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 Jobs, Precincts and Regions

(DJPR)

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1   Executive summary

Sagittaria platyphylla and *Sagittaria calycina* (Alismataceae) are emergent aquatic herbs native to north America that have become serious weeds of shallow ephemeral or permanent water bodies, in natural and ruderal habitats. In Australia, *S. platyphylla* extends from the tropical (Townsville) to the temperate regions of New South Wales, Australian Capital Territory, Victoria, South Australia and Western Australia. It is a serious invader of irrigation channels and drains in south-eastern Australia, forming dense monocultures that impede water flow, increasing risk of flooding and damaging irrigation infrastructure. In natural waterways, extensive infestations threaten native biodiversity and potentially impede the movement of native fish. *Sagittaria calycina* is much less widespread than *S. platyphylla* and is currently only present in NSW where it is a major crop competitor in rice crops of the Murrumbidgee and Coleambally irrigation areas, causing yield reductions of up to 75%, increased production costs and reductions in rice quality. Few effective options are available for the management of *S. platyphylla* and *S. calycina*, particularly in sensitive aquatic habitats or where off-target damage to horticultural and rice crops is a concern.

Sagittaria platyphylla and *S. calycina* were declared targets for biological control in Australia in November 2015 after an in-depth biogeographical study on the genetic, demographic and herbivory differences between native USA and invasive Australian populations concluded that the prospects for successful biological control were high.

The host specificity testing of the sagittaria fruit-feeding weevil, *Listronotus appendiculatus* LeConte (Coleoptera: Curculionidae) was conducted within Agriculture Victoria's quarantine insectary located at AgriBio in Bundoora, Melbourne.

The test list followed the standard phylogenetic approach, concentrating on closely-related native and introduced species within the Alismataceae family. As there are relatively few Alismataceae species present in Australia, the test list was comprised of a total of nine species of which four were native and five were exotic ornamental or naturalised species. An additional two species outside of the Alismataceae that are commonly found in *S. platyphylla* and *S. calycina*-invaded areas, were also tested.

Host specificity testing involved several experimental methods as outlined in Figure. 1, including:
1. Container trial: In these trials, adults were placed in a testing arena (plastic container) with only one (**no-choice single species)** or two test species (**choice-minus-target**), but not with the target. The target species (i.e. *S. platyphylla*) was offered to adults in a separate container. For both no-choice and choice-minus-target trials, bouquets of cut foliage and flowers were presented to adults and assessed for oviposition as well as levels of foliage and fruit herbivory.
2. **No-choice whole plant oviposition trials:** In these trials, Australian native plant species for which some oviposition and egg hatch had occurred in the container trials, were subjected to no-choice whole plant oviposition trials on potted plants contained within gauze sleeves. In these trials, oviposition (egg laying) and survival to adults was assessed.
3. **Whole plant larval development trials:** In these trials, a set number of mature eggs were placed on whole plants to assess the survival rate from egg to adult as well as to assess the damage caused by larval feeding on leaf petioles and flowering stems.
4. **Continuation trial:** As adults had emerged from the native species, *Damasonium minus* in the larval development trials a continuation trial was conducted to assess the reproductive performance of these F_1 adults and hence, the ability of *L. appendiculatus* to maintain a viable population on *D. minus*.

In adult no-choice and choice-minus-target oviposition container trials, oviposition, albeit at very low levels, occurred on the *Sagittaria* species, *S. latifolia* and *S. subulata* as well as on the native species, *D. minus*. No viable eggs were laid on the remaining test species.

In adult no-choice whole plant trials, oviposition (less than four eggs per plant) were laid on the native species, *Alisma plantago-aquatica* and *D. minus*, while no eggs were laid on the remaining test species.

In no-choice whole plant larval development trials, adult emergence occurred on *S. latifolia* and *D. minus*, albeit at much lower levels than on *S. platyphylla* and *S. calycina.* No larval development was supported on the native species, *A. plantago-aquatica* or *Caldesia acanthocarpa*, or the ornamental species, *Echinodorus cordifolius*.

In the continuation trial, first generation adults reared from *D. minus* plants laid very few eggs and were unable to survive in sufficient numbers after subjected to a winter diapause treatment. The analysis of the population growth rate based on egg production and larval survival over two generations predicted that *D. minus* would be unable to maintain viable populations of *L. appendiculatus.*

Field studies in the native range, indicated that the preferred hosts of *L. appendiculatus* were *S. platyphylla* and *S. calycina*. Molecular tools were used to confirm that *L. appendiculatus* did not utilise other closely-related species such as *S. latifolia* or *Echinodorus* species growing near *S. platyphylla* or *S. calycina* plants. Furthermore, there was no evidence of biotype differences between *L. appendiculatus* collected from either *S. platyphylla* or *S. calycina*.

In summary, the results of the quarantine-based host testing and native range molecular studies demonstrate that *L. appendiculatus* has a high degree of specificity for the target weeds, *S. platyphylla* and *S. calycina* and that the risk of off-target damage to native and ornamental species in Australia is low. The native plant, *D. minus* was able to support the development of some larvae, however emerging adults showed low fecundity and survival, and was therefore a substantially inferior host for *L. appendiculatus*. The impact caused by larval feeding on *D. minus* fruit was minimal and unlikely to cause population-level impacts on this widespread species, itself a troublesome weed of rice crops. If approved for release, *L. appendiculatus* might cause some adult-feeding damage to the ornamental species, *S. latifolia* and *S. subulata*, however these species are of minor value to the Australian horticulture industry and are banned for sale in states where the *Sagittaria* genus is declared noxious. The decision tree outlined in Figure 1.1 shows the level of risk likely for each test plant species based on the series of trials undertaken in this study.

