategies is one approach to identify a successful combination of tactics. Models that incorporate multiple processes such as weed population dynamics, disturbance and biological and non-biological control tactics can also assist in determining the long-term impact of agents within a broader integrated management strategy for the target weed (see the mimosa case study).

Site rehabilitation

Follow-up interventions, such as various management and revegetation activities, are necessary if a biologically-controlled target weed is replaced with other non-desirable plant species. Lack of natural recovery of desirable species at controlled sites may be due to the loss (or depletion) of their seedbank or to some other factors that facilitate continued weed invasion (eg disturbance, high nutrient levels). While this is beyond the scope of biological control programs, it is important to alert land managers to these issues to avoid a treadmill of continuous weed invasion.

Compiled by

Adele Reid and Louise Morin, CRC for Australian Weed Management, CSIRO Entomology, Canberra, ACT.

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Mimosa: a leguminous shrub

Mimosa (*Mimosa pigra*) is an invasive prickly shrub that grows up to 6 m high and forms impenetrable thickets over vast stretches of the Northern Territory in tropical Australia. It grows all year round and produces pods from February to April, which release seeds until June. Its dense stands threaten native biodiversity in Kakadu National Park and other wetland areas and also interfere with human land use by affecting pastures, livestock and access to water.

Challenges faced to monitor impact of mimosa biological control agents at some sites in the Northern Territory include difficulty in access, pictured here, and many others such as crocodiles, mosquitoes, leaches and humidity. Photo: CSIRO

A total of 13 insects and two fungi have been released on mimosa in Australia, of which at least seven have established. Of these, six agents cause obvious damage to mimosa: the seed-feeding bruchid (*Acanthoscelides puniceus*) released in 1983, the stem-mining moths (*Neurostrota gunniella* and *Carmenta mimosa*) both released in 1989, the flower-feeding weevil (*Coelocephalapion pigrae*) released in 1994, the leaf-eating beetle (*Malacorhinus irregularis*) released in 2000 and the defoliating moth (*Macaria pallidata*) released in 2002.

Post-release impact evaluation

Several factors inherent to the biological control of mimosa make it difficult to evaluate impact of established agents. Firstly, limited pre-release data were collected before agents were released. Most agents rapidly dispersed throughout the weed's range after their release, making comparison of sites with and without agents difficult (however see the exception below). Regular monitoring is difficult to undertake in the wet season due to flooding, which restricts access to field sites. The culling of feral Asiatic water buffalos in the late 1980s to early 1990s, may have contributed to reduced mimosa invasiveness, potentially confounding the impact of agents. Lastly, mimosa is a long-lived woody shrub, which means impact of agents at the weed population level may not be detectable for decades.

Mimosa infestation in the Northern Territory.
Photo: CSIRO

The post-release impact evaluation of mimosa agents has so far primarily involved the use of four different approaches.

1. Before- and after-release assessments – comparing quantitative data

From 1984 to 1986, before most agents were released, data on leaf fall and seed and pod production were collected at one site along transects set up from the edge to the centre of a dense mimosa stand. Similar data were collected again at the same site from 2001 to 2003, years after populations of released agents had built-up. The density of the various agents and damage levels were also recorded.

From 2001 to 2003, leaf litter fall was 20% higher and seed rain 47% lower than data collected in the mid 1980s. From these data, the flower-feeding weevil *C. pigrae* was estimated to destroy only 10% of flowers. Seed rain was found to have declined by ~60% where the highest densities of the stem-mining moths *N. gunniella* and *C. mimosa* occurred. The density of agents and reductions in seed rain were highest at the edge of the mimosa stand. This study however, could not attribute with certainty the decline in weed reproductive outputs to the agents as the removal of buffalos may have contributed to the suppression of mimosa recruitment at this site and hence a decline in seed production over time.

A stem-mining (*Carmenta mimosa*) larva eating into a mimosa stem.
Photo: CSIRO

Another survey conducted at this site in 2005, after the release and establishment of the defoliating moth *M. pallidata* revealed that mimosa seedbanks have continued to decline and are now approximately 10% of the levels before the release of biological control agents.

2. Comparing sites with and without agents

Litter and seed fall, seedbanks, vegetation cover, size, density and age structure of mimosa stands at nine sites where the stem-mining moth *C. mimosa* was present were compared with similar data taken at eight other sites where the agent was absent. This evaluation method was appropriate for this agent because it is a slow disperser, allowing sites free of the agent to be easily located. None of the sites were free of the other agents, which dispersed rapidly after release, and consequently densities and damages caused by all agents had to be surveyed and correlated to plant performance across all sites.

