Biocontrol of weeds

Project wrap-up

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The $13 million, four-year New biocontrol solutions for sustainable management of weed impacts to agricultural profitability project (2016–20) has identified new biocontrol options for eight out of 10 target weeds, which are significant on a national level.

The target species for which the new options have been identified include five Weeds of National Significance (African boxthorn, cabomba, sagittaria, silverleaf nightshade and prickly acacia) and the most significant herbicide-resistant weed for grain production systems in Australia (fleabane), a serious environmental and pasture weed (ox-eye daisy), and another major pasture weed (giant rat’s tail grass).
A promising suite of options:

The New biocontrol solutions for sustainable management of weed impacts to agricultural profitability project identified the following potential biocontrol agents for:

- African boxthorn (Lycium berteronianum): a rust fungus (Puccinia rapides) on leaf-chewing beetles, (Cassida distinguenda and Cleto eckloni) and a leaf-feeding weevil (Neoplatygaster serietuberculata).
- Cabomba (Cabomba caroliniana): the cabomba weevil (Hydrotmetes natans).
- Fleabane (Conyza bonariensis): a rust fungus (Puccinia coniceps-alerecii) and a stem-galling moth (Trupanea bonariensis).
- Mother-of-millions (Calandrinia delagoensis): a stem-galling moth (Ox-eye daisy (Leucanthemum vulgare): a leaf-feeding moth (Dichrorampha aeratana).
- African boxthorn (Lycium berteronianum): a rust fungus (Puccinia rapides) on leaf-chewing beetles, (Cassida distinguenda and Cleto eckloni) and a leaf-feeding weevil (Neoplatygaster serietuberculata).
- Ox-eye daisy (Leucanthemum vulgare): a leaf-feeding moth (Dichrorampha aeratana).
- Silverleaf nightshade (Solanum elaeagnifolium): a tingid (Argaphia arizonica) and a mite (Aceria sp.).
- Sagittaria (Sagittaria platyphylla and S. calycina): a fruit-feeding weevil (Listronotus appendiculatus), a crown-boring weevil (L. sordidus) and a tuber-feeding weevil (L. frontalis).
- Giant rat’s tail grass (Sporobolus pyramidalis and S. natalensis): a fungus (Ustilago sporoboli-indica) and a wasp (Tetrameson sp.).
- Prickly acacia (Vachellia nilotica): a gall thrrip (Acaciothrips ebneri), a gall mite (Aceria sp.) and a stem-galling fly (Notomma mutilum).
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Farmers directly affected by the weeds targeted with these new biocontrol agents will see a gradual reduction in their control costs, as the released agents build-up their populations and cause increasing damage on the weeds.

At its completion, the project had identified 17 potential biocontrol agents for eight of the 10 target weeds.

Despite extensive surveys and testing, no suitable agents for sowthistle were identified during this project, due to lack of host specificity.

Applications have been submitted, or are in preparation for, the release of agents for sagittaria, cabomba, ox-eye daisy, african boxthorn, fleabane and prickly acacia.

Further host-specificity testing of candidate agents will continue during the new Rural R&D for Profit project: Underpinning agricultural productivity and biosecurity by weed biological control (2020 – 22). Specifically this will involve candidate agents for African boxthorn, giant rat’s tail grass, prickly acacia, fleabane and silverleaf nightshade. Further host-specificity testing of mother-of-millions also will continue.

More details on the outcomes of this project, relating to individual weeds, can be found on the following pages.
How was the project carried out?

While the methodology followed in this project was tailored to the situation of each target weed, in general, the following steps were followed.

**STEP 1. Stakeholder engagement**

The target weeds were identified through consultation with primary industry and affiliated stakeholders, spanning sectors, states and territories. Early during the project, clear goals for managing each target weed were developed in consultation with key stakeholders, to delineate a role for biocontrol in achieving these goals. The subsequent research was carried out within the context of expectations for biocontrol as part of broader integrated weed management (IWM) strategies. This guided the search for biocontrol agents to deliver the required level of management and the ideal locations for releases of any agents.

