First release and establishment of the biological control agent *Cecidochares connexa* for the management of *Chromolaena odorata* (L.) R.M. King & H. Rob (chromolaena) in Australia

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Summary *Chromolaena odorata* (L.) R.M. King & H. Rob (chromolaena or Siam weed) is a scrambling invasive shrub native to tropical America that significantly impacts terrestrial systems in Africa, SE Asia and, more recently, northern Australia. After its northern Queensland detection in 1994, *C. odorata* was part of a national cost-share eradication program until 2012 when eradication was deemed unfeasible. Anticipating the risk of *C. odorata* to the Australian environment, host testing commenced on the stem-galling fly *Cecidochares connexa* (Macquart), which was approved for release in 2018. This is Australia's first biological control agent for *Chromolaena odorata*.

Keywords: *Chromolaena odorata,* Siam weed, biological control agent, Australia's first, Queensland, Northern Territory.

INTRODUCTION

Chromolaena odorata (L.) R.M. King & H. Rob (chromolaena) was first detected in Australia, near Bingil Bay, Queensland (QLD) in 1994 (Waterhouse 1994) and subsequently in July 2019 in the Northern Territory (NT) (NT Govt. 2020).

Chromolaena is a fast-growing multi-stemmed perennial shrub of the Asteraceae family. Plants grow two to three metres unsupported, and up to 10 metres when supported by other vegetation (Zachariades *et al.* 2009). Chromolaena can form dense thickets that can prevent the movement of livestock in grazing lands, affect agricultural crops and plantations, permanently alter ecosystems and contribute to hotter fires which destroy native vegetation (Zachariades *et al.* 2009). Chromolaena can be toxic to cattle and stock (QDAF 2020).

Chromolaena reproduces vegetatively and via seeds which are produced following the peak flowering period of May to August in Australia. Seed dispersal occurs through wind, attachment to machinery, animals or clothing and the movement of plant material along watercourses (QDAF 2020).

Chromolaena was a target for national cost-share eradication in Australia until 2012, when eradication was no longer deemed feasible. Subsequently, chromolaena was identified as a target for biological control and the stem-galling fly *Cecidochares connexa* was selected as the most feasible agent. Host testing commenced at Ecosciences Precinct in Brisbane, QLD, by the QLD Department of Agriculture and Fisheries (QDAF) in 2012. In 2018 release of *C. connexa* in Australia was approved by the Australian Government Department of Agriculture and Water Resources. The overall likelihood of off-target effects and potential consequences associated with the release of *C. connexa* was determined as being negligible (Australian Government Department of Agriculture and Water Resources 2018).

C. connexa was first released as a biocontrol agent in Indonesia in 1995 (McFadyen *et al*. 2003). It has since been released or detected in 11 countries in Africa, the Americas, Asia, and Oceania (Winston *et al*. 2014, Day *et al*. 2016). Mass-rearing commenced in 2019 at QDAF's Tropical Weeds Research Centre (TWRC) in Charters Towers, QLD. Shortly after, a collaborative breeding program was set up between QDAF and the NT Department of Environment, Parks and Water Security (NTDEPWS) Weed Management Branch. Releases began in both QLD and the NT in November 2019. This paper reports on the mass-rearing and release program of *C. connexa* in Australia.

AGENT BIOLOGY

C. connexa is a small stem-galling fly (Diptera: Tephritidae) native to central America (McFadyen *et al.* 2003). Adults are between three and five millimetres long and females can be distinguished from males by the presence of an ovipositor at the end of the abdomen (Figure 1). Females use their ovipositor to deposit eggs into the stem tips (growing points) of chromolaena plants. Once these eggs hatch, the larvae feed on plant material inside the stem. Over the next 30-45 days, galls form around the larvae and these galls act as nutrient sinks, limiting

the plant's ability to flower and produce seed, reducing its reproductive potential.

One to ten adults can emerge from the gall depending on the size of the gall and number of eggs laid. Emergence is through tunnels created prior to pupation ending with an emergence 'window' (Figure 2). The lifecycle from egg to adult takes approximately 50-80 days depending on climatic conditions. During hotter and wetter conditions, the lifecycle duration decreases and increases during cooler conditions.

