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Release and establishment of weed biological control agents

Why improve release strategies?

The aim of a biological control program is to successfully manage the target weed and reduce its impacts. The time and financial resources devoted to the selection, testing and release of a biological control agent can be significant. Research on a potential agent can be stopped at any time for a number of reasons. However, if work continues to the release stage and the agent fails to establish, there is no return on this substantial investment.

In this guide we outline ways to enhance establishment of weed biological control agents following their release. Case studies are provided to highlight the principles and different approaches used. Note: in this document, use of the term insect includes true insects and mites used as biological control agents.

Agent selection influences establishment

The selection of suitable agents is a crucial step in a weed biological control program. The agents should possess the necessary biological characteristics to have the greatest chance of establishing, thriving in the new environment and suppressing target weed populations. Agents collected in the native range, in areas that climatically match those of the introduced range where the target weed is present, may have more chances of establishing following their release.

Students from Avenel Primary School, Victoria participating in a biocontrol agent redistribution field day. Here they can be seen using a beating tray to collect Paterson's curse pollen beetles (*Meligethes planiusculus*) from flower stalks.
Photo: Raelene Kwong

Example of agent selection influencing establishment

A chrysomelid beetle (*Chrysolina quadrigemina*) was released against St John's wort (*Hypericum perforatum*), in Australia. These beetles were reared from individuals originally collected from England and did not establish. Beetles collected from the Mediterranean area of Europe were then used to rear another cohort for release. These insects established readily and continue to contribute to the control of this weed.

Chrysomelid (*Chrysolina quadrigemina*) beetles on St John's wort.
Photo: CSIRO

Careful matching of the target host (weed) and agent is essential to enhance the chances of establishment after release. This is important for some insect species but particularly crucial for highly host-specific agents such as rust fungi which often consist of a number of strains highly adapted to specific genotypes of the host. Consequently, more than one strain of the pathogen often has to be selected and released in the introduced range if the target weed comprises several genotypes or closely-related taxa. The release strategy may then involve an additional pre-release step to identify plant genotypes present at selected sites to determine which strains should be released at these sites. Alternatively, all selected strains may be released at any one site, irrespective of the genetic structure of the weed population, although only the strains that are pathogenic on the weed genotypes present will establish (see blackberry and lantana case studies).

Rearing agents for release

Once an agent is cleared for release from quarantine, an effective method to produce a large number of individuals is devised. The rearing protocol for insect agents involves good hygiene practices to ensure that the colony is maintained under optimum conditions. Fungal or bacterial infections are promptly recognised and treated. Where indoor or outdoor plants are used to rear the agent, unwanted pests such as mites and aphids are controlled and plants fertilised regularly to ensure the highest quality host for agent reproduction. Presence of endosymbionts such as the bacterium *Wolbachia* in the rearing colony may be inevitable with some insect species and may affect fecundity and sex ratios in the establishing population. This may reduce the persistence and rate of increase of the population and overall establishment.

Culturing methods for plant pathogens used as agents aim to produce abundant spores for release. High quality plants are required to culture biotrophic pathogens such as rust fungi that require living plant cells to grow and produce spores. Other types of pathogens can be grown on artificial media, but may require specific conditions, such as exposure to near-ultraviolet light, for abundant sporulation to occur.

Genetically diverse insect rearing colonies are generally established to avoid excessive inbreeding and produce a genetically variable population of individuals for release. Genetic variability in the released population is encouraged because it may increase chances of establishment. In contrast, for pathogens, single or multiple genetically-pure strains are generally mass-produced and released. Each strain is produced separately to ensure genetic integrity during culturing.

Direct release of insect agents collected from the country of origin has been allowed in some instances in Australia. These decisions were made on the basis that it was not feasible to rear the insect under artificial conditions. In these instances, particular care is necessary to ensure no parasites or diseases are released with the field-collected insects, which can involve surface sterilisation of the imported living individuals prior to release.

Agent biology and ecology in a release strategy

Knowledge of the phenology of the agent and its responses to different temperatures and rainfall patterns assists in the design of a release strategy. This includes selection of the best time of the year to perform releases (see Suitable conditions for release). An efficient release strategy will take into consideration that host plant quality can significantly influence mating efficiency, oviposition, development and subsequent fecundity of insect agents. Host plant quality can also affect development of plant pathogens, in particular for rust fungi that cause severe disease symptoms on actively growing and unstressed plants. Planning releases so that the phenology of the agent is synchronised with that of the weed is also very important for some agents, particularly those that feed on flowers and fruits.

Although agents have been selected after careful matching with their target host, minor genetic differences between and within populations of the target weed may influence their performance, due to subtle differences in plant physiology and morphology. This may have implications for agents that do not disperse easily, and thus releases of small numbers of individuals should be made on several plants across a site to reduce the effect of genetic variability on establishment (see gorse case study).

The agent's non-host requirements, eg for mating, pupation, aestivation / hibernation, may also need to be considered in devising a release strategy. The cues for mating in insect agents can sometimes be manipulated to enhance population build-up after release and thus increase chances of establishment. For insects with low mating frequency, it is best to mate them prior to release. In contrast, more frequent releases are recommended for insect species with high mating frequency.