**STEP 2. Literature searches**

Before field surveys for potential agents were carried out, the literature was searched extensively to gather information on the taxonomy of each target weed, its distribution and known natural enemies. Knowledge of the evolutionary centre of origin and diversity of the weed helped plan subsequent field surveys. The centre of origin of the target weed species was sometimes inferred from botanical records obtained from various herbaria when the species distribution is limited to a single country or region. Insect collections, mycological herbaria and web-based databases, were consulted, in addition to the commonly available literature, to develop lists of natural enemies of the target weed, in both the putative centre of origin and invaded range. However, as many more natural enemies have been described worldwide from plants of economic importance than from those of no commercial interest, it was common to find some previously undescribed species on target weeds during field surveys in the native range.

**STEP 3. Surveys for candidate biocontrol agents**

Molecular characterisation of some of the target weeds, using efficient cutting-edge technologies (e.g., genome-wide polymorphic molecular marker systems, such as genotyping-by-sequencing) was used to determine the weed genetic structure, which helped identify the weed’s native range. To further refine these areas, the species distribution modelling tool, CLIMEX, was used to characterise and compare the climate of the weed’s native and invaded ranges. By comparing meteorological data from the different regions, specific areas of native range were identified where potentially best climatically suited candidate biocontrol agents could be found. The results from molecular characterisation of the weeds and bioclimatic models informed field surveys for candidate agents. These surveys involved spatially extensive and temporally intensive surveys between 2017 and 2020, resulting in a catalogue of candidate agents.

**STEP 4. Host-specificity testing of promising agents**

Host-specificity testing is necessary to determine the potential range of plants (hosts) that could be attacked by a candidate agent following its release in Australia. Agent rearing and propagation methods were explored before host-specificity testing commenced. Experimental investigations were generally undertaken in quarantine facilities in Australia (including obtaining export and import permits from the relevant regulatory authorities in the native range and Australia), and in some cases were also performed in the field and collaborators’ laboratories overseas.

**STEP 5. Prepare and submit application for release**

Where required, the individual weed was formally nominated through the Invasive Plant and Animal Committee (IPAC; Now Environment and Invasives Committee), which assesses possible conflicts of interest before endorsing it as a biocontrol target. Applications to release candidate agents were submitted to the relevant authorities, if results of host-specificity testing demonstrated they would not pose a risk to non-target plant species. This step is a key regulatory requirement before the release of any agents.

**STEP 6. Release of agents**

It was not possible to undertake releases as part of the current project as applications were still pending approval. Agent release will be a core part of the new Rural R&D for Profit project: Underpinning agricultural productivity and biosecurity by weed biological control.
African Boxthorn
(Lycium ferocissimum)

Introduced to Australia from South Africa during the mid-1800s as a hedge plant, African boxthorn has since spread into pastures, neglected areas, roadsides, railways and waterways. It is regarded as one of the worst weeds in Australia because of its invasiveness, potential for spread, and economic and environmental impacts.

There are several Lycium species in South Africa and significantly more genetic diversity within Lycium ferocissimum in South Africa than Australia. Early project investigations revealed all local specimens to be Lycium ferocissimum, with no evidence of hybrids. The research team determined the closest genetic match in South Africa for the Australian populations, supporting the search for suitable biocontrol agent candidates.

Puccinia rapipes shows promise as a candidate biocontrol agent. At the landscape scale, minimising the disruptions of biocontrol agents by other control tactics (e.g. avoiding spraying plants in nursery sites with herbicides) will require coordination among land managers recommending and deploying management tactics for African boxthorn. In addition, there are likely to be many circumstances where the pathogen (and other biocontrol agents) may work successfully in combination with physical and chemical management. For example, it could be of value to trial the use of the pathogen as a follow-up treatment to control recruiting seedlings in place of herbicide applications. Similarly, the timing of releases of the pathogen with periods of regrowth of the weed following physical treatment, may also aid the integration of biocontrol to be another chronic stressor for this weed.

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Current distribution of African boxthorn in Australia
Source: GBIF.org 24 July 2018

ABOVE: African boxthorn is primarily spread by birds eating its fruits and can form impenetrable, spiny thickets.

Project outcomes
A comprehensive survey for diseases and insect herbivores on African boxthorn at 28 sites across the Eastern and Western Cape provinces of South Africa identified the rust fungus, Puccinia rapipes as a promising candidate agent. The fungus was imported into a quarantine facility in Australia for host-specificity testing.

In total, 96 insect species comprising 1315 individuals were collected from both South African provinces. Of these 96 species, three were prioritised as potential biocontrol agents based on their distribution, abundance, preliminary biology studies, pilot in-field host-specificity studies (in South Africa) and feeding preference — the leaf-chewing beetles Cassida distinguenda Spaeth (Chrysomelidae) and Cleta eckloni Mulsant (Coccinellidae) and the leaf-mining weevil Neoplatygaster serietuberculata Gyllenhali (Cucullionidae).

