Biological Control of Sagittaria Update: June 2017

*Sagittaria (*Sagittaria platyphylla*) and arrowhead (*S. calycina*), are declared targets for biological control. This fact sheet provides an overview of preliminary host specificity testing of two potential biocontrol agents: a fruit-feeding weevil and a crown-boring weevil. The tests were conducted by Agriculture Victoria to assess their safety for release into Australia.*

Sagittaria plants produce thousands of seeds.

BACKGROUND

There are currently no satisfactory means of effective long-term control of sagittaria (*Sagittaria platyphylla*) or arrowhead (*S. calycina*), particularly in sensitive aquatic habitats and difficult-to-access locations. The measures presently in use are expensive, non-selective and not always effective. Biological control may assist in reducing sagittaria impacts and spread, although it may not be effective in all situations, and must therefore be considered one tool within an integrated weed management framework.

A biogeographic study that compared native populations of *S. platyphylla* with invasive populations in Australia and South Africa found three key differences: (1) invasive populations were more dense, occupying over three times more percentage cover than native populations, (2) the sexual reproductive capacity was significantly greater in invasive plants, producing 40% more seed (achenes) per fruit than native plants, and (3) invasive populations were devoid of any natural predators, while in the USA plants were attacked by a suite of insects and pathogens (Kwong et al., 2017). The release of *S. platyphylla* plants from its natural enemies during its introduction into Australia has most likely led to its invasive success here.

The aim of the project, Biological Control of Sagittaria – Phase 2 (2014/15 to 2016/17), was to undertake the host specificity testing of two of the three candidate agents that had been prioritised during Phase 1 of the biological control program. The fruit-feeding weevil, *Listronotus appendiculatus,* is a pre-dispersal seed predator, and its release into Australia would assist in reducing the prolific seed production of sagittaria. The larvae of the crown-boring weevil, *Listronotus sordidus,* can kill plants rapidly, and if released into Australia would cause a decline in the density of sagittaria populations.

Fruit-feeding weevil, *Listronotus appendiculatus.*

Economic Development,
Jobs, Transport
and Resources

METHODS

Host specificity testing conducted within Victoria's AgriBio quarantine laboratories assessed the potential host range of the weevils against a range of native and exotic (mostly ornamentals) species. The majority of the species tested represented taxa closely related to *S. platyphylla,* from within the Alismataceae plant family. This is a modest list of species compared to other biological control programs that may test over 50 plant species, but is appropriate for *S. platyphylla*. The Alismataceae is a relatively small plant family with 17 genera worldwide containing approximately 100 species (cf. the Asteraceae with approximately 1,620 genera and more than 23,600 species). In Australia, the Alismataceae is represented by five native genera (four of which were tested), and three naturalised genera (two of which were tested). All native Alismataceae species, as well as three distantly-related species (two native and one crop (rice)) that have distributions that overlap *S. platyphylla* and *S. calycina* in temperate Australia were included.

Crown-boring weevil, *Listronotus sordidus.*

RESULTS

We have completed preliminary testing of closely related and valued plant species with biogeographic similarities with sagittaria. The tests provide evidence that four distantly related plant species (*Cycnogeton procerum*, *Oryza sativa*, *Eleocharis sphacelata* and *E. dulcis*) are not at risk of off-target damage from either the crown-boring or fruit-feeding weevil.

Host specificity tests found that the crown-boring weevils could lay eggs on all native plant species within the Alismataceae family, albeit at much lower levels for most species, but the survival of larvae was significantly lower than on *S. platyphylla*. The results for two of the native species, *Damasonium minus* and *Caldesia oligococca,* were inconclusive during the larval development tests as the plants died during the experiment and it was not clear whether this was due to water levels being too low, or from insect damage (although no adults emerged from these plants). This trial is being repeated using both high and low water levels, to ascertain whether crown-boring weevils can survive on these two native species. Presently, there is insufficient evidence to conclude that the crown-boring weevil is of negligible risk to some Australian plant species and therefore an application to Department of Agriculture and Water Resources (DAWR) and Department of Environment (DoE) at this point is premature.

The host specificity testing of the fruit-feeding weevil produced very promising results. No oviposition or larval development was recorded on the native species, *Alisma plantago-aquatica*, *Caldesia oligococca* and *Caldesia acanthocarpa*. Eggs were laid, minor damage was done to the foliage and petioles, and larvae developed on the fourth native species, *D. minus*, but at rates much lower than the two *Sagittaria* species. This is a common artefact of no-choice, laboratory testing and is not expected to occur in the field. Importantly, oviposition of the F1 generation adults reared on *D. minus* was very low, indicating that this plant is a poor host and unlikely to maintain viable, successive generations. An application for release of *L. appendiculatus* has been prepared and will be circulated to collaborating biocontrol scientists for peer review prior to submission to DAWR and DoE.

A larva of the fruit-feeding weevil feeding on a sagittaria fruiting head.

FUTURE DIRECTIONS

Funding has been secured through the Rural Research and Development for Profit program (2016/17-2019/20) to complete the host specificity testing of the crown-boring and fruit-feeding weevils and to initiate testing of the third agent. The tuber-feeding weevil, *Listronotus frontalis* was imported into AgriBio in December 2016 and host testing will commence this spring (2017).