An agent's dispersal mechanism(s) and efficiency will influence the release strategy and number of releases to be carried out. For example, fewer releases will be required for wind-borne plant pathogens such as rust fungi, which have robust spores that can travel long distances under certain conditions. For insects with a tendency to disperse before mating, alternative strategies are to release large numbers of individuals at any one site or make continuous releases of smaller numbers at several sites. With some species, it may be beneficial to mate and feed the insects before their release, while for others an initial release within large field cages containing fresh plant material will prevent dispersion and ensure

A fine gauze cage used to contain leafhoppers (*Zygina* sp.) over an infestation of bridal creeper after release.
Photo: Raelene Kwong

Releasing gorse spider mite (*Tetranychus lintearius*) by lodgement of infested cuttings.
Photo: John Ireson

mating occurs. The latter approach can be useful for insects with a tendency to disperse before oviposition. Providing additional food sources (eg pollen and nectar) at time of release may also help prevent insects dispersing too rapidly and thus increase the chances of mating and improve agent survival in some instances.

Suitable conditions for release

Based on information gathered on the biology and phenology of an agent and its distribution in the native range, it is possible to determine, often with the assistance of computer modeling, the most suitable climatic areas where agent establishment is more likely to occur (see lantana case study).

Climate can also affect plant phenology, which can dramatically influence agent establishment and persistence. For example, seasonal variation in fruit production can lead to agent starvation. Consequently multiple releases across several climatic zones where the target weed occurs may be necessary (see bellyache bush case study).

Agents can be vulnerable to adverse weather conditions at the time of release. Releasing insects in cold, wet conditions may reduce their ability to seek protected or sheltered locations and more importantly, may reduce survival of individuals already stressed by transport. Releasing larvae in the heat of the day can result in their desiccation or even predation, as many larvae normally go down into the leaf litter during the day and feed at night, so releases may need to be conducted in late afternoon. If the adult is nocturnal, it may be necessary for a release to occur at night or dusk. Alternatively, releasing insects in quiescent stages of development, such as eggs or pupae, can be considered, but this will depend on having a good understanding of the agent and weed biology, and on taking steps to reduce predation risks (see Predation and parasitism).

Plant pathogen agents often have specific humidity requirements, such as 6–8 continuous hours of moisture on foliage, to infect the target weed. Releases are thus generally performed during a misty or rainy day, or at the end of a day to take advantage of the natural dew formation at night. Alternatively, release protocols can include steps such as misting plant parts sprayed with the spore suspension and covering with plastic bags overnight to provide the required moisture during the early infection phase (see blackberry case study).

Example of suitable conditions for release

The adult Paterson's curse crown-boring weevil (*Mogulones larvatus*) lies dormant over summer and emerges in autumn in response to decreasing day lengths. In drier climates where Paterson's curse (*Echium plantagineum*) germination occurred after the adult emergence, the weevil failed to establish. The Australian national release strategy therefore targeted regions with higher rainfall. In addition, landholders were advised to provide supplementary water to the release site in mid-autumn to encourage Paterson's curse germination, particularly during years when the autumn rains were late.

A crown-boring weevil (*Mogulones larvatus*) on a Paterson's curse flower.
Photo: CSIRO

Selecting a release site

Biological considerations

Suboptimal nutritional quality and the growth rate of the target weed can have a dramatic negative impact on agent establishment so releases should be made in habitats that support optimal growth of the weed. Agents may also perform better in either dense or scattered infestations of their host or on the edge of infestations. Information on the agent's most common native range habitats may guide the release strategy in the introduced range. The overall size and density of the weed infestation at a release site also influences the number of agents that can be released. Large infestations are generally required to provide sufficient food for agents released in large numbers (see Effective release size).

Searches for potential release sites should be made during the driest time of the year (unless the insect agents have a diapause mechanism that allows them to survive without food). Soil type and soil moisture can be important for survival of soil dwelling stages and thus soil conditions must be considered. This is especially important if the number of agents available for release is limited (see lantana case study).

Microhabitats within sites can be exploited to increase chances of establishment. For example, agents that prefer cool temperature will likely benefit from release in a shaded, cool microhabitat, while an agent such as a pathogen that requires moisture to infect its host, may be best released in sheltered gullies with high moisture retention (see lantana case study).

Different habitats can also affect predation and parasitism on the released agent (see Predation and parasitism). Large homogeneous infestations of the weed most likely have low parasitoid numbers and less predation toward the centre of the infestation, which is where the optimal release site will be.

In situations where other agents of the target weed have previously been released and established, the new agent should be released at sites free of the other agents if possible, to avoid competition. This is particularly important if the agents occupy the same broad ecological niche.

Social considerations

There are many social issues to consider when selecting a release site, particularly when releasing agents on private land. Failure to adequately educate landholders about biological control, including what to expect, how it works, how long it takes etc, can lead to misconceptions and possibly result in the destruction of the release site. For example, if landholders cannot recognise the agent or the nature of their impact, they may not realise that the agents failed to establish. This could result in the weed infestation getting worse if biological control was being relied on by the landholder as their only weed management tool. Or, if public or private land managers have not been told how to manage the release site, they may not realise that grazing the area or using herbicides may threaten the survival of the agent.

One approach that may be useful in managing the relationship with landholders is an 'Agreement of cooperation'. Some landholders are motivated to use biological control for the wrong reasons, particularly when they view biological control as an excuse for not controlling the weed by other more expensive or labor intensive methods. Other landholders may have unrealistic expectations about the level of control this method can provide or the time required for it to work, while others can be unaware that the release site may need special management to assist the establishment of agents. This is where an 'Agreement of cooperation' can ensure landholders are aware of their responsibilities in having release sites on their properties. These responsibilities may include maintaining weed management practices on the rest of the property to minimise weed spread from the release site, or including specific management techniques to minimise disturbances to the release site or to maximise establishment of the agents.