Puccinia rapipes shows promise as a candidate biocontrol agent and, pending final testing and approval for its release, is likely to be an important component of landscape-scale management of African boxthorn. This fungal pathogen is likely to establish and perform optimally in wetter or more humid parts of the Australia affected by this weed, and in wetter years. Sites that fit these criteria might make effective nursery sites, from where the agent could initially establish and natural or assisted dispersal of the agent could occur.

Further host-specificity testing will be carried out on candidate agents as part of the new Rural R&D for Profit project: Underpinning agricultural productivity and biosecurity by weed biological control.
Cabomba is regarded as one of the worst aquatic weeds in Australia because of its invasiveness, potential for spread, and economic and environmental impacts. It is choking waterways along Australia’s east coast. This fast-growing weed produces a large amount of vegetative material and can significantly reduce water storage capacity and taint drinking water supplies. Water treatment costs can be increased by up to $50 a megalitre. Heavy infestations can also raise water levels to a point where overflows and heavy seepage losses occur.

Project outcomes

Over the course of the project, surveys of natural enemies of cabomba were undertaken in the native range (guided by the bioclimatic modelling). These surveys, which validated earlier surveys, indicated there was only a small number of insect herbivores present on this submerged aquatic weed: four Coleoptera, three Lepidoptera, three Diptera and one Hemiptera species. Based on the published literature, most of these were likely to be too general in their host range for consideration as potential biocontrol agents for cabomba in Australia. The exception was the cabomba weevil (*Hydrotimetes natans*).

The cabomba weevil was prioritised and imported from Paraguay and Argentina into a quarantine facility in Australia for comprehensive host-specificity testing. Results from a suite of laboratory and glasshouse-based field observations and host-specificity testing in the native range and in the quarantine facility in Australia demonstrated the weevil has a high degree of specificity towards cabomba. Based on these results, a release application for the cabomba weevil was submitted to the Department of Agriculture, Water and the Environment (DAWE) on 10 March 2020.

Building numbers

Multiple importations of the cabomba weevil were carried out because of difficulties with establishing colonies in the quarantine facility. Detailed investigations helped to characterise the life cycle of this species, which was crucial for the host-specificity studies and formed the basis for developing a mass-rearing protocol. If approved for release, the mass-rearing protocol will be refined further to enable water asset managers to set-up their own colonies.

Pending approval, the cabomba weevil will be released at two primary nursery sites: Lake MacDonald (Noosa Shire, Queensland) and Kingfisher Lagoon, Ross River, Townsville. These sites are among the larger infestations of the weed in Australia. Additional infested sites in the Northern Territory and Victoria have also identified for the next establishment phase. The mass-rearing protocol will enable landholders to maintain weevil colonies on farm dams impacted by Cabomba.

The project team has worked closely with key water asset managers to distil context-specific IWM guidelines for cabomba.

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Although present for a long time in Australia, flaxleaf fleabane has only recently become a widespread weed of cropping systems, due to the development of herbicide resistance.

As a small-seeded weed, which requires several days of moist surface soil to germinate, it favours conditions in no-till, stubble-retention farming systems. Many fleabane populations in Australia are glyphosate resistant, making them extremely difficult to control during a summer fallow.

**Project outcomes**

A comprehensive review of the literature identified a total of 19 fungi, including several rust fungi, and 16 insects recorded on fleabane in the native range in South America and nearby areas, Central America and the southern USA. The natural enemies of most interest for biocontrol were various rust fungi and two gall flies: *Trupanea bonariensis* and *Eutreta rhinophora* (Tephritidae).

Samples of fleabane from different regions across Australia and the native range were genetically analysed, and bioclimatic modelling was performed to identify optimal locations for carrying out native range surveys for potential biocontrol agents.

Of the potential fungal agents investigated, the mycrocyclic rust fungus *Puccinia cnici-oleracei* (ex. *Conyza*) was the most promising candidate. Export and import permits were obtained and the fungus was imported into a quarantine facility in Australia on 20 November 2018. A single-telium isolate was generated from the material imported and a culture established in quarantine.

