MASS REARING AND RELEASING OF BIOLOGICAL CONTROL AGENTS FOR WEEDS. WHAT'S IT ALL ABOUT?

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ABSTRACT

The process of mass rearing and releasing biological control agents involves mass producing and releasing approved post-risk analysis introduced agents, at facilities such as Queensland's Department of Primary Industries (QDPI) Tropical Weeds Research Centre (TWRC) in Charters Towers. The purpose of a program is to disseminate these agents, achieve establishment and spread, whilst impacting invasive weed species. This involves a web of interconnecting factors.

This paper will discuss the processes, challenges and considerations used at TWRC to mass rear and release biological control agents for the management of targeted invasive weed species for northern Australia over the last 20 years.

Key words: Tropical Weeds Research Centre, invasive weeds, northern Australia, Queensland.

INTRODUCTION

Mass rearing of biological control agents for control of invasive weeds in Australia started in 1903. Since then, over 270 agents have been released to focus on invasive weed control within Australia (Cullen *et al*. 2023). Queensland's Department of Primary Industries has been involved in mass rearing since the beginning. In 1985, Tropical Weeds Research Centre (TWRC) in Charters Towers opened, to enable weed research and mass rearing of biological control agents for northern Queensland and Australia. Since then, 37 agents have been mass reared and released from the facility to target 11 invasive weed species using funding from the Land Protection Fund or external funding bodies. Over the years the numbers of agents being mass reared have varied, with 23 species between 1985-1994 (Pukallus, 2016), and 10 agents in the last 20 years (Table 1). This paper will focus on the last 10 agents and the processes used at TWRC to develop a successful biological control mass rear and release program.

INFRASTRUCTURE

TWRC infrastructure includes three 12m x 9m compartmentalised glasshouses (Figure 1a). Each compartment is evaporatively cooled, has a heating system, louvred windows, automatic roof vents, overhead misting system and fluorescent lights. Inside conditions are thermostatically regulated to achieve a temperature range of 22°C - 27°C.

TWRC also has numerous enclosed and open shade houses (19m x 9m) with overhead irrigation and concrete flooring. Both the glasshouses and shade houses are covered with 70% shade cloth, with the glasshouse's being retractable. Galvanised

metal benches (1.8-2.4m x 0.6-0.9m) are used to hold the rearing cages or plants (Figure 1b) within both structures.

Figure 1: TWRC glasshouse and shade house (a-left), *Lantana camara* plants on glasshouse benches (b-centre), rearing cage (c-right).

MASS REARING

Host plants

Host plant material is needed as a constant food source for agents. This process starts six months before the agent arrives to ensure plants are at their optimum life stage and growth for colony requirements. Seeds, plant material or plants are sourced from various locations to ensure varietal and genetic diversity is achieved. 400-500 of each plant species are used in rotation. Eaten or defoliated plants are removed from rearing cages weekly and are cut back severely as shown in Biosecurity Queensland[b] (2018). The plants are fertilised, insecticide and fungicide treated and allowed to regrow, ready for use later. All plants are invasive weeds species and are kept under a prohibited or restricted matter permit to meet our legal obligations under the *Biosecurity Act 2014*.

Basic horticultural knowledge is required to ensure TWRC plants are in ideal life stage for each agent, e.g. flowering and seed producing plants (*Agonosoma trilineatum*). Field collected plant material e.g. *Opuntia species* stems and pads for *Lagocheirus funestus* have also been used (Figure 2a). Artificial diet has been used minimally over the years at TWRC.

Maintaining clean host plants, free from pathogens and pests, is a constant battle. Use of insecticides or fungicides can compromise the colony, so natural predators such as ladybeetles or spraying with white oil, a less toxic product are used. Occasionally systemic insecticides are required, and plants are isolated for longer before returning into use.

Rearing Techniques

Mass rearing requires constantly producing agents on a larger production scale, efficiently and economically. The average duration for a program is three-four years (Table 1), but program times can vary depending on funding provisions. This time frame encompasses mass rearing, releasing, monitoring and reporting. The duration of at least three years, allows for climatic and seasonal variances, which could either benefit or impede field establishment.

Table 1: Biological control agents mass reared at Tropical Weeds Research Centre 2003-2025.