This document presents information supporting an application seeking the field release of *L. appendiculatus* for the biological control of *S. platyphylla* and *S. calycina* in Australia.
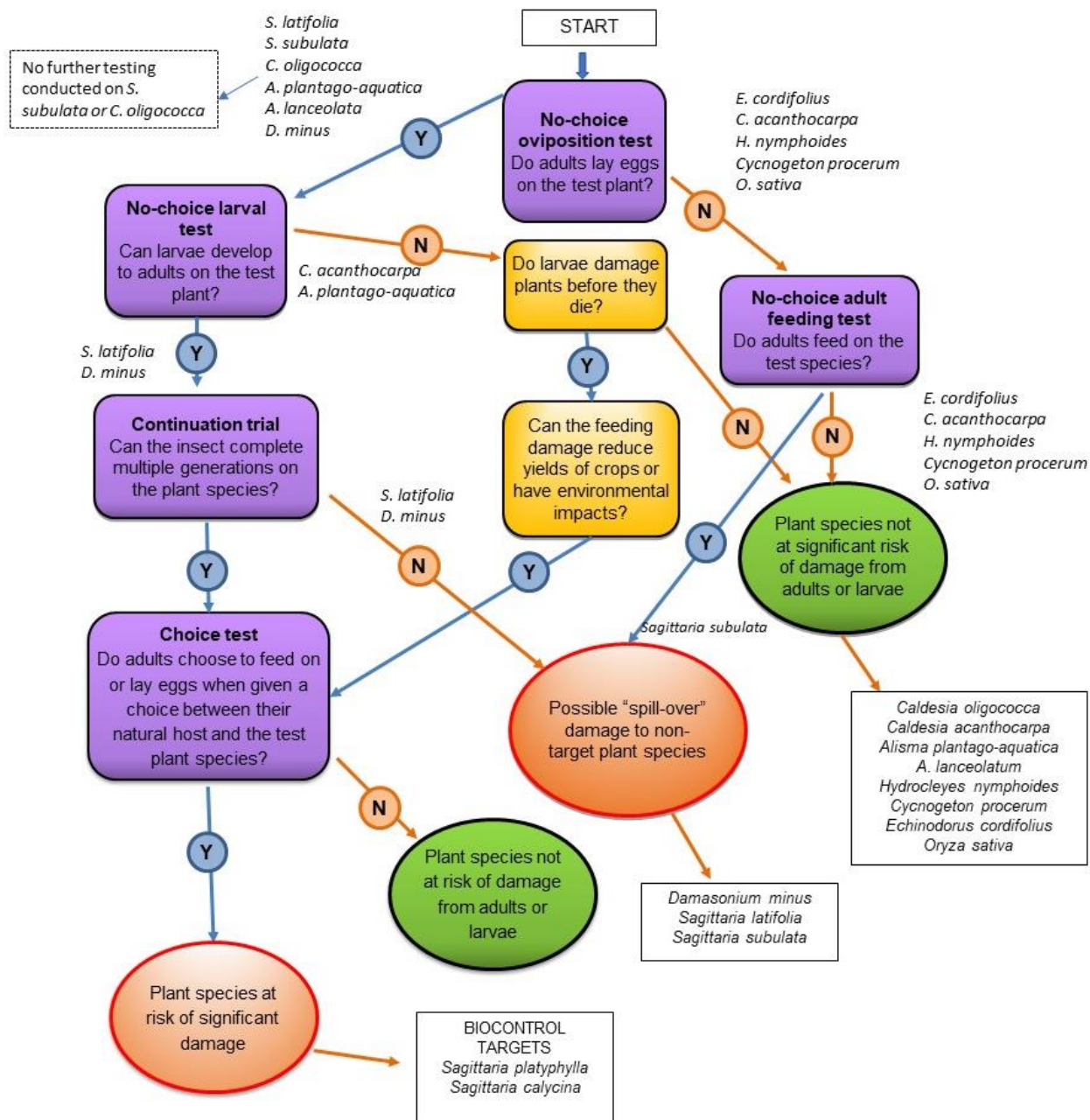
Figure 1.1. Decision tree used to determine the types of host specificity tests to be undertaken for *Listronotus appendiculatus* for the target species, Sagittaria platyphylla and *S. calycina*. The outcome for each test plant species is show in the rectangular boxes.

2 Information about the Target species

2.1 Taxonomy

Class: Liliopsida
Subclass**:**   Alismatidae
Order**:**   Alismatales
Family**:**   Alismataceae

2.1.1 *Sagittaria platyphylla* (Engelmann) J.G Smith

Common names

Delta arrowhead (USA), Sagittaria (Australia)

Synonyms:

- *Sagittaria graminea* Michaux var. *platyphylla* Engelmann in A. Gray. Man. Bot. ed. 5: 494. 1867.
- *Sagittaria recurva* Engelmann *ex* Patterson, Checklist 130. 1887.
- *Sagittaria mohrii* J.G.Smith, Mohr Bull. Torrey Club 24: 19, 1897.

Description:

The following description is provided by Keener (2005).

Perennial, glabrous, emergent aquatic herb, to 150 cm; rhizomes absent; stolons present; tubers present. Leaves phyllodial and petiolate; phyllodial leaves submersed or emersed, flattened, 5–28 × 0.5–2.7 cm; emersed leaves petiolate (Figure 2.1).

Petioles: 10–70 cm long, blades linear to ovate, 4–18 × 0.4–8 cm, occasionally with 1–2 short basal auricles.

Scapes erect, emersed; peduncles 15–54 cm long; inflorescences racemose bearing 3–8 nodes, each node with 2–3 flowers, 4.5–18 × 2–9 cm; lower nodes bearing carpellate flowers; upper nodes bearing staminate flowers; nodal bracts scarious, connate more than 1/4 total length, lanceolate, tip acute, 3–6 mm long.

Flowers: Carpellate flowers pedicellate; pedicels spreading to ascending, to 3 cm long, cylindric, not distinctly thicker than upper staminate pedicels in flower but sometimes becoming so in fruit, cylindric, spreading to recurved in fruit; sepals spreading to reflexed, lanceolate, 3.7–5.5 × 2–3.5 mm. Staminate flowers pedicellate; pedicels ascending, cylindric, to 2 cm long; sepals lanceolate 4–5 × 1.5–3 mm; filaments dilated, longer than to more or less equal to anthers, pubescent; anthers yellow.

Fruiting heads to 1.5 cm diameter, not enclosed by sepals; achenes oblanceolate, 1.5–2.2 × 0.8–1.2 mm, beaked; adaxial margin slightly keeled, keel entire; abaxial margin slightly keeled, keel entire; faces ridged to slightly winged, wing entire, resin canals absent; beak laterally attached, obliquely emerging, to 1 mm long.

S. platyphylla was previously considered one of seven varieties of *Sagittaria graminea* Michaux (*S. graminea* var. *platyphylla*) (Bogin, 1955), but was later assigned to specific rank based on the studies

of Wooten (Wooten, 1973). *S. platyphylla* can be distinguished from *S. graminea* by the presence of fruiting heads on recurved pedicels which are distinctly thicker in diameter than the staminate pedicels. *S. graminea* produces erect to spreading carpellate pedicels which are more or less the same diameter as the staminate pedicels (Godfrey and Wooten, 1979).

The leaf shape and size of *S. platyphylla* are highly variable and are influenced by a range of environmental and management factors (Flower, 2004; Sainty and Jacobs, 1981). Three main leaf forms are recognized: broad-leaf emergent, narrow-leaf emergent, and the submersed phyllodial leaf form. The broad-leaf emergent form produces erect linear to ovate, acuminate blades. This type of leaf morphology tends to occur in nutrient-rich, slow moving water bodies in which plants produce vigorous stolons. The narrow-leaf emergent form produces erect, narrowly tapered blades. Plants of this form occur following nutrient stress or herbicide application and generally produce weaker, depleted stolons. The submersed phyllodial form produces linear strap-like leaves and is mostly found in deeper water than emergent plants. In the early juvenile stages of growth, both phyllodial and emergent leaves may occur on the same plant.