Host-specificity testing of *P. cnici-oleracei* (ex. *Conyza*) is underway and providing results do not identify unacceptable risks, an application for release will be submitted to the Department of Agriculture, Water and the Environment (DAWE).

The stem-gall-forming tephritid fly, *T. bonariensis*, was imported to a quarantine facility in Australia in November 2019 and February 2020. Colonies are being established as a precursor to detailed biological studies and host-specificity testing. This work will be undertaken as part of the new Rural R&D for Profit project: *Underpinning agricultural productivity and biosecurity by weed biological control*.

**Integrated control approach**

Chemical approaches can manage flaxleaf fleabane in cropping systems effectively, but at a cost. Biocontrol agents, such as *P. cnici-oleracei* (ex. *Conyza*) could serve as chronic stressors to the weed in fallows and outside cropped areas, limiting reproductive output, reducing the risk of seedbank build-up in fallows and the risk of spreading from surrounding non-cropped areas.

The utility of biocontrol in suppressing weed performance in unmanaged contexts (i.e. beyond crop fields and fallows) could further limit the rate of in-crop incursion of weed seeds within the growing season.

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Sowthistle is a widespread weed of grain crops and cotton, which has developed herbicide resistance during recent years, making it extremely difficult to manage with currently available methods. It has flourished in no-till situations and can germinate and set seed year round.

Sowthistle can produce up to 25,000 seeds per plant with the seed being readily dispersed by wind.

A comprehensive literature review revealed 23 fungi and seven insects recorded on sowthistle in Australia. A PhD study affiliated with the project, which combined literature and field surveys, documented 17 insect species, mostly generalists of exotic origin, could feed and develop on sowthistle in Australia.

In Europe, 22 fungi and 75 insects, different to those reported in Australia, have been recorded in the literature as infecting and feeding on sowthistle. Among the fungi found on sowthistle in the native range, several species were singled out as having the most promise as potential biocontrol agents for sowthistle in Australia. Several insect species were also of interest, because of their potential restricted host range.

A range of genetic analyses was carried out to determine the origins of sowthistle introductions into Australia in order to guide field surveys for natural enemies with potential for biocontrol. These analyses revealed the most likely invasion scenario was an initial introduction from Northern Europe and a more recent, introduction from Southern Europe or North Africa.

Bioclimatic modelling identified northern Africa and the Southern European edge of the sowthistle range as most climatically similar to regions where the species is a problem in Australia. As such, native range surveys were concentrated in southern Portugal, southern France, northern Italy, the Balkans and Greece, with a particular focus on Morocco and southern Spain. Surveys were also performed in the Canary Islands, in the Macaronesian region off the northern African west coast.

Although several fungal pathogens and insects with potential for biocontrol underwent initial host-specificity testing at the CSIRO European Laboratory in Montpellier, France, none were found to be specific enough to be pursued further as possible biocontrol agents in Australia.

While there may be additional natural enemies that could be found to have potential for biocontrol, this scenario is unlikely considering the considerable survey efforts undertaken in this project.

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Mother-of-millions, introduced into Australia during the 1940s from Madagascar, is a major weed in Queensland, New South Wales, Victoria, South Australia, Western Australia and Norfolk Island. Mother-of-millions is adapted to dry conditions and can survive long periods of drought, increasing the plant’s potential to persist and spread. Seed production is prolific, with each inflorescence able to produce about 20,000 seeds. However, the species is best known for its vegetative reproduction. Six to eight daughter plantlets are produced at the terminal ends of each phyllose. Since its introduction it has invaded thousands of hectares of grazing land. Mother-of-millions is toxic when ingested by livestock and is also poisonous to humans and household pets. The toxic effects of these plants are due mainly to bufadienolides (a type of cardiac glycoside) which cause heart failure. The toxins are present in all parts of the plant.

Established infestations are difficult and expensive to control mechanically or chemically. Infestations can increase by up to 20-30% per year if left uncontrolled. For control of large infestations, the integrated use of herbicides, grazing management and fire can be effective. However, continued use of herbicides and fire can bring about deleterious changes to the composition of native vegetation.

Project outcomes

This project aimed to improve available control options for farmers, building on an earlier program, which identified three insect species from Madagascar that show potential as biocontrol agents: the stem-boring weevil Ospihilia tenuipes (Coleoptera: Curculionidae), the phytophagous wasp Eurytoma bryophylli (Hymenoptera: Vespidae) and the phyllose- and bulbil-feeding beetle Rhembastus sp. (Coleoptera: Chrysomelidae).

