

The tale of the *Jatropha* leaf-miner, *Stomphastis thraustica* in Australia

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Summary Imported into quarantine from Peru in late 2014 for the biological control of bellyache bush, *Jatropha gossypifolia*, the path to the release in Australia of the *Jatropha* leaf-miner has been thwart with twists and turns. Initially thought to be a new species to science, in 2021 the identity of the insect was determined to be *Stomphastis thraustica*. Around the same time a leaf-miner found on bellyache bush in Australia was also identified as *S. thraustica*, though DNA analysis later demonstrated that it is a separate *Stomphastis* species. Given the time and effort already committed to the project by this point, the decision was made to proceed with the release of *S. thraustica*. Approval to field release the leaf miner was granted in September 2022. Field releases commenced in October 2022. To date, the leaf miner has been released at 33 sites across Queensland and the Northern Territory. Repeated releases are being made at these sites. The moth is believed to be an adept disperser and once it has established it will readily disperse to other infestations. *Stomphastis* species cannot be easily differentiated morphologically. The undescribed *Stomphastis* sp. already present in Australia is widespread, found at nearly all sites where *S. thraustica* has been released in Queensland, and the Northern Territory. Trials where the two species were forced to interbreed resulted in progeny only when *Stomphastis* sp. females were crossed with *S. thraustica* males and these progeny produced offspring. To confirm the establishment of *S. thraustica* and whether interbreeding occurs between the two species in the field, we will collect larval samples from release sites towards the end of the wet season in 2025 (≥ 2 years after initial releases) for DNA analysis.

Keywords bellyache bush, weed biological control, Gracillariidae.

INTRODUCTION

Jatropha gossypifolia L. (Euphorbiaceae), commonly known as bellyache bush, is a serious weed of rangelands and riparian zones in northern Australia (Randall *et al.* 2009). It forms dense thickets, reducing

the usefulness of land for pastoral and grazing purposes and has been declared a Weed of National Significance. Bellyache bush has been the target of biological control since 1996 with the release of the seed feeding jewel bug *Agonosoma trilineatum* (F.) (Heteroptera: Scutelleridae), which failed to establish (Heard *et al.* 2012).

As part of a renewed biological control effort, exploration in central South America identified several potential agents, the most promising of which was a leaf miner commonly found on bellyache bush in northern Peru (Dhileepan *et al.* 2014). The leaf miner was imported into a quarantine facility in Brisbane for host specificity testing and biological studies in 2014.

Specimens of the leaf miner were sent to a Gracillariidae expert who tentatively identified it as a new species to science. However, the moth was ultimately determined to fit within the description of *S. thraustica* (Meyrick) (De Prins *et al.* 2023). Molecular work confirmed that the leaf miner from Peru is *S. thraustica*. *Stomphastis thraustica* is known to attack *J. gossypifolia* and *J. curcas* L. in Africa and Asia (and now South America; De Prins and De Prins 2024).

During the quarantine testing of the leaf miner from Peru, samples of a leaf miner known to occur on bellyache bush in Australia and previously identified as *Epicephala* sp., (Wilson 1997) were also sent to the Gracillariidae expert. Morphologically these samples fit within the species description of *S. thraustica*, however genetic work demonstrated that it was not *S. thraustica*, but rather an undescribed *Stomphastis* sp. (De Prins *et al.* 2023).

By the time we received the confirmed identification of the two leaf miners, host testing of the leaf miner from Peru had been completed (with 50 non-target species), with only bellyache bush and congener *J. curcas* supporting complete development of the insect. Due to the time and effort already committed to the project by this point, the decision was made to proceed with the release of *S.*

thraustica. Approval to field release the leaf miner was granted in September 2022.

In this paper we discuss the release program of *S. thraustica* to date and other post-release studies with *S. thraustica* and the undescribed *Stomphastis* sp. present in Australia.