(a) (b)

Figure 2.1. *Sagittaria platyphylla*; (a) infestation within the Broken Creek, Numurkah, Victoria, and (b) fruiting heads with achenes that dislodge readily when mature.

2.1.2 *Sagittaria calycina* Engelmann

Common names:

Arrowhead, hooded arrowhead.

Synonyms:

- *Sagittaria calycina* var. *fluitans* Engelmann *in* Torrey, Bot. Mex. Bound. 212. 1859.
- *Sagittaria calycina* var. *maxima* Engelmann *in* Torrey, Bot. Mex. Bound. 212. 1859.
- *Sagittaria calycina* var. *media* Engelmann *in* Torrey, Bot. Mex. Bound. 212. 1859.
- *Sagittaria calycina* var. *grandis* Engelmann *in* A. Gray, Man. Bot., ed. 5. 494. 1867.
- *Lophiocarpus calycinus* (Engelmann) Micheli *in* A. & C. DC., Monogr. Phan. 3: 61. 1881.
- *Lophotocarpus calycinus* (Engelmann) J.G. Smith, Mem. Torrey Club 5: 25. 1894.
- *Lophotocarpus californicus* J.G. Smith, Missouri Bot. Gard. Rep. 11: 146. 1899.
- *Lophotocarpus depauperatus* Engelmann ex J.G. Smith, Missouri Bot. Gard. Rep. 11: 148. 1899.
- *Lophotocarpus fluitans* (Engelmann) J.G. Smith. Missouri Bot. Gard. Rep. 11: 145. 1899.
- *Sagittaria montevidensis* subsp. *calycina* (Engelmann) Bogin, Mem. New York Bot. Gard. 9: 197. 1955.

Description:

The following description is provided by Keener (2005).

Annual or perennial, glabrous, emergent aquatic herb to 80 cm tall; rhizomes present or absent; stolons absent; tubers present (Figure 2.2).

Leaves phyllodial and petiolate; phyllodial leaves submersed or emersed, flattened, linear, 2.5–45 × 0.5–2 cm; petiolate leaves emersed, rarely floating; petioles, 8–55 cm long; blades sagittate, hastate or elliptic, 3–20 × 2.5–12.5 cm.

Scapes erect, recurved or decumbent, emersed or partly submersed; peduncles 1.5–40 cm long; inflorescence racemose, bearing 2–12 nodes, each node with 1–3 flowers, 1–17.5 × 1–15 cm; lower nodes bearing perfect flowers; upper nodes rarely bearing staminate flowers or functionally staminate with rudimentary carpels; nodal bracts opaque with scarious margins or scarious, distinct or connate at base less than 1/4 of total length, lanceolate to ovate, tip obtuse to acute, 2–34 mm long.

Flowers: Carpellate flowers pedicellate; pedicels spreading to erect, to 7 cm long, cylindric, distinctly thicker than upper staminate pedicels, cylindric, recurved in fruit; sepals erect, appressed, ovate, 5–12 × 4–9 mm. Staminate flowers pedicellate; pedicels ascending, cylindric, to 4 cm long; sepals lanceolate to elliptic, 2.5–4.5 × 3–5 mm; filaments cylindric, longer than anthers, glabrous or pubescent; anthers yellow.

Fruiting heads to 2.5 cm diameter, enclosed by sepals; achenes oblanceoloid, 1.8–2.5 × 0.8–2 mm, beaked; adaxial margin keeled, keel entire; abaxial margin keeled, keel entire; faces smooth to slightly ridged, resin canals present or absent; beak laterally attached, horizontally emerging, to 0.5 mm long.

S. calycina was previously considered one of four subspecies of *Sagittaria montevidensis* Cham. & Schltdl.: *S. montevidensis* subsp. *montevidensis*, *S. montevidensis* subsp. *chilensis* (Cham. & Schltdl.) Bogin, S. *montevidensis* subsp. *calycina* (Engelmann) Bogin, and *S. montevidensis* subsp. *spongiosa* (Engelmann) Bogin. Using molecular data, Keener (2005) elevated *S. montevidensis* subsp. *calycina* to specific status as *S. calycina* Engelmann *in* Torrey, recognizing two varieties, *S.*

calycina var. *calycina* and *S. calycina* var. *spongiosa* Engelmann *in* Gray.

Historic reports of *Sagittaria montevidensis* Cham. & Schltdl. in Australia may stem from the previous taxonomic treatment of *S. calycina* as a subspecies of *S. montevidensis*. The two species share similar morphology and can sometimes be mistaken for each other. *Sagittaria montevidensis* is native to tropical and subtropical areas in south central and western South America and has naturalized in south eastern US and western Africa. It is usually more robust with purple spots at the base of the petals. These spots are absent or clear in *S. calycina*. Some herbarium records of larger *S. calycina* specimens could be confused with *S. montevidensis* without adequate flowering material to examine for the purple spots. Until a specimen is discovered in Australia that clearly has a purple spot on the petal, *S. montevidensis* should be excluded from the Australian flora (Adair *et al.* 2012).

(a)   (b)

Figure 2.2. *Sagittaria calycina* (a) infestation in Griffith, NSW, and (b) line drawing (accessed 13 June 2017 https://plants.usda.gov/core/profile?symbol=SACAC).