Mimosa seed rain was reduced by more than 90% at sites colonised by *C. mimosa* where the highest damage severity caused by the agent was recorded. Seedbanks were reduced at *C. mimosa*-infested sites compared with sites where the agent was absent. The cover of other vegetation also increased under stands where *C. mimosa* was present, inhibiting mimosa seedling establishment and also apparently increasing the susceptibility of mimosa to fire, by boosting fuel loads beneath stands. It was concluded that *C. mimosa* has the potential to reduce dramatically the abundance of mimosa on Northern Territory floodplains, provided overgrazing does not reduce the level of fuel for fires.

Limited impact could be attributed to the other biological control agents *C. pigrae*, *N. gunniella* and *A. puniceus* during this study. Mimosa stands however, were only surveyed annually, which may be insufficient to detect an impact from these other agents. Admittedly, because sites with and without *C. mimosa* were not randomly chosen, results may have been influenced by differences in site characteristics that affect agent-plant interactions.

3. Agent exclusion experiments

Although attempted, insecticide exclusion experiments were not successful to assess impact of insect agents on mimosa. In early trials, an even application of insecticide onto fully grown shrubs was difficult to achieve due to their size and consequently shrubs were not maintained totally free of agents. The use of insecticide was also found to disrupt pollinator insects and hence mimosa reproductive outputs. Another insecticide exclusion experiment was later performed using mimosa seedlings transplanted to the field, but unfortunately, several months after initiating the experiment, the systemic insecticide used was found not to be effective in excluding *C. mimosa*. It is worth emphasising that insecticide exclusion experiments, using newer, more specific insecticides and different application methods may be found to be appropriate to assess agent impact on mimosa.

4. Demographic modelling

Models based on the two most effective agents, the stem-mining moths *N. gunniella* and *C. mimosa* predicted that it would take from 12–29 years to reduce the cover of mimosa from 90% to <5% at sites where the agents have established. Explicit modelling of integrated weed management strategies then revealed that other approaches such as burning and mechanical control were needed to reduce the short- to medium-term detrimental impacts of mimosa.

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Parthenium: an annual pasture weed

Parthenium (*Parthenium hysterophorus*) is an annual herb, with a deep tap root and an erect shoot reaching up to 2 m high. It germinates in spring–early summer and flowers within 6–8 weeks. A fully grown plant can produce more than 15,000 seeds and the seedbank persists in soil for long periods, with nearly 50% of seeds remaining viable for up to 6 years. It generally senesces in late autumn, but under suitable conditions such as high rainfall, it can grow and reproduce all year round. Parthenium is mainly found in Queensland, where it out competes useful pasture species and causes significant human health problems such as hay fever and asthma.

Nine of the 11 biological control agents released on parthenium in Australia since 1980 have established: the leaf-feeding beetle (*Zygogramma bicolorata*) released in 1980, the seed-feeding weevil (*Smicronyx lutulentus*) released in 1981, the stem-galling moth (*Epiblema strenuana*) and stem-boring weevil (*Listronotus setosipennis*) both released in 1982, the leaf-mining moth (*Bucculatrix parthenica*) released in 1984, the winter rust fungus (*Puccinia abrupta* var. *partheniicola*) released in 1991, the stem-galling weevil (*Conotrachelus albocinereus*) released in 1995, the root-boring moth (*Carmenta ithacae*) released in 1998 and the summer rust fungus (*Puccinia melampodii*) released in 1999. Of these agents, *Z. bicolorata* and *E. strenuana* appear so far to have the greatest impact.

Post-release impact evaluation

Four main approaches have been used to evaluate post-release impact of the key agents released for parthenium biological control. They explored how climatic, spatial and temporal variability, plant competition and multiple agents affect parthenium at the plant (including different life stages) and population levels. Studies have been performed at sites where livestock was excluded to avoid potential confounding effects due to grazing pressure, which positively interacts with parthenium invasiveness.

1. Before- and after-release assessments – photopoints

Photographs of a field heavily infested by parthenium taken before, during and after an outbreak of the leaf-feeding beetle *Z. bicolorata* showed a decline in weed population and the recovery of desirable pasture species (Fig. 2). This series of photographs provided a visual impression of the impact of this agent, which was supported with subsequent quantitative impact studies.