Neither the Rhembastus sp. beetle nor the E. bryophylli wasp were found, despite repeated field surveys over the life of the project. However, with the help of a post-graduate student from the University of Antananarivo (Madagascar), the stem-boring weevil, O. tenuipes and a new species of root-feeding beetle, Bikasha sp., were discovered and tested in the native range and in Australian quarantine facilities. Although Bikasha sp. significantly impacts the growth of mother-of-millions, testing carried out in both Australia and Madagascar indicates the beetle is not host specific.

During testing in the native range, O. tenuipes significantly reduced the growth of mother-of-millions. Host-range testing carried out in Australia and Madagascar indicated the stem-boring weevil could feed and lay eggs on two other species of interest to Australia (other than mother-of-millions), but to a lesser extent. One of these species is a Madagascan ornamental (Kalanchoe blossfeldiana) and the other is a naturalised species (Kalanchoe spathulata). Before a final risk analysis can be performed for O. tenuipes, continuation trials (on the abovementioned species) will be carried out in Madagascar and Australia to fully understand the risks to these species. A proposal is currently being developed, to be submitted for the Australian Government DAWE Advancing Pest Animal and Weed Control Solutions funding call to continue this work.

Four mother-of-millions field study sites were established in Australia – two in NSW (Wee Waa and Turrawan) and two in Queensland (Dalby and Inglewood). The data from these studies has established a good pre-release database of the weed in Australia. If a biocontrol agent is approved in the future, these sites could be used to establish long-term monitoring sites to evaluate impact.

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Ox-eye daisy is a serious environmental weed in Australia, with the potential to become a problem for primary producers (as observed in America and Canada). Biological control in environmentally sensitive areas (containing threatened species), where herbicides cannot be readily sprayed will be an important future management strategy.

Ox-eye daisy is a rhizomatous perennial herb, native to Europe that has invaded more than 40 countries (including Australia and New Zealand). Seed longevity is high and up to 80% of propagules are viable for six years. The weed is not palatable to cattle and affects pastoral lands by reducing carrying capacity. Dense infestations exclude other plant species, leading to soil erosion and depletion of soil organic matter.

Ox-eye daisy is invasive in Victoria (where it is a declared noxious weed), New South Wales (where one of the more alarming infestations is in Kosciuszko National Park), South Australia, ACT and Tasmania. This weed species thrives in disturbed areas, however, of greatest concern is its ability to aggressively invade areas of conservation importance.

While mechanical and chemical control can be implemented to manage localised infestations of ox-eye daisy, there is an urgent need for the sustainable management of this invasive plant at the landscape level, especially in conservation areas.

Two biocontrol options are showing promise for ox-eye daisy, including the root-feeding moth, Dichrorampha aeratana (ABOVE) and root-feeding weevil, Cyphocleonus trisulcatus (not shown).

Since these initial investigations, D. aeratona, has been collected, reared and tested in Australian quarantine and at CABI in the native range. A release application is currently being prepared.

A second insect, the root-feeding weevil, Cyphocleonus trisulcatus, was also initially studied during this project in Switzerland. Additional research will see further testing of the weevil in Switzerland and NSW from July 2020 onwards, with funding through the Environmental Trust of NSW.

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Target weeds

Giant rat’s tail grass
(Sporobolus spp.)

Giant rat’s tail grasses (Sporobolus spp.) belong to a group of invasive grass species, often referred to as ‘weedy Sporobolus grasses’. Five of the introduced Sporobolus species are invasive plants in Queensland and New South Wales: giant rat’s tail grass (Sporobolus pyramidaldis and Sporobolus natalensis), American rat’s tail grass (Sporobolus jacqueumontii), Parramatta grass (Sporobolus africanus) and giant Parramatta grass (Sporobolus fertilis).

Current distribution of giant rat’s tail grass in Australia

Source: Atlas of Living Australia

Two biocontrol options are showing promise for giant rat’s tail grass, including an endemic pathogen, Ustilago sporoboli-indici (LEFT) and a larvae of a stem-galling wasp Tetramesa sp (ABOVE).

Current control efforts for giant rat’s tail (GRT) grasses centre on the use of chemical and mechanical control, plant competition and pasture management. Despite the production of a best-practice manual for GRT grasses and the widespread use of the recommended control strategies, control has not been achieved and the weeds continue to spread into new areas.