2.2 **Close relatives native in Australia:**

The Alismataceae is a primitive mono-cotyledon family consisting of aquatic or semi-aquatic herbs with erect or floating leaves (Haynes and Holm-Nielsen, 1994). The family comprises 12 genera (Haynes et al., 1998) of approximate 95 species (Les et al., 1997). In Australia there are seven indigenous alismataceous species; *Alisma plantago-aquatica* L., *Damasonium minus* (R.Br.) Buchanan, *Caldesia oligococca* (F.Muell.) Buchenau, *C. reniformis (= C. parnassifolia)* (L.) Parl. *C. acanthocarpa* (F. Muell.) Buchenau, *Astonia australiensis* (Aston) S. W. L. Jacobs (Jacobs 1997), and *Butomopsis latifolia* (D.Don) Kunth (Jacobs and McColl, 2011).
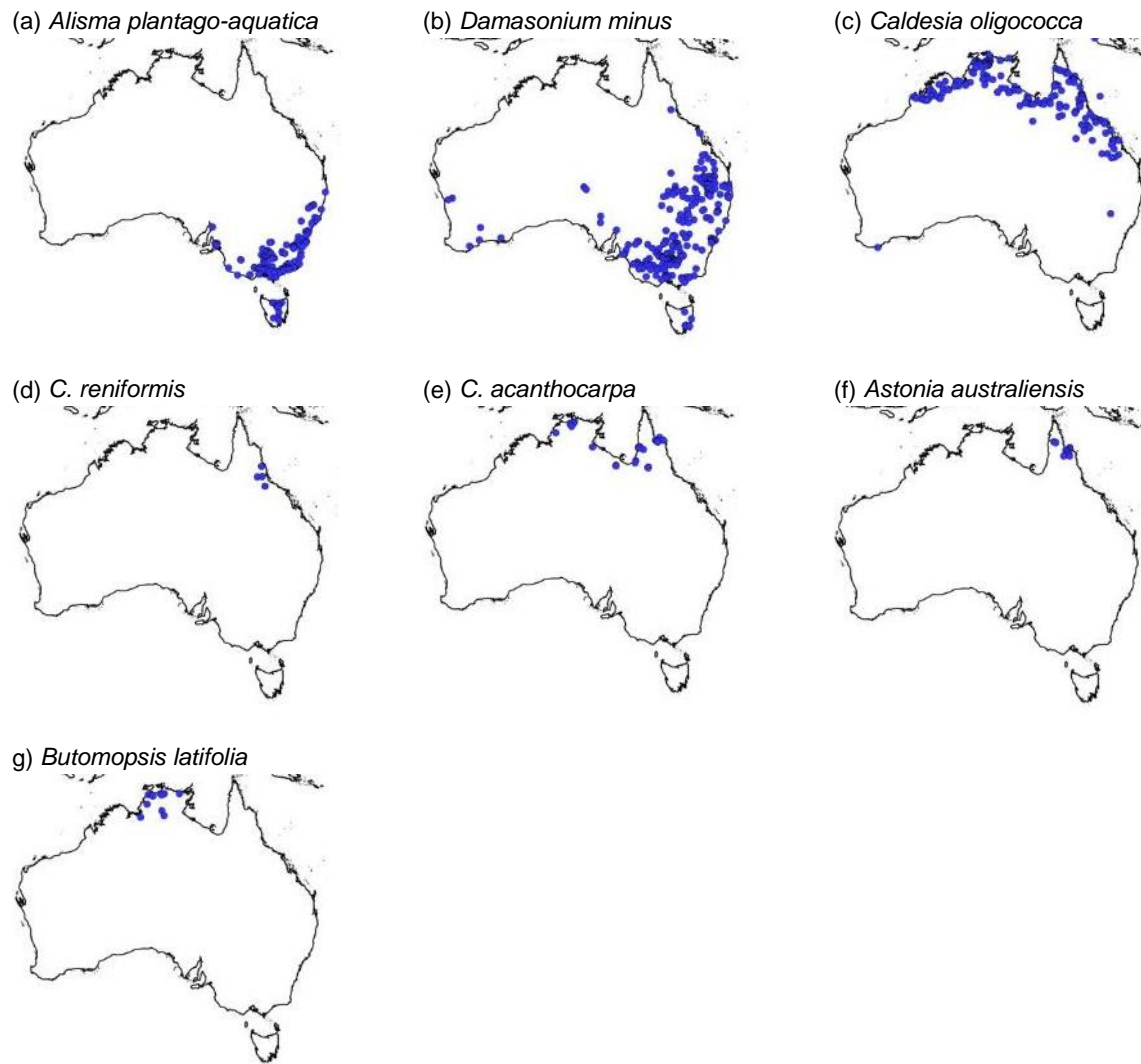
Alisma plantago-aquatica (water plantain) is a widespread temperate species occurring across Eurasia and from North Africa to Tanzania. It is considered to be native in parts of Australia, where it is widely distributed throughout Victoria, Tasmania and New South Wales (Fig. 2.2.1a). It is considered a weed of rice crops in southern NSW (Ash et al., 2008). It is not listed as a nationally-threatened species under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

Damasonium minus (starfruit) occurs in all Australian states and territories except the Northern Territory (Fig. 2.2.1b). It grows as an annual or short-lived perennial herb in slow-moving and shallow water. In agriculture, *D. minus* has been called "the most important broadleaf weed in the Australian rice crop" being difficult to control due to herbicide-resistance and tolerance (Ash et al., 2008). It is not listed as a nationally-threatened species under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

Caldesia. Three species of *Caldesia* are native to Australia and occur through the tropical regions of Western Australia (WA), the Northern Territory (NT) and Queensland (QLD). *C. oligococca* is an annual with floating leaves and emergent inflorescences flowering from March to October. It grows in lagoons, billabongs and riverbanks in northern WA, NT and QLD (Fig. 2.2.1c). *C. reniformis* is a perennial or annual with emergent and floating leaves and emergent inflorescences flowering during the wet season. It has a discrete distribution, occurring in north-eastern Queensland (Fig. 2.2.1d). *C. acanthocarpa* is an annual (sometimes perennial) with floating leaves and emergent inflorescences that flower during the wet season. It is native to Kakadu and Arnhem Land in the NT and Cape York Peninsula, QLD (Fig. 2.2.1e). None of the *Caldesia* species are listed as nationally-threatened under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

Astonia australiensis is endemic to the Cape York Peninsula, north Queensland (Fig. 2.2.1f). It grows in freshwater shallow lagoons and flowers during the wet season (Jacobs, 1997). It is not listed as a nationally-threatened species under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* but is declared an Endangered species under the Queensland Government's *Nature Conservation Act 1992* (*Astonia australiensis*, WetlandInfo, 2014).

Butomopsis latifolia is native to tropical Africa, Southeast Asia and northern Australia. In Australia, it occurs in ephemeral floodplain swamps in the wet/dry tropics in NT and QLD (Fig. 2.2.1g) (Jacobs 1997). It is not listed as a nationally-threatened species under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and is listed as 'of least concern' under the Queensland Government's *Nature Conservation Act 1992.*

(a) *Alisma plantago-aquatica*

(b) *Damasonium minus*

(c) *Caldesia oligococca*

(d) *C. reniformis*

(e) *C. acanthocarpa*

(f) *Astonia australiensis*

g) *Butomopsis latifolia*

Figure 2.3. Australian distribution of indigenous Alismataceae. Maps generated from AVH (2014). Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria, (http://avh.chah.org.au), accessed 4 April 2014.

2.3 **Close relatives introduced to Australia:**

In addition to *S. platyphylla* and *S. calycina* there is evidence from herbarium records that an additional three species may have naturalised in Australia; *S. filiformis* J.G. Smith, *S. macrophylla* Zuccarini and an uncertain sagittate-leaved species (Adair *et al.* 2012), however their status is unknown. Several other *Sagittaria* species are utilised and traded in Australian ornamental horticulture but are not known to be naturalised Table 2.1. The current precise number is unknown as some species are being traded under incorrect names. For example, *S. latifolia* is commonly sold under the name of *S. sagittifolia* and *S. subulata* under the name of *S. natans*. *S. platyphylla* has been known to be sold as *S. lancifolia* (Watergarden Paradise Aquatic Nursery, personal communication, 2014). Non-naturalized *Sagittaria* in Australia (according to nursery catalogues) include *S. lancifolia* Linnaeus, *S. latifolia* Willdenow, *S. natans* Pallas, *S. sagittifolia* Linnaeus and *S. subulata* (Linnaeus) Buchenau (Adair *et al.* 2012). Other closely related species within the Alismataceae are currently available through the aquarium trade including a number of *Echinodorus* species and their hybrids. Naturalised species within the Alismataceae family include *Alisma lanceolatum* With. and *Limnocharis flava* (L.) Buch.