2. Comparing plots with and without agents

Three parthenium-infested sites were surveyed for 2–3 years to primarily evaluate impact of the leaf-feeding beetle *Z. bicolorata*, although the other agents, *E. strenuana* and *L. setosipennis* were also prominent at the sites. At each site, parthenium patches were selected based on whether or not *Z. bicolorata* was present and defoliating plants. Within each patch, ten 0.25 m^2 quadrats were randomly selected and a range of plant and agent measurements recorded: plant height, plant density, flower production, total plant biomass, viable soil seedbank, *Z. bicolorata* defoliation levels and, incidence of *E. strenuana* galls and *L. setosipennis* larval damage. Each year, measurements were made in different parthenium patches.

Figure 2: Series of photographs of a parthenium-infested field at Mt Panorama in Central Queensland taken at a fixed reference point before (November 1996), during (January 1997) and after (July 1998) an outbreak of the leaf-feeding beetle (*Zygogramma bicolorata*).
Photos: K Dhileepan

In patches defoliated by *Z. bicolorata*, parthenium density, height, biomass, flower production, germinable seeds from soil samples and seedling emergence, were reduced by up to 100% percent compared to that of unattacked patches. Nonetheless, because plants measured at each site were not randomly chosen and the agents were not experimentally manipulated, it is difficult to claim with certainty that changes in plant performance were only due to the agents. For example, plants in some patches may have been more susceptible to attack by *Z. bicolorata*, potentially leading to confounded results. The negative effect of *Z. bicolorata* defoliation on plant performance parameters however, was observed over several sites and years, increasing confidence in results.

3. Cage exclusion experiments

The effect of the stem-boring weevil *L. setosipennis* on different growth stages of parthenium was assessed using a cage exclusion experiment in the field. Three insect-proof cages (12 m x 4 m) were set up outdoors and each cage was further divided into eight sub-cages using insect-proof partitions. Six parthenium seedlings were transplanted into the ground of each sub-cage. Two sub-cages were randomly allocated to a control treatment, which remained insect-free. Thirty insects were introduced in each of the other six sub-cages when parthenium was at the rosette, pre-flowering or flowering stages (two replicate sub-cages per growth stage). At the first signs of parthenium senescence, all plants were harvested and height, root length, stem diameter, and number of branches, leaves, flowers, oviposition and larval feeding sites were recorded. Root, stem and leaf dry weight biomass was also measured.

Flower production was reduced by 38% percent when the agent was introduced at the rosette stage. No reduction was observed when insects were introduced at the pre-flowering and flowering stages of parthenium. The impact on flower production measured in this experiment however, was only about half of that measured in previous experiments involving potted plants in a glasshouse. The stem-boring weevil did not have any impact on total plant height and biomass at the end of the experiment, irrespective of the parthenium stages at which insects were introduced.

Similar field cage exclusion experiments were also performed to measure impact of *E. strenuana* and *Z. bicolorata*, although for *E. strenuana* both potted plants and parthenium seedlings, which grew naturally from seeds in the field, were used instead of transplanted seedlings.

The field cage experiments for the three agents however, produced different results to those obtained in insecticide exclusion experiments (see below), with cage exclusion results generally indicating a higher impact than insecticide exclusion for *E. strenuana*, lower impact than insecticide exclusion for *L. setosipennis*, and no difference in impact between cage and insecticide exclusions for *Z. bicolorata*. The discrepancy observed for two of the agents may have been due to differences in insect population levels, duration of experiments, influence of cages on the system and the use of potted plants and seeded plants in the cage exclusion experiment instead of a natural field infestation of the weed.

4. Pesticide exclusion experiments

A pesticide exclusion experiment was carried out between 1996 and 2000 at two sites infested by parthenium

The leaf-feeding beetle (*Zygogramma bicolorata*) and the stem-galling moth (*Epiblema strenuana*) so far appear to have the greatest impact on controlling parthenium. Here (left) is a close-up of *Z. bicolorata* as an adult, feeding on a parthenium leaf, and (right) the larva of the stem-galling moth within a parthenium stem.
Photos: K Dhileepan

Close-up of a parthenium stem-boring weevil (*Listronotus setosipennis*) adult.
Photo: K Dhileepan

to measure impact of agents, primarily *E. strenuana* and *Z. bicolorata*. At each site, eight 2.25 m^2 plots were maintained free of agents by monthly applications of insecticides (monocrotophos [Azodrin®] a systemic organophosphate applied as a foliar spray and carbofuran [Furadan®] a broad spectrum carbamate applied as granules to soil). A fungicide (Mancozeb) was also applied at one site in 2000 following an outbreak of the rust *P. melampodii*. Another eight plots at each site were left exposed to the agents and sprayed with water only.