Project outcomes

Building on the findings of previous research, the project team carried out field surveys both across infested areas of Australia and the native range in Africa for promising agents.

Field surveys within the native range in eastern South Africa yielded more than 50 insects and pathogens, with at least three insects, all stem-boring wasps (two Tetramesa spp. and a Bruchophagous sp.) showing potential. Laboratory testing of the three wasps, collected at two sites in the Limpopo Province, was carried out at Rhodes University, South Africa. Based on field observations and preliminary host-specificity testing on selected plants, closely related to the two target Sporobolus species, Tetramesa sp. A was deemed to be suitably host specific to warrant further investigation. Impact studies in the field indicate Tetramesa sp. significantly reduces height, survival and reproductive output of GRT. Import permits are being organised to introduce the insect for more detailed host specificity testing. Here a wide range of native and economic grasses will be tested to determine if Tetramesa sp. A will attack and damage any of the species.

The exploration stage for endemic GRT pathogens identified 44 fungal genera on both Australian native and naturalised Sporobolus host plants. The Sporobolus leaf smut, Ustilago sporoboli-indici, found on GRT in Queensland is having a significant impact on the invasive grass species. Infected plants are stunted, void of seedheads and easily pulled out of the ground.

Field trials have demonstrated the benefits of adopting an integrated control approach, using a slasher, heavy grazing, targeted application of herbicides to GRT tussocks using wick wipers and the leaf smut. Indications suggest this pathogen could be an excellent biocontrol option as part of an integrated weed management strategy, with populations of GRT at the trial site being reduced by almost 40% in three years. The addition of the GRT leaf smut at the trial site has meant almost all (>95%) GRT flower-heads found are sterile. Feedback from landholders incorporating the use of weed wick wipers is supporting the research results but, with a viable soil seedbank of 8 to 10 years, control efforts will need to be sustained until endemic and classical biocontrol options are optimised.

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Field site
Laboratory

Photo credits: Joseph Vitelli, QDAF

For more information on the current distribution of giant rat’s tail grass in Australia, see the Atlas of Living Australia.
Silverleaf nightshade, is one of the most difficult agricultural weeds to kill. An extensive root system enables the plant to draw moisture and nutrients from a large volume of soil and compete effectively against other species. Silverleaf nightshade competes with summer-growing crops and pastures, and reduces the production of winter crops, such as cereals. The plant’s spiny leaves and coarse stems can lower the quality of hay taken from infested areas, resulting in contaminated product, which may be rejected for sale. All parts of the plant are toxic to animals.

A potential barrier to biological control of silverleaf nightshade has been uncertainty surrounding the dual provincial and national permitting processes for exporting biocontrol agents from Argentina. FuEDEI has now advised that La Rioja Province has issued collection permits for Aceria sp. nov. for biological studies at FuEDEI and potential studies in Australia. The necessary provincial and national permits should be issued in time for a 2020 shipment to Australian quarantine. When confirmed, import permits will be sought from Australian regulators in time for the 2020 importation.

New recommendations for selecting cultivars used in host-specificity testing and a cultivar-selection tool were developed during the project to address identified gaps. This work resulted in a revised list of potato cultivars for host-specificity testing. The decision tool has broad application in weed biological control risk assessment, is easy to use, can account for uncertainty, is adaptable to different species, and is suitable for both small and large cultivar groups irrespective of complexity. If adopted, it could result in more transparent, defensible and reproducible cultivar selection practices leading to greater confidence in biological control risk assessments.

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Sagittaria is an aquatic plant native to North America, introduced as an ornamental plant. It was first identified in Australia during 1959 near Brisbane, and then in Victoria during 1962. During the 1980s Sagittaria spread rapidly and it is now widely dispersed across southern NSW, particularly in the Murray Irrigation District, and is common in waterways around Sydney and Newcastle.

In natural systems the vigorous, choking habits of Sagittaria threatens aquatic flora and fauna. Dense infestations restrict water flow and can substantially affect biodiversity and stream health. In irrigation systems it can reduce flow and efficacy of water delivery. Infestations also have detrimental impact on recreational activities and reduce visual amenity of waterways.

The fruit-feeding weevil (Listronotus appendiculatus) is set to form a key role in the integrated management of Sagittaria populations across Australia.