Table 2.1. Members of Alismataceae in Australia (native, naturalised and currently traded in horticulture).

Genus + species	Distribution and habitat	Status	Horti-culture
Caldesia oligococca F.Muell.	Tropical WA, NT, Qld. Grows in lagoons, billabongs and along riverbanks.	Native	No
Caldesia reniformis (D.Don) Makino (= *Caldesia parnassifolia*)	Tropical eastern Qld. Grows in spring-fed creeks and permanent swamps.	Native	No
Caldesia acanthocarpa F.Muell.	Kakadu and Arnhem Land (NT) and Cape York (Qld). Grows in lagoons and billabongs.	Native	No
Butomopsis latifolia (D.Don) Kunth	Old World tropics and wet/dry tropics in Australia (NT and Qld).	Native	No
Damasonium minus (R.Br) Buchenau	Temperate endemic species widespread in SE Australia	Native	No
Astonia australiensis (Aston) S.W.L.Jacobs	Endemic to Cape York Peninsula, NE Qld. Flowers during the dry season.	Native	No
Alisma plantago-aquatica L.	Eurasia, North Africa, New Zealand. Considered native to parts of Australia (Tas, Vic, NSW).	Native	Yes
Alisma lanceolatum L.	Native to Europe, Africa and Asia. Introduced into southern Australia and established in WA, Vic, Tas, and NSW. A weed of irrigation and rice.	Naturalised	No
Helanthium tenellum (Mart. ex Schult.f.) J.G.Sm.	A South American species not known to be naturalised in AUS.	Not naturalised	Yes
Helanthium bolivianum (Rusby) Lehtonen & Myllys	A South American species not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus cordifolius (L.) Griseb.	A North American species naturalised in wetlands and pond margins in south east Qld.	Naturalised	Yes
Echinodorus uruguayensis Arechav. (sold as *E. africanus*)	A South American species from Brazil to south central Chile, not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus grisebachii Small (sold as *E. parviflorus*)	Native to Cuba, Central and South America, is not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus 'Rose'	A hybrid between *E. horemanii* and *E horizontalis*.	Not naturalised	Yes
Hydrocleys nymphoides (Humb. & Bonpl. Ex Willd.) Buchenau	Native to South America. Naturalised in Qld, NSW and Vic.	Naturalised	Yes
Limnocharis flava (L.) Buch.	Native to Mexico, the Caribbean, Central America and tropical South America. Naturalised in Qld.	Naturalised	No
S. lancifolia L.	Native to the south eastern USA, Mexico, Central and South America. This species is not yet available for sale in Australia.	Not naturalised	No
S. latifolia Willd.	Native to southern Canada, USA, Mexico and Central America. The form currently sold in Australia is a sterile form, producing only male flowers.	Not naturalised	Yes
S. natans Pall	Native to northern Europe and Asia. Despite labelled as *S. natans*, the plants sold in Australian nurseries are more likely to be *S. subulata*. It is uncertain if *S. natans* is present in Australia.	Not naturalised	Uncertain
S. sagittifolia L.	Native to Asia and Europe. The form currently sold in Australia does not flower.	Not naturalised	Yes

| Genus + species	Distribution and habitat	Status	Horti-culture
Caldesia oligococca F.Muell.	Tropical WA, NT, Qld. Grows in lagoons, billabongs and along riverbanks.	Native	No
Caldesia reniformis (D.Don) Makino (= *Caldesia parnassifolia*)	Tropical eastern Qld. Grows in spring-fed creeks and permanent swamps.	Native	No
Caldesia acanthocarpa F.Muell.	Kakadu and Arnhem Land (NT) and Cape York (Qld). Grows in lagoons and billabongs.	Native	No
Butomopsis latifolia (D.Don) Kunth	Old World tropics and wet/dry tropics in Australia (NT and Qld).	Native	No
Damasonium minus (R.Br) Buchenau	Temperate endemic species widespread in SE Australia	Native	No
Astonia australiensis (Aston) S.W.L.Jacobs	Endemic to Cape York Peninsula, NE Qld. Flowers during the dry season.	Native	No
Alisma plantago-aquatica L.	Eurasia, North Africa, New Zealand. Considered native to parts of Australia (Tas, Vic, NSW).	Native	Yes
Alisma lanceolatum L.	Native to Europe, Africa and Asia. Introduced into southern Australia and established in WA, Vic, Tas, and NSW. A weed of irrigation and rice.	Naturalised	No
Helanthium tenellum (Mart. ex Schult.f.) J.G.Sm.	A South American species not known to be naturalised in AUS.	Not naturalised	Yes
Helanthium bolivianum (Rusby) Lehtonen & Myllys	A South American species not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus cordifolius (L.) Griseb.	A North American species naturalised in wetlands and pond margins in south east Qld.	Naturalised	Yes
Echinodorus uruguayensis Arechav. (sold as *E. africanus*)	A South American species from Brazil to south central Chile, not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus grisebachii Small (sold as *E. parviflorus*)	Native to Cuba, Central and South America, is not known to be naturalised in AUS.	Not naturalised	Yes
Echinodorus 'Rose'	A hybrid between *E. horemanii* and *E horizontalis.*	Not naturalised	Yes
Hydrocleys nymphoides (Humb. & Bonpl. Ex Willd.) Buchenau	Native to South America. Naturalised in Qld, NSW and Vic.	Naturalised	Yes
Limnocharis flava (L.) Buch.	Native to Mexico, the Caribbean, Central America and tropical South America. Naturalised in Qld.	Naturalised	No
S. lancifolia L.	Native to the south eastern USA, Mexico, Central and South America. This species is not yet available for sale in Australia.	Not naturalised	No
S. latifolia Willd.	Native to southern Canada, USA, Mexico and Central America. The form currently sold in Australia is a sterile form, producing only male flowers.	Not naturalised	Yes
S. natans Pall	Native to northern Europe and Asia. Despite labelled as *S. natans*, the plants sold in Australian nurseries are more likely to be *S. subulata*. It is uncertain if *S. natans* is present in Australia.	Not naturalised	Uncertain
S. sagittifolia L.	Native to Asia and Europe. The form currently sold in Australia does not flower.	Not naturalised	Yes
Sagittaria subulata (L.) Buchenau	Native to the south eastern USA, Columbia and Venezuela. Can mistakenly be sold as *S. natans*.	Not naturalised	Yes

2.4 Habitat

2.4.1 Native geographic range and climatic and edaphic variation between sites within range. Limits to distribution where known.