Densities of rosettes, pre-flowering and flowering plants, plant height and flower production were recorded in all plots at monthly intervals during the growing season. The proportion of plants with *E. strenuana* galls and the number of galls per plant, as well as the proportion of plants with *Z. bicolorata* damage and an estimate of leaf area defoliated, were also recorded. Soil samples were collected four times per year to determine the number of viable parthenium seeds present; a crucial parameter to measure in an annual weed whose populations fluctuate widely between years. In mid autumn, at the end of each growing season, parthenium plants were pulled from the ground and total dry weight biomass assessed. The associated pasture grass in plots was also cut at ground level and its dry weight measured.

The effectiveness of *E. strenuana* and *Z. bicolorata* was significantly affected by weather conditions, with major impacts on parthenium seen in only 1 of the 4 years, due to below average rainfall. By the end of the experiment, a significant reduction in the soil seedbank of plots where agents were present was observed at one of the sites. In 1997, a 40% increase in associated grass biomass was observed at one site as a result of heavy defoliation and galling of parthenium by *Z. bicolorata* and *E. strenuana*. At the other site, grass biomass increased by 52% in 1998 mostly due to parthenium galling by *E. strenuana* and by 45% in 2000 because of the combined effects of *E. strenuana* and the rust *P. melampodii* on parthenium.

The variability in agent effectiveness due to climate would not have been detected if the experiment had not been conducted over multiple years. Maintaining pesticide exclusion treatments for 4 years however, was both expensive and labour intensive. The pesticides also had to be applied more frequently during warm or wet weather, which further increased the costs of the experiment.

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Bridal creeper: a geophyte climbing vine

Bridal creeper (*Asparagus asparagoides*) is a climbing vine that smothers large areas of natural vegetation and threatens biodiversity. It is also found climbing trees in irrigated orchards in New South Wales and Victoria, where chemical control is difficult to implement. It develops extensive below-ground mats of rhizomes and tubers, which provide energy for shoot growth as well as a buffer against adverse conditions. In winter rainfall areas, it begins to grow in late summer – early autumn, produces fruits in mid to late spring and above-ground foliage naturally senesces at the beginning of summer. Bridal creeper is common in the temperate zone of Australia.

Three biological control agents of South African origin have been released on bridal creeper in Australia since 1999. The leafhopper (*Zygina* sp.) released in 1999 and rust fungus (*Puccinia myrsiphylli*) released in 2000 have successfully established across most of the range of the weed. In contrast, the leaf beetle (*Crioceris* sp.) released in 2002, has so far established at only a few sites.

Post-release impact evaluation

Studies to evaluate impacts of biological control agents on bridal creeper had to take into consideration the weed's climbing habit, which makes it difficult to distinguish between individual plants. Consequently, percentage cover and number of shoots and biomass produced per surface area were recorded instead of the usual plant density measurements. Artificial trellises or stakes had to be used to support climbing shoots on which most fruit production occurs. Peak measurements and sampling had to be performed in early to mid spring before fruits had ripened, to prevent removal by birds, and before shoots had started to senesce. Due to difficulties in sampling the below-ground biomass, standardised potted plants were transferred to the field in one experiment to assess agent impact on rhizome and tuber production.

Three different approaches so far have been used to evaluate impact of agents, primarily the rust fungus, on bridal creeper.

1. Before- and after-release assessments – comparing quantitative data

One to 3 years before releasing the leafhopper and / or rust fungus in the early 2000s, support structures (trellises), 2 m in height and 90 cm wide made with meshed wire attached to two steel fence pickets, were set up at the edge of 3 m^2 plots (three to four plots per site) at 15 bridal creeper infested sites across southern Australia. Since then, bridal creeper growth and reproductive parameters, such as percentage cover, number and dry weight of shoots climbing trellises and growing within a randomly selected, permanent 1 m^2 quadrat, and number of fruits produced have been recorded in mid-spring each year. The incidence and severity of damage caused by the agents have also been measured each year since their release.

Bridal creeper populations have drastically declined since the release of the rust fungus at most sites located in coastal areas (Fig. 3). Assessments made in 2007 revealed that most bridal creeper at these sites has been replaced by native plants or other weeds. A similar decrease in bridal creeper populations however, has not been recorded at drier inland sites since release of the agents. Lower incidence and severity of damage caused by the rust fungus has consistently been observed at these sites, where bridal creeper growth and reproductive parameters have considerably fluctuated from year to year. Although present at most sites, leafhopper populations have been

The rust fungus (*Puccinia myrsiphylli*) adversely impacts bridal creeper across most of its range. Photo: CSIRO

Figure 3: Example of a trellis set up at Broulee, NSW, where bridal creeper has been monitored before and since the release of the rust fungus (*Puccinia myrsiphylli*) in 2001.
Photos: CSIRO

erratic and patchy across sites and over time, and thus may only play a minor role in regulating bridal creeper populations. Results from these assessments are currently being analysed.