Further testing of the crown-boring weevil will focus on three areas: (1) insect behavioural studies to look more closely at whether the insect sensory system is capable of detecting these non-target species from a distance, (2) thermal

physiology and habitat studies to determine if there are phenological differences between sagittaria and non-target species that may reduce the risk of weevil damage, and (3) ecological host range studies using genetic barcoding techniques to determine if the weevils attack a broad range of plant species in their native range.

Testing of the fruit-feeding weevil is almost complete, with only minor tests to be completed on *C. oligoccoca* and *D. minus*. Should these results prove favourable, the draft application for release will be finalised and submitted to DAWR and DoE.

Given the stringent requirements associated with the Import Risk Analysis undertaken by DAWR and DoE, the objective of this new project is to obtain sufficient evidence to demonstrate that the sagittaria agents are sufficiently host-specific as to pose negligible or very low risk to Australia's agriculture and environment.

Reference

Kwong, R.M., Sagliocco, J.L., Harms, N.E., Butler, K.L., Green, P.T., Martin, G.D., 2017. Biogeographical comparison of the emergent macrophyte, *Sagittaria platyphylla* in its native and introduced ranges. Aquatic Botany 141, 1-9.

Project Team:

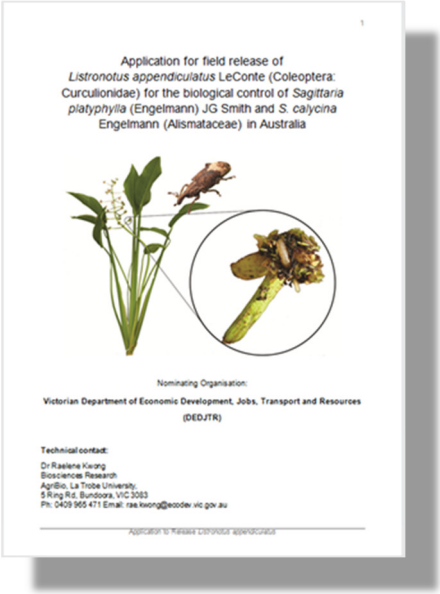
Dr Raelene Kwong, Senior Research Scientist
Email: rae.kwong@ecodev.vic.gov.au

Jackie Steel, Research Scientist
Email: jackie.steel@ecodev.vic.gov.au

Disclaimer

Two natural enemies of sagittaria under investigation in Victoria's AgriBio quarantine facility: the fruit-feeding weevil, *Listronotus appendiculatus* (left) and the crown-boring weevil, *Listronotus sordidus* (right).

Summary of Achievements

Project Objectives	Key Achievements
Obtain administrative approval to proceed with the importation and host-range testing of two biocontrol agents	
	*Sagittaria platyphylla* and *Sagittaria calycina* were declared targets for biological control by the Invasive Plants and Animals Committee on the 26 November 2015. Applications to import the fruit-feeding and crown-boring weevils, *L. appendiculatus and L. sordidus,* from the USA into quarantine were approved by the Department of Agriculture and Water Resources (DAWR) and Department of Environment (DoE). With assistance from USA collaborators in Alabama, Mississippi, Texas and Tennessee, sagittaria natural enemies were collected and imported into AgriBio's quarantine facilities on 29 January 2015 (crown-boring weevil) and 5 October 2015 (fruit-feeding weevil).
Conduct host range testing to ensure that potential biocontrol agents are specific to sagittaria and no threat to other plant species	
	A host-test list was compliled consisting of native and ornamental plant species closely related to *S. platyphylla*. Many of the native species were field collected from locations as far away as Darwin (NT) and Cape York Peninsula (Qld), while the temperate species were collected from NSW and Victoria. A large aquatic plant propagation facility was established at AgriBio to supply sufficient plants for rearing the biocontrol agents and for host specificity testing. An extensive series of host specificity tests were conducted in quarantine over two years to determine if the weevils were specific to *S. platyphylla* or are able to feed and reproduce on other plant species.
Obtain administrative approvals for the release of sagittaria biocontrol agents into Australia	
 Application for field release of *Listronotus appendiculatus* LeConte (Coleoptera: Curculionidae) for the biological control of *Sagittaria platyphylla* (Engelmann) JG Smith and *S. calycina* Engelmann (Alismataceae) in Australia Nominating Organisation: Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Technical contact: Dr Raelene Kwong Biosciences Research AgriBio, La Trobe University, 5 Ring Rd, Bundoora, VIC 3083 Ph: 0409 965 471 Email: rae.kwong@ecodev.vic.gov.au	The host specificity testing of the crown-boring weevil indicated that adult weevils could lay eggs on a range of plant species but only a few species supported the development of immature stages (larvae) through to adulthood. No plants outside of the Alismataceae family were suitable hosts. The survival of larvae on the native species, *Damasonium minus* (star fruit) and *Alisma plantago-aquatica,* necessitates further testing in order to adequately assess the level of risk posed by the crown-boring weevil to these native plants. A water-depth experiment revealed that inundation levels influence the ability of crown-boring weevils to kill *S. platyphylla* plants. If approved for release, this insect would suit *S. platyphylla* infestations occurring in shallow and riparian habitats. Testing of the fruit-feeding weevil indicated that adults were very selective in the plant species on which they lay their eggs. None of the native species were acceptable hosts for larval development, except for star fruit (*D. minus*). Further studies showed that adult weevils reared on star fruit laid very few eggs, confirming that star fruit is unlikley to maintain viable populations of the fruit-feding weevil. Based on these results, an application for release of the fruit-feeding weevil has been prepared and will be circulated amongst biocontrol scientists for peer-review prior to submission to DAWR and DoE.