The following key points should be considered when involving landholders in agent releases:

- Establish a good rapport with landholders and ensure that biological control is a suitable management option for their situation.
- Ensure that the landholder understands the limitations of biological control and is particularly aware that biological control will not eradicate the weed.
- If land managers are conducting releases on the behalf of an agency, they will need to know what constitutes a suitable site and what is an unacceptable site, as well as how to release the agent.
- An 'Agreement of cooperation' with the landholder may assist in reinforcing the landholder's responsibilities in having a release site on their property. In particular, the landholder should be encouraged to continue other methods of weed management on the rest of the property and to maintain a buffer zone around the release site to minimise disturbance.
- Release sites should be in areas that are a low priority for control by other means.
- Checks should be made with the local weed management authority to ensure that releases on the property will not conflict with weed compliance programs.

Effective release size

The number of individual insects in the original population can be critical for agent establishment and will vary according to agent biology (see Agent biology and ecology in a release strategy). The release of approximately 1,000 individuals has often been used as an initial minimum release size for many insect agents, but field establishment may be achieved with smaller or higher numbers. The initial release size is often dependent on the number of individuals available for release, which depends on the ease of rearing, and time and resources available for rearing.

Effective release size is not applicable for pathogen releases. These releases generally involve spraying or dusting plants with a suspension of spores, or the transfer of infected potted plants to release sites from which spores naturally spread to the field infestation (see blackberry case study). Ideally several plants should be infected at a release site to increase chances of an epidemic developing.

A major decision is whether to opt for a large number of small releases, a small number of large releases, or a mixed strategy involving both options. Such decisions are partly dependent on the number of agents available, with the optimal release strategy being a trade-off between release size and the number of releases through space and time. Insect agent establishment has been shown to relate to release size, with small, newly released populations being more vulnerable to extinction than larger ones. Nonetheless, a point will eventually be reached beyond which increasing the initial release size does not increase the chance of establishment (see gorse case study).

The relationship between release size and establishment will vary depending on a range of factors. These include demographic randomness (chance variation in births and deaths), environmental variability, and Allee effects (reduced population growth rate at low densities), particularly during the first year after release. Genetic variation within plant populations of the same species can also affect the relationship by producing variations in the performance of the insect on each individual plant. Habitat size, higher dispersal rates or increased levels of parasitism or predation at higher release densities could also be determinants. The optimum release size is the one that produces the highest mean number of successful establishments for a given number of agents. This release size and optimal release strategy can be determined by conducting field trials to compare the establishment achieved when releasing a range of sizes (see gorse and bellyache bush case studies), or from experience obtained from 'trial and error' field releases. Using a mixed strategy in the early stages of a release program will improve the chances of finding the approach that will maximise establishment.

If the agent is easy to rear and there are thousands of individuals available for release, then releases should be made across a range of habitats and climates. If the agent is difficult to rear and only small numbers are available for release, the number of release sites, their location and the method of release will need to be chosen more carefully. In both cases, monitoring agent establishment will provide valuable information for the refinement of future release strategies.

Experiment to determine the influence of predators and parasitoids on the bridal creeper leaf beetle (*Crioceris* sp.).
Photo: CSIRO

Predation and parasitism

Many insect agents are consumed by predators. It is generally assumed that insects with external feeding stages are most vulnerable to predation. In Australia, ants are particularly active predators and have been known to consume vulnerable stages of insect agents, eg eggs, larvae, and pupae (see lantana case study). Birds and mammals are also known to attack agents. Although pupae and eggs are more easily transported, adult insects are most often released because they require less protection against predation. Some insect agents also attract generalist parasitoids shortly after release but it is often difficult to predict whether an agent will become

parasitised. Parasitism can reduce the population size to such a degree that dispersal and population growth are considerably diminished.

It may be useful to conduct pilot releases to determine if predation occurs in the field, what organisms are responsible, and when these events occur. Habitat can influence the agent's vulnerability to predation so these trials should be conducted in different habitats. Once it has been established that predation is possible at a level that necessitates management, then a management plan can be devised. There may be value in performing additional pilot studies or small scale experiments to develop a strategy prior to mass release. Small trial releases can be used to test the vulnerability of different life stages of the agent, eg eggs, larva, pupa and adults. Predators can be excluded in such studies by chemical, physical and biological means. To reduce the number of predators, pesticides and baits (against ants) can be applied in the release area prior to the release of the agents. Agents can also be placed in cages with insect proof netting, though there is a tradeoff in their use. While this may reduce predation, it prevents dispersion of the agent, increases competition, and consequently may decrease population growth. Or it may retain predators in the cage with the agent, thus increasing predation.

An alternative approach is to compensate for predation through the release of very large numbers of agents and to make releases at times when populations of predators are less active. If this approach is used, it is important to consider the other parameters influencing the release strategy, such as agent dispersal behaviour and the stage of the agent most likely to establish. Once different approaches have been trialed, the cost / benefit of each approach can be determined and compared to the cost of rearing larger numbers to compensate for predation.

Assessing establishment of the leaf-rolling moth (*Tortrix* sp.) on bitou bush.
Photo: CSIRO

For pathogens, although predation and parasitism are known to occur, neither has been reported to prevent their establishment.