MATERIALS AND METHODS

Rearing The mass-rearing of *S. thraustica* has been undertaken at the Ecosciences Precinct in Brisbane in a temperature-controlled glasshouse (28/20°C, 65% RH, 14:10 day length). The life cycle of *S. thraustica* under glasshouse conditions is around 20 days. Larval mines are visible after 4-5 days with pupation occurring after 13 or so days. (unpublished data).

Newly emerged *S. thraustica* adults were removed from quarantine between September and November 2022 for colony establishment outside of quarantine and initial field releases. For colony establishment, up to 20 adults (including up to 10 females) were released into each 45 x 45 x 90 cm gauze covered cage, containing four potted bellyache bush plants. Pupae were collected from these cages twice a week into a plastic takeaway container with a gauze window in the lid and emerging adults used to establish new cages. As a precaution the colony in quarantine was maintained until June 2023, when the remaining individuals were destroyed.

Releases *Stomphastis thraustica* has been released as pupae and/or adults. During the week leading up to a release trip, pupae were collected every couple of days into takeaway containers lined with moist paper towel and with a gauze window in the lid. Pupae collected earlier in the week were placed into 1 L containers (for adult releases) and pupae collected later in the week were placed into 0.5 L containers (for pupal releases). Up to 600 pupae were collected into a single container (Figure 1.a). Small vials of sports drink with a piece of sponge as a wick were placed in the large containers to provide sustenance for emerging adults. Initially adults were collected into vials and then released into a container, but this proved to be very time consuming.

Release sites were selected in consultation with a range of stakeholders including local councils and cattle properties. Sites were selected that had a decent amount of bellyache bush, were not subject to other control options, were accessible and were at least 10 km from the nearest release site.

Pupae were released by placing an opened 0.5 L container into a delta trap which is hung in the shade on a high bellyache bush branch or tree branch close to bellyache bush plants (Figure 1.b). A sticky insect barrier was painted onto the hanging wire to prevent ant predation. Adults were released by opening the 1 L container and agitating the container and paper towel until they all disperse.

At sites where follow-up releases were made and late instar larvae and pupae were evident, bellyache bush leaves were opportunistically collected to look for evidence of parasitism.

Preliminary cross-mating trials Bellyache bush leaves with *Stomphastis* sp., larvae were collected from either Springsure (central Queensland) or Gregory (western Queensland) prior to the release of *S. thraustica* in the area. Leaves were placed into a partially opened décor container between sheets of moistened paper towel until larvae had pupated. Pupae were collected into individual containers and monitored for adult emergence.



Figure 1. a. A container of adult *Stomphastis thraustica* for release. b. Pupal release.

Between 2-10 newly emerged unmated *Stomphastis* sp. adults of one sex were placed into a cage with a single bellyache bush plant and 2-3 newly emerged unmated *S. thraustica* adults of the opposite sex. Plants were checked periodically for evidence of larvae, pupae and F1 adults. Five replicates were completed for female *Stomphastis* sp. x male *S. thraustica* and four replicates for female *S. thraustica* x male *Stomphastis* sp.

RESULTS

Field releases of the *S. thraustica* commenced in October 2022 with a release near Ayr in northern Queensland. Releases have generally been made between late October and May, as bellyache bush plants lose their leaves during the dry season at many locations. To date 58 releases have been made at 30 sites across Queensland, including one *J. curcas* site (Table 1; Figure 2). Releases of *S. thraustica* have recently been made in the Northern Territory at three sites (Table 1; Figure 2).

Table 1. The number of *S. thraustica* release sites and releases in Australia until July 2024.

Region	Sites	Releases
Northern Qld	13	30
Central Qld	5	10
Western Qld	7	12
Far northern Qld	5	6
Northern Territory	3	3

The number of individuals per release generally ranged from 300-500 pupae (up to 1500) and an estimated 100-300 adults (up to 500; the number of adults that emerged from the delta traps has been conservatively estimated to be 50% of the pupae). Two or more releases have been made at most sites. Pupae alone have been released at 11 sites, adults alone at 12 sites and a combination of adults and pupae at 10 sites.