**Project outcomes**

The key objective of this project was to undertake host specificity testing of three candidate agents, the fruit-feeding weevil (Listronotus appendiculatus), the crown-boring weevil (L. sordidus) and the tuber-feeding weevil (L. frontalis) to assess their safety for release into Australia as biocontrol agents for Sagittaria.

The three weevil species were imported into quarantine from the southern USA for host specificity testing. Testing was successfully completed for the fruit-feeding weevil, enabling an application for its release to be submitted to the then Department of Agriculture. Host testing of the crown-boring and tuber-feeding weevils indicated several ornamental Sagittaria species and two native Alismataceae were acceptable hosts. Further studies are required to determine if the native species, Alisma plantago-aquatica and Damasonium minus are at risk of attack under natural conditions.

Staff from Goulburn-Murray Water visiting the quarantine facilities at AgriBio (left to right: Danielle Ick, Stuart Nield, Tim Nitscke, Mark Finlay (GMW) Raelene Kwong, Jackie Steel (AgVic)).

Molecular barcoding used in this project has revealed the fundamental host range of potential biocontrol agents, from laboratory host-specificity testing, can overestimate the ecological host range of these agents in their native range. The physiological suitability of a plant species to support the life cycle of an insect does not always predict the plant species that will be used as a host in the field.

"Molecular approaches to field surveys can detect a greater host range and can identify biotypes that may have different host ranges," said project lead, Raelene Kwong, DJPR. “The barcoding approach allows researchers to ensure they are testing a single biotype of the candidate agent, and that the biotype is the one associated with the target weed.”

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Prickly acacia infests more than six million hectares of natural grasslands and more than 2000 km of bore drains across Queensland, with potential to spread throughout northern Australia. Prickly acacia trees form dense impenetrable thickets, restricting stock access to watercourses, compete with native pasture species, and prevent growth of plants beneath the canopy. Prickly acacia costs primary producers about $9 million per year in lost pasture production.

**Project outcomes**

Native range studies for the prospective biocontrol agents for prickly acacia were conducted in Ethiopia, Senegal, Tanzania, Kenya, Nigeria, India and South Africa. Based on field host range, geographic range and damage potential, a gall thrip (*Acaciothrips ebneri*), a gall mite (*Aceria* sp.), and a stem-galling fly (*Notomma mutilum*) were prioritised as prospective biological control agents in Australia.

The three prioritised agents were exported from the native range into quarantine facilities in Australia and South Africa for identification, colony establishment and host specificity testing. The gall thrip from Ethiopia was imported into a high-security quarantine facility in Brisbane for colony establishment and host specificity testing. The colony of thrips was established and maintained on Australian prickly acacia plants grown from prickly acacia seeds collected from north Queensland.

Testing of the gall thrips against 56 plant species has been completed for a minimum of five replications and there is no evidence of gall development on any of these non-target species. Only two more species remain to be tested. Tests for the remaining test plant species are likely to be completed during 2020.

The test plant list for host specificity testing for prickly acacia biocontrol agents has been revised as the *Acacia* genus has undergone great change during the past 20 years, which has ramifications for host specificity testing for weed biocontrol agents for this weed.

A gall mite from Shewa Robbit, Ethiopia was exported to a quarantine facility at Plant Health and Protection (PHP), Pretoria, South Africa in April and December 2017. A colony of the leaf-galling mite was established in the quarantine, on potted prickly acacia plants, grown from seeds sourced from prickly acacia populations in Australia. Control and test plants were checked after six weeks for evidence of gall development.

A trip to Ethiopia in February 2020 was planned to collect a large consignment of mite galls for export to South Africa, to facilitate the host specificity testing of a large number of test-plant species at once. The trip was delayed due to the current coronavirus outbreak. Host specificity testing will continue when it is safe to travel, although, a possible alternative being explored is to have collaborators collect the agent.

A release application for the gall thrip is currently being prepared for submission to the Department of Agriculture, Water and the Environment (DAWE) for approval.

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This project enabled a diverse coalition of stakeholders, representing land managers, extension agencies, research and development (R&D) corporations and research providers working together to develop and deliver needs-based RD&E in the context of weed management.

A network of international collaborators spanning Australia, Europe, Africa, South America, Asia and North America joined forces with research teams to identify potential agents in their native range and work through testing procedures and regulatory processes for importation.

Industry project partners committed significant resources, both financially and in-kind. These established partnerships will facilitate widespread adoption of the project findings, both within and between industry sectors and between agricultural and environmental stakeholders.
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