Evaluating establishment

Evaluating establishment of agents is one of the most important aspects of any release program. It not only provides direct feedback to researchers on the success of the program in terms of agent selection and release strategy, including site selection, size and timing of individual releases and release methods, but may serve as a guide for future release programs with other agents. It also provides an initial indication of the potential of agents to have long-term impact on the weed. Monitoring establishment of agents can begin within weeks of the first releases and extend, in some form, well beyond the end of the release program (see gorse case study).

What constitutes establishment within the early stages of a release program can be difficult to define. Evidence of damage and, in the case of insect agents, sightings of adults and / or immature stages within the first few weeks or months of release, though encouraging, does not necessarily provide a good indication of establishment. Survival of initial populations, especially where relatively small numbers have been released, is strongly influenced by a number of factors (see bellyache bush case study). Thus, long-term survival of agents across years, at one or more sites, combined with evidence of population increase and spread, are good indicators of establishment. Conversely, establishment of agents within the first few years of release may be extremely difficult to detect, with some successful establishments initially being deemed failures over all or part of the release area.

Example of evaluating establishment

The first outbreak populations of the rubber vine moth (*Euclasta whalleyi*) were seen throughout north Queensland more than 4 years after an apparently unsuccessful release program was terminated. Similarly, establishment of a chrysomelid beetle (*Calligrapha pantherina*) in sida infestations (*Sida acuta* and *S. rhombifolia*) near Charters Towers and Townsville, Queensland, was confirmed years after the final releases were made. These areas were initially thought to be too dry for the insect to establish and persist.

The establishment of the chrysomelid beetle (*Calligrapha pantherina*) on sida in Queensland was confirmed years after its release.
Photo: CSIRO

Visits to release sites can involve visual inspections for agents and their damage and include trapping methods for insect agents such as the use of sweep nets, beating trays or sheets, and light traps. Destructive sampling may also be used to find very small agents or internal feeders in roots, stems and seeds. The timing and frequency of visits to release sites is influenced by their distance from the research centre, their accessibility at different times of the year, the time allocated to the project, and, importantly, when the agent is most likely to be detected. Networks of interested stakeholders, provided with information on the lifecycle of the agent, descriptions of life-stages and damage, can provide valuable follow-up monitoring at release sites (see Getting communities involved).

Redistribution of agents from nursery sites

Redistribution uses field-established populations of agents as a source for further releases. A redistribution program aims to maximise the agent release phase by establishing designated nursery sites at strategic locations. These nursery sites serve as collection points, enabling the harvesting of field-hardy agents in large quantities or on multiple occasions. It is often easier to collect insects in the field than rear them in the laboratory and harmful in-breeding is minimised. Community groups and landholders can participate in agent redistribution by managing nursery sites and coordinating agent collection and redistribution.

Cages may be used at nursery sites to contain insect agents within an area and expedite the harvesting process. If not managed properly however, cages can adversely affect the survival of the agent population as the agent may completely consume or destroy the host plant supply within the cage.

When developing and implementing an agent redistribution program, the following key points should be considered:

- When selecting locations to set up nursery sites, ensure that the sites will be easily accessible for future redistribution activities.
- If the nursery site is to be located on private land, ensure that the landholder agrees to public access to the property on agent-harvesting field days.
- Monitor the nursery site periodically to determine when it is ready for harvesting. In general, a nursery site is ready when large quantities of the agent can be collected in a relatively short period of time. Be careful not to deplete the agent population to critically low levels.
- Determine which life stage of the agent is the most easily identified and practical to harvest, transport and release. Stages of the agent that are the most robust and able to survive for up to two or three days before being released are favoured. Use best practices when making the releases.
- If caging the nursery site, it will be necessary to remove the cage before the weed supply is exhausted or to use a large enough cage to sustain the insect agent population.
- Consider potential biosecurity risks when developing a redistribution technique. For example, plant material collected from nursery sites may contain other weed seeds or plant pathogens and pests, or the agent may be parasitised. Ensure good hygiene practices are followed, such as the cleaning of vehicles to remove weed seeds and minimising the release of agents with field-collected plant material. It may be necessary to separate the agent from plant material prior to release, or to inspect the harvested, infested foliage for any signs of unwanted contaminants.
- The transport of agents over long distances may expose them to other natural enemies and different environmental conditions that may require re-evaluation of the release strategy at the new locations.

Getting communities involved

Community groups, especially those involved in natural resource management, can greatly assist in the release of agents. They can help in finding suitable release sites, erecting fencing or release cages, conducting releases, monitoring agent establishment and redistributing agents from established sites. In some programs, schools have taken on the rearing of agents in the classroom and supply agents to the local community.

To involve the community and landholders effectively in agent releases, it is imperative that participants are given adequate training and supporting

literature that clearly and simply explains the techniques and processes involved, including managing sites. Training provided through practical displays, such as demonstrating agent release techniques in a field situation, has proven to be highly successful. When community members receive hands-on experience, they often feel more confident to continue without much further assistance.

The benefits of community participation can be enormous as potentially many more releases can be conducted, thereby speeding up the delivery of biological control to end users. In addition, the community gains a greater understanding of biological control and a sense of ownership of the program through direct involvement in its implementation.