Sagittaria platyphylla is native to southern North America from Kansas to Texas across to Georgia and western Florida. The species is also native to central Mexico. (Keener 2005). The possible centre of origin is the Mississippi Delta (Figure 2.4a). It inhabits swamps, margins of lakes and ponds, sluggish streams and wet ditches from sea level up to 900 m. It has a broad ecological tolerance but is restricted to warm-temperate regions. Substrates high in potassium and organic matter are associated with *S. platyphylla*, suggesting specificity to certain soils (Wooten 1973). Frosts may damage top growth, but regrowth occurs from submerged or subterranean organs.

Sagittaria calycina is native to central North America in the United States (Figure 2.4b), ranging south into Mexico. It has a broad climatic range within its native distribution where it occupies temperate to subtropical wetland habitats in the Nearctic region (Adair et al., 2012).
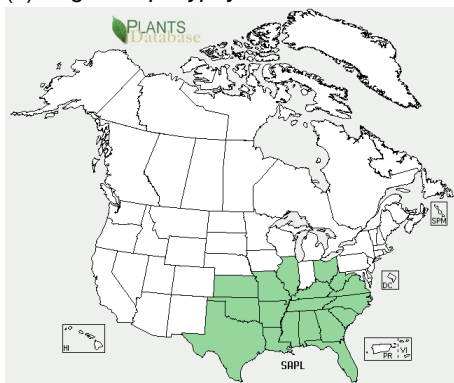
(a) *Sagittaria platyphylla*   (b) *Sagittaria calycina*
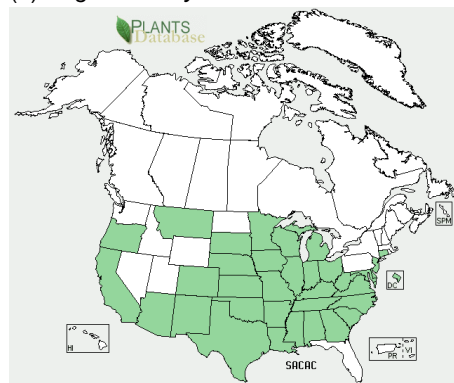
Figure 2.4. Native distribution of (a) *Sagittaria platyphylla* and (b) *Sagittaria calycina* in the United States of America. Source: United States Department of Agriculture Plants Database: http://plants.usda.gov/core/profile?symbol=SAPL.

2.4.2 Current Australian distribution

Sagittaria platyphylla inhabits shallowly flooded or marshy areas associated with rivers, streams, natural swamps and wetlands (Parsons and Cuthbertson, 2001) and is a weed of irrigation and drainage channels, ditches and permanent swamps associated with irrigation and drainage systems in south-eastern Australia. Main infestations occur in the Murray, Goulburn, Ovens and Edward Rivers, and irrigation and drainage networks in northern Victoria and southern NSW (Chapman and Dore, 2009). Isolated occurrences of *S. platyphylla* occur in South Australia on the Murray River from Mannum to the Younghusband and Bowhill areas, and in Western Australia's south west in Albany and the Canning River in Perth (Sage et al., 2000) (Figure 2.5.a). The rate and extent of spread of *S. platyphylla* is increasing, particularly in south-eastern Australia (Sagliocco et al., 2007). Records for *S. platyphylla* occur in near-coastal areas of Queensland and the species is reported as locally common in the Brisbane and Noosa areas. The most northern infestations occur along the Ross River in Townsville, where scattered populations were first recorded in 2012. The species is presently absent from Tasmania and the ACT, with a single population recently recorded in a constructed pond in the

Northern Territory. The species' potential distribution includes waterways and wetlands throughout eastern and southern Australia (Figure 2.5b). In other parts of the world, *S. platyphylla* is naturalized in the former USSR, Indonesia, the Panama Canal, along the Gulf of Mexico coast, and in South Africa (Adair *et al.* 2012) and New Zealand (according to online Flora of NZ).

(a) current distribution

(b) potential distribution

Legend - Potential Distribution

Unsuitable
Marginally suitable
Moderately suitable
Highly suitable
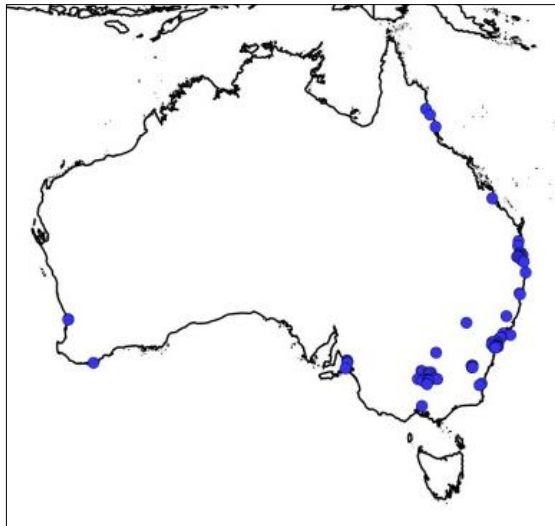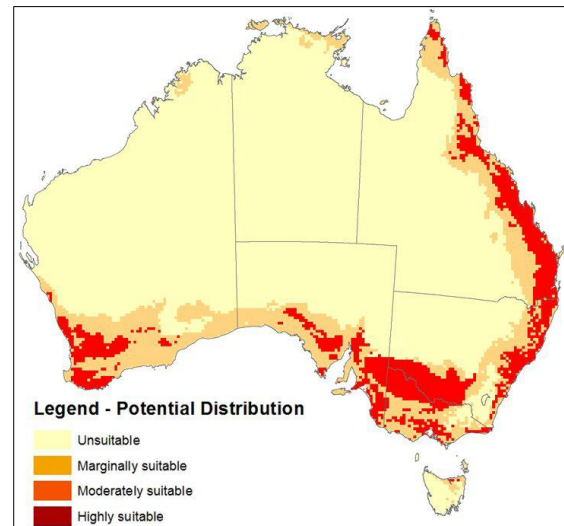
Figure 2.5 Current and potential distribution of Sagittaria platyphylla in Australia. *(a) Map generated from AVH (2014).* Australia's Virtual Herbarium*, Council of Heads of Australasian Herbaria, <http://avh.chah.org.au>, accessed 4 April 2014; (b) map taken from the* National Sagittaria Strategic Plan 2012-2017.