Stomphastis sp. was found at almost every release site in Queensland and the Northern Territory with damage varying from low at some sites (e.g., Barcaldine) to high at others (e.g., Mt Isa). Evidence of parasitism has been found in the pupal cases of unidentified *Stomphastis* collected in northern Queensland. Evidence of larval predation from the leaf mines has been seen at many sites in Queensland and the Northern Territory.

In all *Stomphastis* sp. females x *S. thraustica* males replicates eggs were laid and larvae developed and pupated. Two replicates were continued until adults emerged and one was continued until second generation adults emerged. No eggs were laid in any of the *S. thraustica* females x *Stomphastis* sp. males.

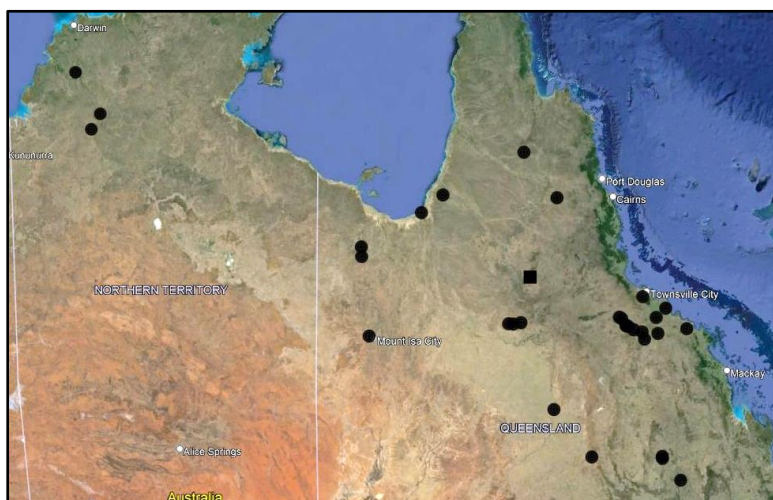


Figure 2. Releases of *Stomphastis thraustica* in Australia. All releases were on bellyache bush except one (denoted by a black square) which was on *Jatropha curcas*.

DISCUSSION

Releases of *S. thraustica* have been in progress since October 2022. The moth has a short generation time and high fecundity and, like other Gracillariidae, is predicted to be an adept disperser (Taylor *et al.* 2017). These characters indicate that the leaf-miner should successfully establish and spread to other infestations, just as the undescribed *Stomphastis* sp. has done without human intervention.

Evidence of leaf-miner damage has been observed at all except one of the release sites that we have returned to (Figure 3). However, the identity of the *Stomphastis* species present cannot be determined visually. *Stomphastis* species can only be distinguished by examining internal genitalia of adults (De Prins *et al.* 2023). To confirm the establishment of *S. thraustica*, larvae will be collected from release sites towards the end of the wet season in 2025 (≥ 2 years after initial releases) for DNA analysis.



Figure 3. *Stomphastis* leaf mines.

The leaf miner present in Australia prior to the release of *S. thraustica* has been more prevalent than previously thought. This raises the question of how the two species will interact in the field. Forced mating trials indicate that hybridization is possible but only between *Stomphastis* sp. females and *S. thraustica* males. Murray *et al.* (2023) also found asymmetrical mating in no-choice trials with the two *Eueupithecia* moths released for parkinsonia. Pheromone traps set in the Northern

Territory prior to the release of *S. thraustica* with newly emerged unmated *S. thraustica* females failed to attract *Stomphastis* sp. males. This supports the result from preliminary cross-mating trials. Expanded mating and pheromone trials are planned to compare *S. thraustica* x *S. thraustica*, *Stomphastis* sp. x *Stomphastis* sp., *S. thraustica* females x *Stomphastis* sp. males, *Stomphastis* sp. females x *S. thraustica* males. Genetic material from these lab-based mating trials will be compared with field collected material.

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