Agent	Target weed species	Program years	Established achieved
Agonosoma trilineatum	*Jatropha gossypiifolia*	2003-2007	no
Cometaster pyrula	*Vachellia nilotica*	2004-2007	no
Ophiomyia camarae	*Lantana camara*	2008-2012	yes
Aceria lantanae	*Lantana camara*	2014-2017	yes
Eueupithecia cisplatensis	*Parkinsonia aculeata*	2013-2018	yes
Eueupithecia vollonoides	*Parkinsonia aculeata*	2019-2024	yes
Cecidochares connexa	*Chromolaena odorata*	2019-2024	yes
Acaciothrips ebneri	*Vachellia nilotica*	2023-current	yes
Calligrapha syn. Zygogramma bicolorata	*Parthenium hysterophorus*	2023-2025	*redistribute
Lagocheirus funestus	*Opuntia species*	2024-current	*redistribute

The main rearing technique is housing host plants or plant material and agents in gauzed rearing cages on benches in the glasshouses. Cages are H90cm x W87cm x L80cm with two outward opening doors, a solid aluminium base and the sides and roof fitted with interchangeable polyester gauze material (Figure 1c). Cages are maintained to prevent escapees and cleaned daily and wiped with methylated spirits, to minimise disease and fungal build (Biosecurity Queensland[b] 2018). *Bacillus* fungus can build up quickly in the rearing cages from insect excrement and moisture and kill a colony, especially in the larval stage (*Cometaster pyrula* or *Eueupithecia sp.)*. Any infected insects are removed immediately from cages to prevent disease spread. Once a cage use is finished, they and any associated equipment are washed and sterilised with chlorine. To additionally minimise disease or colony collapses, colonies are spread between glasshouses. Another rearing methodology involves housing plants and agents on open benches which are harvested directly (*Aceria lantanae)*.

Mass rearing continues all year round with summer and spring being the largest production times, due to the warmer weather creating shorter insect lifecycles and host plants growing quicker.

Agents

Exactly when TWRC receives a new agent, is determined by the prior rigorous host specificity testing and the risk analysis approval process through Australian Government's Department of Agriculture, Fisheries and Forestry (DAFF 2025). The completion and approval time can vary. The number of agents being mass reared concurrently at TWRC can vary from one to three agents (Table 1). Agents may be in different stages of their programs, which allows overlap of facility requirements. Agents can also be in redistribute stage, as a follow up from a previous program to supply new areas or increase field numbers (*Calligrapha bicolorata* and *L. funestus*) (Table 1).

Knowledge of agent biology is gained from host specificity reports, literature reviews or talking with host-testing researchers. From this information we can determine lifecycle timeframes, glasshouse setup and rearing requirements for the new agent.

Lifecycle timeframes can range from months (*L. funestus),* compared with days (*Acaciothrips ebneri)*. This is then the starting point for our mass rearing timelines and host plant requirements and determines the area of glasshouse space and rearing methodology.

The lifespan and fecundity levels of reproductive adults influence how many newly emerged adults are put into a cage or adult box to achieve an output of optimum egg numbers. If females lay eggs directly into the host plant material (*Cecidochares connexa* and *Ophiomyia camarae)* (Pukallus *et al.* 2022), adults are collected and placed directly into cages with host plants. Whereas, if they lay egg rafts directly onto the host plant material (*A. trilineatum),* they are placed into adult boxes (Figure 2b). Newly hatched juveniles are then collected and placed with host plants into rearing cages. The number of adults used can change throughout the program, due to agent shortage or increased knowledge on numbers required. Short-lived adults (a week), require the males and females to be collected quickly after emergence and put into cages or adult boxes to ensure maximum egg production.

Figure 2 Cut *Opuntia tomentosa* stems for *Lagocheirus funestus* rearing (a-left), adult box for *Agonosoma trilineatum* (b-centre), *Cecidochares connexa* packed for delivery (c-right).

We aim for a spread of life stages throughout the colony, to ensure a constant supply of agents for releases and to prevent a boom-bust situation at any stage. Males tend to emerge first within a colony and having a spread of development stages, allows equal numbers of males and females at any given time. A surplus of females isn't an issue, but a surplus of males does not sustain a colony.