Figure 1. Female *C. connexa* (left) have a black abdomen and ovipositor, males (right) have a brown abdomen and no ovipositor.

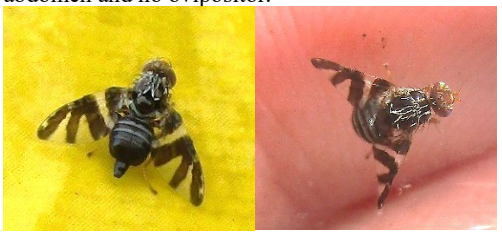


Figure 2. Multiple emergence 'windows' on glasshouse plant gall (left). Female *C. connexa* recently emerged from an emergence hole formed in a mature gall within an established release site (right).

REARING PROCEDURE

At the primary breeding facility in QLD, TWRC, adult flies were collected following their emergence from galled stems in holding cages. Six to eight adult flies of each sex were placed in rearing cages containing three 200mm potted multi-stemmed chromolaena plants (Figure 3). These rearing cages were kept in temperature-controlled glasshouses where temperatures ranged from 27°C day to 22°C night and with natural lighting.

Figure 3. *Chromolaena odorata* plants in a rearing cage at TWRC.

In the NT similar female:male and plant ratios were used, however the rearing cages were housed in an outdoor shaded area exposed to natural temperatures throughout the year ranging from 38°C day to 15°C night.

Three weeks after cage set-up date, the plants were removed from the cages and cages were washed and sterilised with chlorine solution. Each plant was labelled, and the plants continued to grow and develop galls over the following six to eight weeks. Leaves were removed periodically to assist with pest control and improve gall detection.

Once emergence windows appeared, galls were collected by cutting stems approximately 10-15cm below the gall. All remaining green leaf material was removed, and any pests hosed off. Galled stems were placed in water filled glass jars within galled stem holding cages. Most flies emerged during the first few weeks following collection. Stems were kept watered for approximately one month until they turned brown and dried off. Flies emerged from the dried stems in the months following, but in smaller numbers. Several holding cages were used concurrently, with different collection dates, ensuring a consistent emergence of flies.

RELEASES

Two methods were used for releasing *C. connexa*: galled stems or adults. Releases in QLD started with galled stems and then moved to adult releases as the main method. Adults were easier to send and release, and oviposition started immediately once released. Releases in the NT began with adults sent over from QLD and then moved into a combined adult/gall release system once the local colony became sustainable.

Suitable release sites need to have actively growing plants, with new shoots and limited flowers or seeds, in full or partial sun. Initial sites were targeted based on eco-climatic suitability modelling for *C. connexa* in QLD (Day *et al*. 2016). Releases

were conducted on at least a 20m x 20m area containing a minimum of 20 larger chromolaena plants, spread relatively evenly across the site. Sites adjoining another chromolaena infestation were preferred for establishment and to increase spread potential. In the NT, a single suitable release site in the core of the infestation was used in the first year. The release site was also chosen as it was likely to be accessible during monsoonal rains.

Releases have taken place on private property, national parks and reserves, Defence land, local government and state land, in roadside verges, forestry plantations, quarries, riparian areas, open paddocks, gullies, hillsides, and rocky outcrops. Stems or flies were overnight couriered to clients for release or released directly by QDAF or NTDEPWS staff.

Releases from November 2019 to March 2022 (28 months), totalled 27,534 flies and 3,357 galls in QLD and 1,319 flies and 2,982 galls in the NT (Table 1). The total number of release sites for Australia is 114. This encompasses seven Local Government Areas in QLD and the Western Top End region in NT (Figure 4).

Table 1. Release data of *C. connexa* from November 2019 to March 2022 in Australia, showing release numbers and locations within each state.