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Example of getting communities involved

An extensive community-based approach has been implemented in Australia to fast-track the release and redistribution of two agents, a leafhopper (*Zygina* sp.) and a rust fungus (*Puccinia myrsiphylli*), for the environmental weed, bridal creeper (*Asparagus asparagoides*). The program involves biological control practitioners establishing distribution networks consisting of community groups, weed officers, national park rangers and schools. Extensive training and resource materials are provided to network participants during field days. At these training days, participants are instructed on how to select suitable release sites, and shown how to conduct a release and the signs to look for to determine if the agents have established. From these 'nursery sites', community groups can then repeatedly harvest agents, enabling further releases to be conducted in the local area. Hundreds of schools across Australia provide community groups with supplies of agents reared in cages within their classrooms. A dedicated website provides information, as well as a comprehensive map of where agents have been released (http://www.ento.csiro.au/weeds/bridalcreeper/index.html).

The Weed Warriors program: involving school students in rearing and releasing biological control agents for bridal creeper.
Photo: Raelene Kwong

Key papers and further reading

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Optimal release strategy for gorse thrips in Australia

The weed and agents

Gorse (*Ulex europaeus*), a native of Europe, is a leguminous, perennial shrub with narrow, spine-like leaves and yellow flowers and is a serious agricultural and environmental weed. Management costs for gorse in agriculture and forestry across Australia has been estimated at $7 million annually. The Australian biological control program for gorse has resulted in the release of the gorse seed weevil (*Exapion ulicis*) in 1939, the gorse spider mite (*Tetranychus lintearius*) in 1998, the gorse thrip (*Sericothrips staphylinus*) in 2001 and the gorse soft-shoot moth (*Agonopterix umbellana*) in 2007. This case study presents investigations to determine an optimal release strategy for gorse thrips.

Close-up of a gorse thrip (*Sericothrips staphylinus*).
Photo: Wade Chatterton

Rearing

Culturing gorse thrips is easy under glasshouse conditions, providing a supply of potted gorse plants is available. In this program the thrips were cultured on potted gorse plants produced from 15 cm cuttings. When plants were about 30 cm high and 20 cm in diameter they were placed in rearing cages in a glasshouse held at approximately 20ºC – 22ºC. A minimum of 200 thrips were then placed on each plant. As densities increased, additional plants were added to the rearing cages to enable the thrips to transfer onto fresh foliage. When the mean population density recorded was about 8 thrips / cm (range 6–13) of new growth, the cultures were harvested. Adult thrips were collected for field release by dislodgment from the plants into a white tray. This was done by lightly beating the sides of the plants. They were then collected using a pooter attached to a vacuum pump into 8 cm x 2.7 cm (height x diameter) plastic tubes for transportation to field sites. The first releases from the glasshouse culture were conducted in Tasmania in January 2001. This culture has also been used to provide stock for field releases in Victoria, New South Wales and South Australia. To avoid excessive inbreeding, the cultures have been replenished annually by thrips collected from field sites and the old cultures destroyed.

Mass rearing of gorse thrips (*Sericothrips staphylinus*) on plants in the glasshouse.
Photo: John Ireson

Optimal release strategy

To determine the optimal release strategy for Australian conditions, a trial similar to that conducted by Memmott et al (1998) in New Zealand was repeated at Stonehenge in the eastern midlands of Tasmania. No thrips had been released in the vicinity of this site prior to this trial.

Thirty-four small gorse bushes were selected at random (average size 92.5 cm x 79.4 cm x 57.7cm; length x breadth x height). In November 2002, thrips were collected from the glasshouse culture and released in replicated release sizes of 10, 30, 90, 270 and 810 individuals per bush. Eight replicates were used for the 10, 30 and 90 release sizes and six and four replicates respectively were used for the 270 and 810 release sizes.

The plants were sampled in January 2004. The seasonal span of the trial (14 months) would have enabled the thrips to enter their third field generation by the time sampling commenced (Ireson et al 2008a). The foliage of each bush was completely removed and the thrips extracted using the methods detailed by Ireson et al (2008b).

In New Zealand, Memmott et al (1998) demonstrated a significant constant relationship between the number of gorse thrips released and the number recovered 1 year after release. The Tasmanian study showed that recovery rates for gorse thrips could be non-constant, with numbers recovered increasing with release size up to a point beyond which there was no increase.

The New Zealand study suggested that the optimum release size that maximises the average number of successful establishments for thrips might be fewer than 100 adults. The Tasmanian study showed that a release of only 10 thrips could achieve establishment. The results of both trials however, were based on only 1 year, so it is possible that more of the populations produced from this small release size could become extinct as the post-release time increases, compared to those produced from a larger release size. The optimal release strategy is a trade-off between release size and the number of releases. A minimum number of 250 adult thrips (sex ratio approximately five females: two males) was chosen for release as it was also the estimated release size to produce maximum population growth. Furthermore, even if establishment is achieved through releasing a smaller number of adults per site, the high production level from cultures and ease of collection, alleviates the need to consider smaller releases if the higher number improves the probability of establishment and increases population growth rates. For instance, it takes only 5 minutes to collect 250 thrips from a collection tray into which several thousand have been beaten from caged plants containing high densities.

Survey results show that newly establishing populations of gorse thrips are slow to increase and disperse, and can be difficult to find when numbers are low. Consequently, post-release surveys may not record an establishing population in the early stages of growth if the release number is too small, resulting in an incorrect assumption of population failure. To avoid this, establishment surveys should be carried out several years after the initial release, or over a period of several years, to give time for the establishing populations to increase to a detectable level.

In Tasmania, field dispersal ranged from less than 1 m to around 250 m, 3 to 6 years post release at the 35 sites surveyed in 2007. Therefore, a large number of releases of approximately 250 adult thrips as opposed to a smaller number of larger releases was considered the best strategy to counter the possible loss of some sites over time (eg through fire events) and maximise geographical coverage. Gorse thrips from glasshouse cultures were released at over 400 sites in Tasmania by the end of 2007.