In Australia, *S. calycina* is known only from the Murrumbidgee and Coleambally irrigation areas of New South Wales (Flower, 2003), where it occurs in permanent or semi-permanent freshwater to *ca*. 1 m deep and is mostly found in rice fields, drainage ditches and the Barren Box swamp west of Griffith (Figure 2.6).
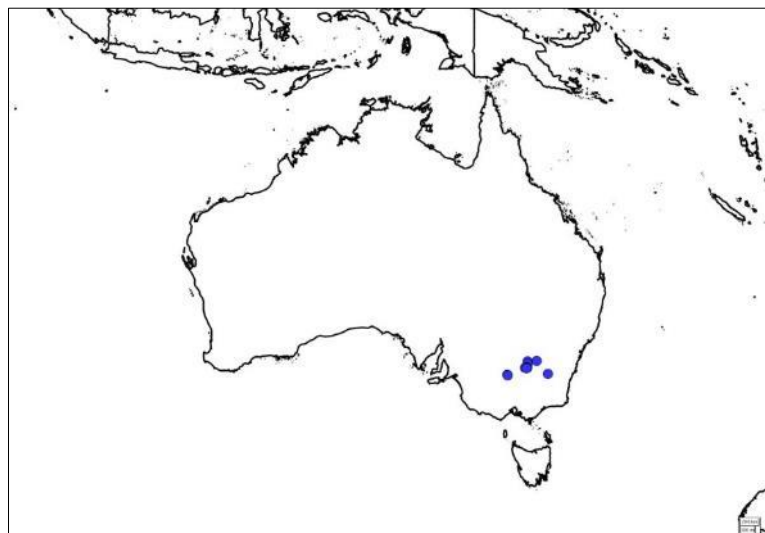
Figure 2.6 Current distribution of *Sagittaria calycina* in Australia. Map generated from AVH (2014). Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria, <http://avh.chah.org.au>, accessed 4 April 2014.

2.5 **Control Methods**

2.5.1 **Herbicides**

In Australia, there are no label recommendations specifically for *S. platyphylla*, but minor usage permits have been issued by the Australian Pesticide and Veterinarian Medicines Authority (APVMA). Several herbicides are registered for the control of *S. montevidensis*, reflecting the formerly used name for *S. calycina*.

Herbicide applications for control of *S. platyphylla* often result in variable levels of control that are not consistent between locations and time of application. Currently, control of *S. platyphylla* is reliant on repeated, high dose applications of the herbicides, glyphosate or 2,4-D amine, under off label permits (Adair 2012). Use of 2,4-D frequently causes abscission of leaves before translocation, resulting in 'chemical mowing', where regrowth usually occurs within 6–12 weeks. In Western Australia, the triazolone herbicide carfentrazone–ethyl 400 g L^{-1} at 250 g ha^{-1} has been advocated for suppression of *S. platyphylla*. In Victorian irrigation channels, Acrolein is used for controlling submerged aquatic weeds but it is ineffective on *S. platyphylla* (Clements et al., 2013).

Early experimentation with dichlobenil (a granular, residual herbicide registered in some aquatic situations) appears to be promising. Dichlobenil kills seedlings of *S. platyphylla* and also prevents growth from tubers and stolons but is not registered for use in water that is used for crop irrigation or livestock and human consumption (Chapman and Dore 2009).

More recently, glasshouse and field trials have been conducted to determine the efficacy of winter applications of endothal and diquat in controlling *S. platyphylla* in static irrigation channels (Clements et al. 2013). Endothal activity persisted for six weeks after treatment and provided excellent control of both emergent and submerged forms of the weed. Diquat was ineffective in turbid irrigation channels at reducing *S. platyphylla* biomass, with and without a gelling agent, during winter conditions.

In the Australian rice growing industry, the sowing of pre-germinated seed distributed into flooded bays provides a favourable environment for *S. calycina* and other alismataceous species. Three main herbicides are utilised for suppression of alismataceous weeds in rice crops: benzofenap, dichlobenil and bensulfuron, and MCPA is utilized to a limited extent on mature *Sagittaria* infestations. Herbicide resistance is reported for alismataceous weeds in rice crop, with 35% of *S. calycina* accessions reported as herbicide resistant, with the potential to cause escalating economic losses (Adair 2012 and references therein).

2.5.2 **Other treatments**

Mechanical control of *Sagittaria* is utilised in channels and drains when the hydraulic capacity of water delivery infrastructure needs to be restored quickly. The technique, which mostly uses excavation machinery, is particularly useful where herbicide application is inappropriate, such as near sensitive crops or where channels are in continual use and cannot be shut down for applications of herbicides. Mechanical control methods can be costly due to high labour and transport costs. They may also fragment *Sagittaria* plants, which may then disperse through water delivery infrastructure. Viable propagules such as seeds, tubers, stolons, rhizomes or crowns may also remain after treatment by mechanical methods, necessitating follow-up suppression activity. In irrigation systems, mechanical control can damage or re-profile drains and channels, leading to leakage or ponding, which can affect water delivery. Design of irrigation channels can influence colonization patterns of *Sagittaria*. Where water levels can be maintained at depths greater than the transition point from submersed to emergent forms of *S. platyphylla* (50 cm), water delivery benefits are obtained. Steeper slopes decrease infiltration of *S. platyphylla* into deeper parts of channel and reduce the impact of damaging emergent forms (Adair et al. 2012, and references therein).

In rice cropping situations Flower (2003) found that various stubble management practices, such as

retention, burning or incorporation have no immediate effect on establishment and growth of *S. calycina,* while nitrogen applications at rates similar to those used for rice (between 20 and 200kg N ha^{-1}) enhanced growth.

2.6 **Response to other human manipulations**

The roles of fire, grazing by domestic stock and slashing have not been evaluated in Australia for suppression of *Sagittaria*, but are unlikely to be of great benefit due to the asexual modes of reproduction in *S. platyphylla,* and non-target impacts in rice growing areas. In natural ecosystems, competitive and desirable native aquatic vegetation may offer potential for the suppression of *S. platyphylla*, particularly if combined with other forms of control such as selective use of herbicides or classical biological control, but formal evaluation is required (Adair et al., 2012).

2.7 **Importance**

2.7.1 **Detrimental**

Agriculture. The most significant impact of *S. platyphylla* invasion is the foliage obstructing water flow in drains and channels causing increased water levels, particularly during periods of high flow or rainfall, and inefficiencies in water delivery. Infestations in irrigation channels affect the reliability of water delivery to farms, reducing capacity to deliver the right amount of water at the right time, which can lead to production losses or increased costs. Damage to irrigation infrastructure can also be significant. In the channel system administered by Goulburn–Murray Water, up to 85% of 14 000 km of creeks, drains and channels have infestations of *S. platyphylla* requiring an annual expenditure of $A250–$500K y^{-1}, depending on the season (Flower 2004, Chapman and Dore 2009). More recently, in the Shepparton and Murray Valley irrigation areas, more than $A2 million y^{-1} is spent on control of *S. platyphylla*. In New South Wales, *S. platyphylla* is recognized as a serious problem in channel systems of the Griffith area.