2. Before- and after-release assessments – response of associated plant communities following agent release

Four bridal creeper-infested sites in Western Australia were surveyed during 2004–2006 to assess the response of plant communities following an epidemic of the rust fungus on bridal creeper. At each site, plots (10 m x 1 m) were established in stands of bridal creeper and in nearby reference areas, within native vegetation with little or no bridal creeper present (two to three paired plots per site). All vascular plant species were identified and percentage shoot cover of the understorey (to a maximum height of 1.5 m) was estimated visually for each species within each plot. In June 2005, approximately 8 months after the first vegetation survey of plots was carried out, the rust fungus was released in the centre of each bridal creeper stand at each site. The sites were revisited in September 2006, before bridal creeper naturally senesced, to perform another series of vegetation surveys.

In both vegetation surveys, the mean number of native plant species and the percentage cover of all native under-storey plants were significantly lower in bridal creeper areas compared to reference areas. Average bridal creeper cover decreased from about 50% at the time of the first vegetation survey to 10% in the last survey, about 15 months after the rust fungus was released within the bridal creeper stands. There was little change in native plant cover in these plots. At one site however, the cover of native climbers increased from 0.07% to 5.0%, while the cover of other exotic species increased from 0.03% to 23.4%.

While there was a reduction in bridal creeper cover due to the impact of the rust fungus during the period plots were surveyed, limited recovery of native vegetation was recorded, possibly due to the persistent below-ground tuberous mats of bridal creeper preventing recruitment. Longer-term monitoring of these plots is required to fully evaluate the response of other plant species to the biological control of bridal creeper and determine whether revegetation will occur naturally. The increase of exotic species in some plots where bridal creeper cover decreased points to possible future hurdles to rehabilitate these sites.

3. Fungicide exclusion experiments

Using potted plants placed in the field: In late autumn bridal creeper plants with a similar number of tubers contained in pots infected or not with the rust fungus, were placed into a natural bridal creeper infestation near Sydney, New South Wales, where agents had not yet been released. This approach allowed for a robust examination of the effects of the rust fungus on the below-ground biomass to be performed in the field. The healthy plants were maintained rust-free using monthly fungicide (Tebuconazole [Folicur®]) applications, while rust-infected plants were sprayed with water only. This fungicide was found not to affect the growth of bridal creeper in a previous experiment. After 6 months, half the plants were harvested in the spring and numbers and dry weight of shoots determined. Tubers were counted, length of rhizomes measured, and total below-ground biomass dried and weighed. The remaining plants were left in the field until they were harvested the following autumn. During that time their foliage naturally senesced at the end of spring and new shoots were produced in late summer.

The above- and below-ground biomass of rust-infected plants harvested in the spring was significantly lower than that of rust-free plants. Many of the rust-infected plants that remained in the field until the following autumn did not re-shoot and had few (if any) tubers, compared to rust-free plants which produced luxuriant foliage and had a well-developed below-ground biomass.

Using plots in a natural field infestation: Permanent 1 m^2 quadrats (10 per site) with a central 100 cm long stake were set up in bridal creeper infestations at six sites across New South Wales and Western Australia where the rust fungus was the dominant, if not sole, agent present. Half of the quadrats at each site were maintained rust-free using monthly fungicide applications. The remaining quadrats were sprayed with water only. The percentage cover of bridal creeper and other plant species within quadrats was measured three times during the growing season. Numbers of bridal creeper shoots, fruits, seeds and seedlings, and dry weight of above-ground biomass were also measured in September before bridal creeper naturally senesced, but after green fruits had developed.

This experiment has so far been conducted for 3 years. Bridal creeper growth parameters in quadrats where the rust fungus was not excluded were significantly reduced compared to measurements made in rust-free quadrats. Bridal creeper cover and the number of shoots produced in rust-infected quadrats was considerably less than in control quadrats and gradually decreased over years, indicating a possible cumulative adverse effect on below-ground organs. There was a consistent increase in leaf litter and bare ground in the rust-infected plots. When other plants did establish, bridal creeper was replaced by both native and exotic species, although other weeds were more prevalent at disturbed sites.

The bridal creeper leafhopper (*Zygina* sp.) can severely damage bridal creeper but its populations have been erratic and patchy across sites and over time.
Photo: CSIRO

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