Surveys in 2008 indicated thrips established at 90% of these sites. Field densities of gorse thrips in Tasmania however, are currently low (<1 thrip per cm of tip growth) so it is impractical to collect and redistribute populations from field sites at present. The ideal field nursery site would be one where the population density is high enough to enable multiple batches of around 250 thrips to be easily collected. The release of numbers lower than 250 for field establishment is an option if field densities remain low once supplies from glasshouse cultures are no longer available.

The release strategy also accounts for the genetic variation that exists within and between populations of gorse. Trials have shown that populations of gorse thrips can develop significantly higher populations on some plants than others, due to the genetic variability between each plant. Such differences in plant suitability could have a major impact on the gorse thrips because it is a sedentary species with poor dispersal abilities. The strategy of making a large number of small releases of the gorse thrips as opposed to a small number of large releases increases the chance of establishment by reducing the effect of genetic variability of gorse.

The life cycle of the gorse thrips is closely synchronised with the phenology of gorse. Once new gorse shoots have matured and hardened by the end of summer, the adult population has entered a reproductive diapause. Over-wintering adults commence egg-laying towards the end of winter. Eggs commence hatching in spring at the time new succulent shoot growth is available as a prime food source. The release of adult egg-laying thrips in early spring is therefore the optimum time to achieve high establishment rates.

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Optimal release strategy for agents targeting lantana

The weed and its biological control program

Lantana (*Lantana camara*) is native to tropical America and was introduced into Europe in the 16th century and bred in glasshouses for several centuries prior to its introduction into Australia and other countries in the mid 1800s. As a result of crossbreeding in glasshouses, there are over 600 varieties of lantana reported worldwide and about 30 present in Australia.

In Australia, lantana is found from the Torres Strait Islands south to the Victorian border, with isolated infestations in the Northern Territory and Western Australia. It occupies a wide range of eco-climatic areas from sea level to altitudes up to 1,100 m and from areas receiving 400 mm annual rainfall to those receiving over 4,000 mm rainfall. It is found on hot, exposed slopes as well as being the dominant understorey species in forests. The Australian lantana varieties appear to be most similar to those from Mexico and the Caribbean.

Of the 31 agents which have been released since a biological control program began in 1914, only 17 (55%) have established. Four of the established agents are exerting some control on the weed.

Exploratory phase and agent selection

The diverse forms and range of lantana means that candidate agents collected from the native range are not necessarily adapted to lantana in the introduced range particularly across all eco-climatic areas. Therefore, research on candidate agents needs to include varietal preference and eco-climatic matching as well as the usual research investigating the biology and ecology of the agent. As expected, several agents show distinct preferences for some lantana varieties over others. For example, only the pink-flowering lantana is susceptible to the rust fungus (*Prospodium tuberculatum*), while the mirid bug (*Falconia intermedia*) and the tingid bug (*Teleonemia scrupulosa*) tend not to attack this form.

The lantana adult mirid bug (*Falconia intermedia*). Photo: Jeff Wright

Mass-rearing

To ensure there are adequate numbers to conduct field releases of agents, mass-rearing cultures need to be set up appropriately. Plants used in rearing need to be healthy, pest free and the appropriate variety for the agent. For many leaf-feeding insects, plants need to be well watered and fertilised so that they have lush leaves. Old, small or hardened leaves are often avoided by adult insects for oviposition and first instar larvae often have trouble feeding on tough leaves. For example, the mirid bug tends not to oviposit on lantana leaves infested with red spider mite and the herring-bone leaf miner (*Ophiomyia camarae*) performs much better on plants with bigger, healthier leaves.

Site selection

Site selection is important because not all agents have the same requirements. The tingid bug, although present from Cairns to Sydney, tends to prefer drier areas and is found in larger numbers on exposed northern slopes while being absent or in low numbers on nearby southern slopes or where lantana is growing under canopy. A leaf sucker agent (*Leptobyrsa decora)* has similar requirements to the tingid bug but is more restricted in its distribution in far north Queensland, being only found on the Atherton Tableland. Likewise, the hispine leaf-mining beetle (*Uroplata fulvopustulata*) has only established in far north Queensland, despite widespread releases.

Rearing cages for the mirid bug (*Falconia intermedia*). Photo: Michael Day

CLIMEX models have been used to assist with area selection of release sites for particular agents. These models are developed using climatic data from the native range of the agent and matching these areas to similar conditions in the country of introduction. Models can also be based on phenological data. The usefulness of models however, depends on the quality of data available to develop them; eg models for the tingid bug, which were based on good quality data, predicted where it actually established in Australia better than the models developed for the lantana leaf-mining beetle (*Octotoma scabripennis*) where the data was not as good.

Sites may vary in quality during the year, being influenced by weather patterns or seasonal conditions and these need to be matched to the agent. For instance, some sites may be lush following rain but during the dry winter months plants can become stressed and leafless hindering the establishment or persistence of populations. For example, the tingid bug prefers open, exposed slopes while the herring-bone leaf miner prefers shaded areas where lantana grows under canopy and humidity is higher. During the drought that gripped eastern Australia recently, the rust fungus was released only in protected gullies which were able to retain moisture. Through careful selection of release sites, initial establishment of the rust increased from 20% to 63%.