Ensuring genetic diversity, as best as possible from one original colony source, is required. When emerged adults are collected for mating, they are mixed from different sources to ensure any genetic diversions are equalled e.g. smaller sizes, reduced fecundity. We have also reintroduced genetic diversity throughout projects by either a reintroduction of agents from their origin county (which is not always possible), reinput from field collected established agents (isolating first to ensure no parasites) or mixing genetics with other mass rear colonies throughout Australia (*C. connexa* and *A. trilineatum*) between Northern Territory and Queensland.

Throughout the mass rear process, voucher specimens of agents are kept for future genetic works, identification and displays or to record colony variations.

MASS RELEASING

Release strategies

As numbers start to build in the mass rear colonies, releases need to start without compromising colony continuation. We always need to have adults coming through to continue the colony, coupled with host plants or material and glasshouse space.

Determining how many agents are required and what life stage to release at a site is based on previous program successes or failures and agent knowledge. This is then coupled with how the agent will be packaged and transported. As the project develops, those numbers can change due to achieved establishment, colony numbers or packaging changes. Agents are usually sent in a Styrofoam box, with a cool brick to prevent overheating and to keep agents still (Figure 2c), and sometimes with a food supply (no reproductive material). This ensures they travel safely, efficiently and arrive healthy. Released life stages have included eggs (*Eueupithecia sp*.) and larval or nymph stages (*A. trilineatum, C. pyrula*, *Eueupithecia sp*.), but they are easily predated or desiccate quickly. Pupal stages (*Eueupithecia sp*. and *O. camarae*) are easily transported and packaged, allowing adult emergence to occur over a few weeks. Released adults (*C. bicolorata, L. funestus, C. connexa*), can initiate egg laying quickly to establish progeny in the field and can escape predation more easily.

Release field cages have been used for agents (*A. trilineatum*), which prevent predation and contain agents for ease of monitoring. Recent agent releases have been open field releases and have included release aids such as delta traps for housing trays of pupae (*Eueupithecia sp.*) or onion bags filled with agents in host plant material (*O. camarae* and *A. ebneri*). The ideal release site is based on the biology of the agent, dispersal habit of the agent and released life stage. If dispersal is by wind (*A. ebneri*), a release location on the edge of the infestation upwind is chosen, enabling the agent to be pushed into the infestation.

Release factors and considerations

Sites which provide actively growing host plants for long periods throughout the year and maintain stable climatic conditions are preferred as a starter area or nursery site. Nursery sites provide a concentrated release strategy focusing on a smaller area but releasing larger numbers of agents. Another option is spreading releases out to achieve a pocket of establishment across several locations, to understand better the ideal agent conditions. Sites that are all-weather and easily accessible, provide timely releases and access for monitoring. Establishment is occasionally hampered by unforeseen external factors, due to sites being destroyed by others, herbicide spray, slashing, branches broken or trees being knocked down.

Short-term extreme weather events can influence release schedules and site accessibility. Long-term weather events, such as drought periods, can influence chances of establishment (*A. trilineatum* and *C. pyrula*), compared to agents released

over wetter years (*C. connexa*) (Table 1). Seasonality also impacts host plant conditions in the field, requiring geographical options for agents throughout a program.

Releases are facilitated by an extensive collaboration of people including staff from QDPI, local and state government agencies, NR&M bodies and landholders. TWRC provides biological control agents for releases throughout northern Australia, encompassing Queensland, Northern Territory and Western Australia.

The use of spatial imagery and satellites for accurate GPS recording has developed over the last 20 years. Targeting release locations based on a combination of host plant location and data climate modelling programs in the initial stages and recording release and distribution data, has proved valuable to recording a program's success.

Records are kept throughout the program of rearing process, numbers and release information. TWRC develops information release sheets collect release site information, which is collated for reporting and mapping and provide release instructions. Instruction YouTube videos (Biosecurity Queensland[a] 2018) have also been created to aid with the release process.

CONCLUSION

The importance of biological control in managing weeds remains a priority for stakeholders across Queensland and Australia. With ongoing collaboration and an extensive understanding of the multifaceted factors required, Tropical Weeds Research Centre in Charters Towers is well-positioned to ensure the continuation of successful biological control mass rearing and release programs in the future.

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