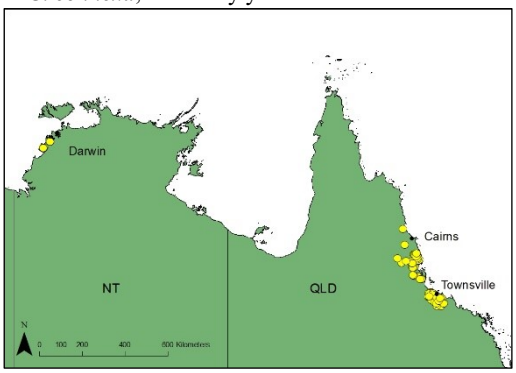
Local Government Area or location	Number of release sites	Number of adults/galls released	Number of sites with galls present/ monitored
QLD			
Burdekin	5	1,864/0	4/5
Cassowary Coast	43	7,814/ 2,510	20/30
Charters Towers	10	2,127/ 110	6/6
Douglas	1	0/101	1/1
Hinchinbrook	9	994/636	4/4
Tablelands	5	1,754/0	2/3
Townsville City	39	12,981/0	23/28
Total	**112**	**27,534/ 3,357**	**60/77**
NT			
Western Top End	2	1,319/ 2,982	2/2
Total	**2**	**1,319/ 2,982**	**2/2**

Galled stems

In QLD, bundles of 40 to 50 stems were gathered and bound with a rubber band towards the base of the stems. Ends were trimmed to a uniform length and inserted through the lid of biodegradable coffee cup. A concentrated mixture of water crystals forming a slurry was placed in the bottom 1/3rd of the coffee cup. Once positioned in the field at the base of chromolaena plants, 5-6m apart, the coffee cups were filled with water. Refilling was sometimes required at weekly intervals. In the NT, bundles of 15 to 30 stems were placed in water-filled 100ml solid plastic containers with sponge around the top. Up to 16 containers were placed in an eight litre plastic bucket with drainage holes drilled in the base. The buckets were hung approximately one metre above ground level and in a shady location within the chromolaena site. The buckets were collected during later monitoring visits.

The flies emerged gradually over the following few weeks. The number of galled stems per release site ranged from 31 - 405 in QLD and 56 – 344 in NT.

Figure 4. Release locations in Northern Australia of *C. connexa*, shown by yellow dots.



Adults

Flies were collected from glasshouse galled stem holding cages over four to five days and placed into 250ml round plastic containers. Large holes in the lids and gauze allowed air into the containers and prevented flies escaping. Approximately 20 females and 20 males were placed in each container, along with pieces of moistened paper towel. Flies were released directly onto plants in the field within seven days of collection and females oviposited straight away. One container was released every 3 to 5 metres. The total number of flies released per site ranged from 28 – 1,223. In the NT similar ratios and spacing were used, however the first four releases

included the addition of a fly-screen cage placed over a single large plant at the release location. This was for the first few weeks after release and restricting all flies onto that plant during oviposition.

The number of releases per site ranged from one to six, except for one NT site that had 18 releases, using either adults, galled stems or a combination of both. The release numbers varied due to site suitability, site access, rearing colony production and establishment detection.

MONITORING

Monitoring of sites commenced one month after adult releases and two months after gall releases. Follow-up monitoring for signs of galls continued over the following months. Establishment is declared at a field site when it contains galls at different stages of maturity over several months. Of the 79 monitored release sites, 62 (78.48%) met these criteria, which is considered a high level of establishment over the initial release period (Table 1).

Gall numbers have fluctuated at sites but persisted throughout the seasons. Plants at sites with average annual rainfall greater than 1,200mm, have longer periods of active growth and have produced more galls throughout the year. Typically, the wet season in northern Australia runs from November to April during which time most of the annual rainfall can occur. In QLD, 87% of releases were conducted during the wet season to capitalize on active growth periods of chromolaena. Outside of these months, plants display leaf drop and stem dieback due to the long period of low rainfall. Whereas in the NT, access to sites is restricted by heavy rainfall during the wet season, making it necessary to release more in the dry season when road conditions are more favourable.

During host specificity testing in Australia, *C. connexa* developed on *Praxelis clematidae* (Day *et.al* 2016). To date, no galls have been detected in wild field populations of *P. clematidae*, even growing within *C. connexa* established chromolaena sites.

Continued releases and monitoring are required for further evidence of *Cecidochares connexa* establishment, spread and impact on *Chromolaena odorata* within Australia.

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