Specific site selection may also need some social considerations. Lantana is commonly found on the edge of roads but these may not be suitable sites for release due to road maintenance. Sites are targeted where other controls or disturbances are minimal. Release sites on private properties need to be protected against clearing or grazing by livestock.

Releasing the mirid bug (*Falconia intermedia*) on lantana, north of Brisbane.
Photo: Michael Day

Release size and stage

The optimal number of individuals to release can vary with the agent and the life stage being released. For the mirid bug, a mixture of adults and nymphs were released and establishment was achieved if about 4,000 individuals were released at any one time. Establishment of the lantana tree hopper (*Aconophora compressa*) was achieved using release sizes of 250–500 individuals. Establishment of the herring-bone leaf miner in South Africa has been successful from release of harvested leaves containing larvae. In Australia, trials are currently underway releasing both adults and mined leaves in climatically suitable areas.

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Release and establishment of rust strains to enhance biological control of European blackberry

The weed

European blackberry (*Rubus fruticosus* agg.) is one of the most important weeds of southern Australia, infesting almost 9 million hectares. In Australia, the aggregate comprises at least 15 species and several different genotypes.

In natural ecosystems, dense infestations reduce biodiversity and wildlife habitat as well as the conservation value of public lands, parks and reserves. In agricultural areas, blackberry thickets replace pasture and exclude livestock. The weed also causes access problems in forests and reduces timber production by competition and preventing natural regeneration.

The agent

The leaf-rust fungus (*Phragmidium violaceum*) that attacks European blackberry is a biotrophic pathogen, which depends on living tissue for development. It is most damaging to young, emerging leaves of blackberry during active shoot growth, but causes limited or no disease symptoms on old leaves. Severely infected leaves have reduced photosynthesis efficiency and fall prematurely. Wind-dispersed spores repetitively produced during the growing season are responsible for epidemics. The rust overwinters on infected plant debris.

The rust is already present in Australia, having been introduced on at least two occasions: an unauthorised introduction of an uncharacterised population in the mid 1980s and authorised releases of a specific strain in 1991 and 1992. Since its introduction, the rust has provided useful control of blackberry in areas with climatic conditions optimal for disease development, but its effectiveness has been limited due to resistance of some blackberry genotypes.

Eight additional strains of the rust, sourced from France, were approved for release in 2004 to increase the genetic diversity and hence potential of this agent. These strains are genetically different to the existing population of the rust in Australia and are capable of infecting the full range of blackberry taxa present. Multiple strains are released simultaneously at any one site, to allow natural selection to take place and the fittest rust genotypes to emerge. The genotypes that will eventually become dominant at each site will vary over time depending on the underlying genetic structure of the blackberry population or other environmental conditions.

A blackberry leaf infected by the leaf-rust fungus (*Phragmidium violaceum*).
Photo: CSIRO

Large-scale release program

At the onset of the program, an 'Expression of Interest' process was undertaken with communities affected by blackberry, to identify suitable release sites for the additional strains. Sites with extensive and dense stands of actively growing blackberry, located in full sun or with limited tree canopy, and near a water course, are given priority for release. Blackberry plants growing under such conditions continuously produce new leaves, which are most susceptible to infection. Sites located in high rainfall regions (> 800mm) with cool summer temperatures (approximately 20°C maximum daily temperature) provide the best climatic conditions for severe rust epidemics to occur.

The strains are mass-produced separately on detached young blackberry leaves contained in Petri dishes under controlled conditions. Spores are collected and then directly used for releases, stored in a fridge for up to 2 weeks or placed in a freezer (-80°C) for long-term storage and later use. Release kits containing guidelines, spores of each strain, a spray bottle and other necessary material are sent or given to participants during field days.

Releases are performed by participants in mid to late spring. Spores of each strain are suspended in water and sprayed onto the under-surface of young blackberry leaves at the end of the day to avoid hot conditions. Inoculated portions of canes are misted with water and covered with plastic

Spores of the blackberry leaf-rust fungus (*Phragmidium violaceum*) are mass-produced on detached leaves contained in Petri dishes.
Photo: CSIRO

bags to retain moisture during the initial phase of the infection process.

The program is greatly benefiting from the participation of land managers, which facilitates the release of the strains at a large number of sites across the entire range of blackberry.

Confirming establishment and persistence

Presence of rust symptoms on inoculated leaves within 3–4 weeks after release of the additional strains has been reported by most participants, demonstrating effectiveness of the release technique. Molecular tools, however, are required to monitor establishment and persistence of these particular strains beyond the growing season of release because they cannot be distinguished morphologically from the existing population of the rust. The strains can also readily cross with other individuals of the population after the overwintering period and thus are not maintained as unique genetic entities over time. To overcome these hurdles, microsatellite DNA markers were developed and are being used to monitor the incorporation of genes unique to the strains, into the gene pool of the field population of the rust. The intense sampling and time required for sample processing preclude the use of these molecular markers across all release sites and therefore monitoring is only undertaken at a small number of representative release sites.

Sampling the blackberry leaf-rust fungus (*Phragmidium violaceum*) at a release site to confirm establishment of additional strains using molecular tools.
Photo: CSIRO

There is no single solution to deal with the blackberry problem in Australia. Consequently any improvement of biological control through the release of additional strains of the leaf-rust fungus is most welcome, particularly at sites where implementation of other control methods is inappropriate or impractical.

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Rearing and release of *Agonosoma trilineatum* for the biological control of bellyache bush

The weed

Bellyache bush (*Jatropha gossypiifolia*) is an aggressive, deciduous shrub, well adapted to the wet / dry climate of northern Australia where it has become widely distributed in the Northern Territory and Queensland. In Queensland, it is a major weed of riparian areas of the Burdekin, Walsh, Palmer, Flinders and Gregory River systems. Its range has expanded in Queensland during recent years. All parts of the plant are toxic, although medicinal properties have also been documented. In northern Queensland, fruiting is primarily rainfall dependent. Seed production commences around September / October and may continue for up to 10 months.

Agonosoma (*Agonosoma trilineatum*), a seed-feeding biocontrol agent for bellyache bush.
Photo: Tim Heard

The agent

Several candidate agents were identified in native range surveys. Of these, the seed-feeding agonosoma (*Agonosoma trilineatum*), collected from Venezuela and Curacao in 1999, was the first agent approved for release in Australia. Initial studies showed that its lifecycle takes approximately 9 weeks from egg to adult with five nymphal instars. Adults live for approximately 9 weeks, their feeding completely destroying the seeds of plants. Development of both nymphs and adults is dependent on seed availability.

Rearing for release

The Berrimah Research Station in Darwin and the Tropical Weeds Research Centre in Charters Towers received shipments of agonosoma in early 2003. The insect was initially container-reared on cut foliage with seed capsules in a laboratory and later on cut foliage in glasshouse cages.

A trial using potted plants in glasshouse cages was unsuccessful. The plants developed powdery mildew and lost leaves. Plants of a size suitable for cages did not have sufficient seeds for many insects. Consequently, insects required regular addition of fresh plants or supplementary seed to complete development. Attempts to find a suitable artificial diet mix for rearing were also unsuccessful.

Fruiting tips were collected from the plants grown at the research centre or from field sites near Charters Towers. Seed from both sources was scarce during the cooler, dry months of June, July and August, reducing the number of insects that could be maintained during this period. In general, difficulties in finding seed during winter, combined with time required to collect and replace cut stems and seeds within cages, restricted the number of insects that could be reared. The majority of releases took place from November onwards after colony numbers increased following the end of the dry season.

Agonosoma (*Agonosoma trilineatum*) adults in a glasshouse rearing cage.
Photo: Kelli Pukallus

The release strategy

A strategy trialing of three different release sizes (small, medium and large) was adopted in June 2003. Release sizes were originally based on the minimum monthly colony production, with two sites receiving a single release of 250–300 adults, two sites receiving two to three consecutive releases of 250–300 adults (total 750–1,000 adults) and two sites receiving multiple consecutive releases of 250–300 adults (total over 2,000 adults). Releases were made as insects became available from the colony. The majority of insects were released as mated adults directly onto plants.

Between June 2003 and July 2004, 19 releases were made at these six sites, with a single release at a seventh site. The target of 2000+ adults released was achieved at only one site, 'Mt Ravenswood' on the Burdekin River. There were no signs of insect establishment at six of the seven release sites. Small numbers of insects were found at 'Mt Ravenswood' in January

2004 but not later in the season, despite further releases in the same area. A strategy of multiple, consecutive releases at fewer sites was therefore adopted.

Between January and July 2005, further releases were made at 'Mt Ravenswood'. Small numbers of adults and nymphs, with feeding damage on seed capsules, were seen in February 2005. However, no signs of insect establishment were found on subsequent inspections in April and May 2005. Extremely dry conditions had reduced the amount of seed at all field sites.

Releases recommenced in November 2005 at one site near Charters Towers and one site in far north Queensland on the Walsh River system. Small numbers of both adults and nymphs were seen near Charters Towers in December 2005 and May 2006 and on the Walsh River system after Cyclone Larry in March 2006 but subsequent inspections later in the dry season failed to find insects.

Agonosoma (*Agonosoma trilineatum*) in a field cage near Charters Towers, November 2005. Photo: Kelli Pukallus

Releases during the remainder of 2006 and in 2007 were concentrated near the mouth of the Burdekin River, identified as the most climatically suited for the insect, and along the Palmer River in far north Queensland. Adults and nymphs were seen in March 2007 at one coastal site and in December 2007 at a second site but it is unknown if these populations will persist.

Field cages were also used unsuccessfully at two release sites in an attempt to promote establishment. Adults were released into 1.5 m x 1.5 m x 2 m gauze covered cages over four to six plants and the cages inspected for oviposition after 1–2 weeks. Unfortunately placement of egg batches was affected, with many laid on cage walls rather than on plants.

Most release sites were inspected for signs of insect establishment within 1–4 weeks of initial release and then at 2–3 monthly intervals. Remote sites on the Walsh and Palmer River systems in far north Queensland were inspected opportunistically by local stakeholders and research centre staff when possible.

Problems with establishment

Current indications are that agonosoma has failed to establish in north Queensland despite small numbers of adults and nymphs being seen post release at some sites, including those identified as most climatically suitable by a bioclimatic model. Reasons for this failure are unclear, although the phenology of bellyache bush in north Queensland has probably been a contributing factor. Agonosoma has a relatively long lifecycle and feeds exclusively on the seed of bellyache bush, so a lack of seed during the dry season restricted the number of insects that could be reared for release and affected adult survival in the field. The majority of individual releases consisted of 500 adults or fewer and there were periods in each year when no releases could be made. Of the 82 releases made in Queensland between June 2003 and December 2007 only four comprised 1,000 or more adults.

Ongoing attempts

Final releases of agonosoma were made at the end of 2007 and further inspections for signs of establishment are planned for early 2008 and the summer of 2009.

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