Similarly, in the southern central area of New South Wales, *S. calycina* reduces water delivery efficiencies by blocking channels and drainage ditches. In southern rice production areas, *S. calycina* is a major crop competitor, along with several other Alismataceae (*Alisma plantago-aquatica*, *A. lanceolatum*, *Damasonium minus*), causing reduced yields by up to 75%, increased production costs and reductions in rice quality. Aerial sowing of rice into flooded bays exacerbates the impact of several alismataceous weeds, including *S. calycina*, by prolonging the inundation period. In North America, *Sagittaria* is an alternative host to the aster leaf hopper, *Macrosteles fascifrons* Stål, (Hemiptera: Cicadellidae), a vector of aster yellows phytoplasma, oat blue dwarf virus and clover phyllody virus. While the leaf hopper is not present in Australia, infestations of *Sagittaria* present a potential biosecurity risk to Australian agricultural and floricultural industries (Adair et al. 2012).

Natural waterways. *Sagittaria platyphylla* threatens native aquatic flora and fauna by invading shallow water bodies, where it competes with native species and reduces plant biodiversity (Chapman and Dore 2009). However, quantitative data demonstrating such impacts are lacking. The endangered Lower Murray Ecological community, iconic wetland areas of Barmah and Gunbower Forests, and northern RAMSAR sites such as the Kerang wetlands and Chowilla flood plain are at risk from invasions of *S. platyphylla* (Chapman and Dore 2009). In coastal New South Wales, *S. platyphylla* invades wetlands and is expanding rapidly in the Porters Creek wetlands, where it causes loss of understorey species in *Melaleuca linariifolia* Sm. forests (Adair et al. 2012).

Sagittaria platyphylla is reported to have negative impacts on recreational activities, particularly fishing, boating and passive recreation. In shallow streams, *S. platyphylla* may act as a barrier to the movement of native fish and provide habitat to populations of European carp (*Cyprinus carpio* L.) (Chapman and Dore 2009). Again, quantitative data on these impacts are lacking. In natural

waterways, *S. calycina* appears to have minimal ecological impacts (because it does not invade natural water ways in Australia), although formal evaluation is also warranted.

2.7.2   **Beneficial**

In Australia, few beneficial outcomes are associated with *S. platyphylla* and *S. calycina*. Both species have been utilised as aquatic ornamentals in jurisdictions where they are not listed under relevant noxious weed legislation (Northern Territory, Australian Capital Territory, Queensland), but are of negligible economic value.

2.8   **Legislation**

Sagittaria platyphylla and *S. calycina* are declared species under State noxious weed legislation in Western Australia, Tasmania, South Australia, New South Wales, Victoria and the ACT.

In South Australia, both species are listed as Class 1 weeds and generally require notification and destruction throughout the State.

In Western Australia, *S. platyphylla* is declared C3 (management) and *S. montevidensis* is listed C1 (exclusion), however S*. calycina* is not listed under th*e [Biosecurity and Agriculture Management Act 2007](#)*.

In Tasmania, both species are declared weeds under the *Weed Management Act 1999* and restriction measures are in place to prevent introduction and establishment.

In NSW, *S. platyphylla* and *S. calycina* are declared noxious under the *Noxious Weeds Act 1993. S. platyphylla* is declared a Control Class 5 noxious weed throughout the state and is also a Control Class 4 noxious weed in certain areas of the state. *S. calycina* is declared a Control Class 4 throughout the entire state.

In the ACT, only *S. platyphylla* was listed in the Pest Plants and Animals (Pest Plants) Declaration 2015 (No1) under the *Pest Plants and Animals Act 2005*, section 7. It is declared under three categories: 'notifiable', 'must be suppressed' and 'prohibited'.

In Victoria, the entire genus, *Sagittaria* L. spp. has been declared Regionally Prohibited in six catchment regions and Regionally Controlled in four catchments under the *Catchment and Land Protections Act 1994*.

In the Northern Territory, *S. platyphylla* is a declared Class A (to be eradicated) and Class C (not to be introduced) weed. *S. calycina* is not declared.

In Queensland, *S. platyphylla* is a restricted invasive plant under the Queensland Biosecurity Act 2014. *S. calycina* is not declared.

2.9   **Stakeholder Consultation**

The only identified stakeholder for *Sagittaria* species in Australia is the nursery industry, however *S. platyphylla* and *S. calycina* are rarely traded in jurisdictions where they are not listed under relevant noxious weed legislation. Other *Sagittaria* species are freely traded as aquatic ornamentals for water gardens and/or aquaria (personal communication Kerry Battersby, Nursery and Garden Industry Queensland, August 2015), (see Table 1 for *Sagittaria* species currently available through retail suppliers of aquatic ornamentals).

Preliminary consultation with Nursery and Garden Industry bodies was conducted in July 2015, but only in Queensland and the Northern Territory where *Sagittaria* are not declared weeds. In-principle

support for the nomination of *S. platyphylla* and *S. calycina* was provided by NGIQ, but no response was provided by the Northern Territory.

2.10 Approval as target species for biological control

The Invasive Plants and Animals Committee approved the Victorian Department of Economic Development, Jobs, Transport and Resources' nomination of *Sagittaria platyphylla* and *S. calycina* as targets for biological control on 26 November 2015 (http://www.agriculture.gov.au/pests-diseases-weeds/pest-animals-and-weeds/invasive/ipac/ipac-meeting-4).

2.11 History of biological control

Neither *S. platyphylla* nor *S. calycina* have previously been targeted for classical biological control in Australia, or elsewhere in the world. In 2005, an evaluation of the potential for classical biological control of *S. platyphylla* in Australia identified several arthropods reported to be associated with *Sagittaria* spp. but no phytophagous insects or plant pathogens were specifically recorded associated with *S. platyphylla* in North America (Sagliocco, 2005).

The first systematic survey for natural enemies in the USA commenced in August 2010, through Mississippi, Tennessee and Alabama, with follow-up surveys conducted across Georgia, South Carolina, Arkansas, Texas and Louisiana in September 2011 and 2012 (Kwong et al., 2014). A total of 32 arthropod and 29 fungal taxa were collected. Of the insect species, 19 were confirmed to be associated with *S. platyphylla*. Leaf spot symptoms were present at 53% of sites but none of the isolated organisms were considered promising candidates as they were either generalist pathogens or secondary invaders. The most common and abundant insect species encountered was the fruit, flower and petiole-feeding weevil, *Listronotus appendiculatus* (Boheman) (Coleoptera: Curculionidae) which was collected at 74% of sites. Two further weevils, *Listronotus sordidus* (Gyllenhal) and *Listronotus. frontalis* LeConte are promising candidates due to the damage they cause to plant crowns, roots and tubers, while *Listronotus lutulentus* (Boheman) adults feed on foliage and their larvae mine the petioles (Figure 2.7).

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)
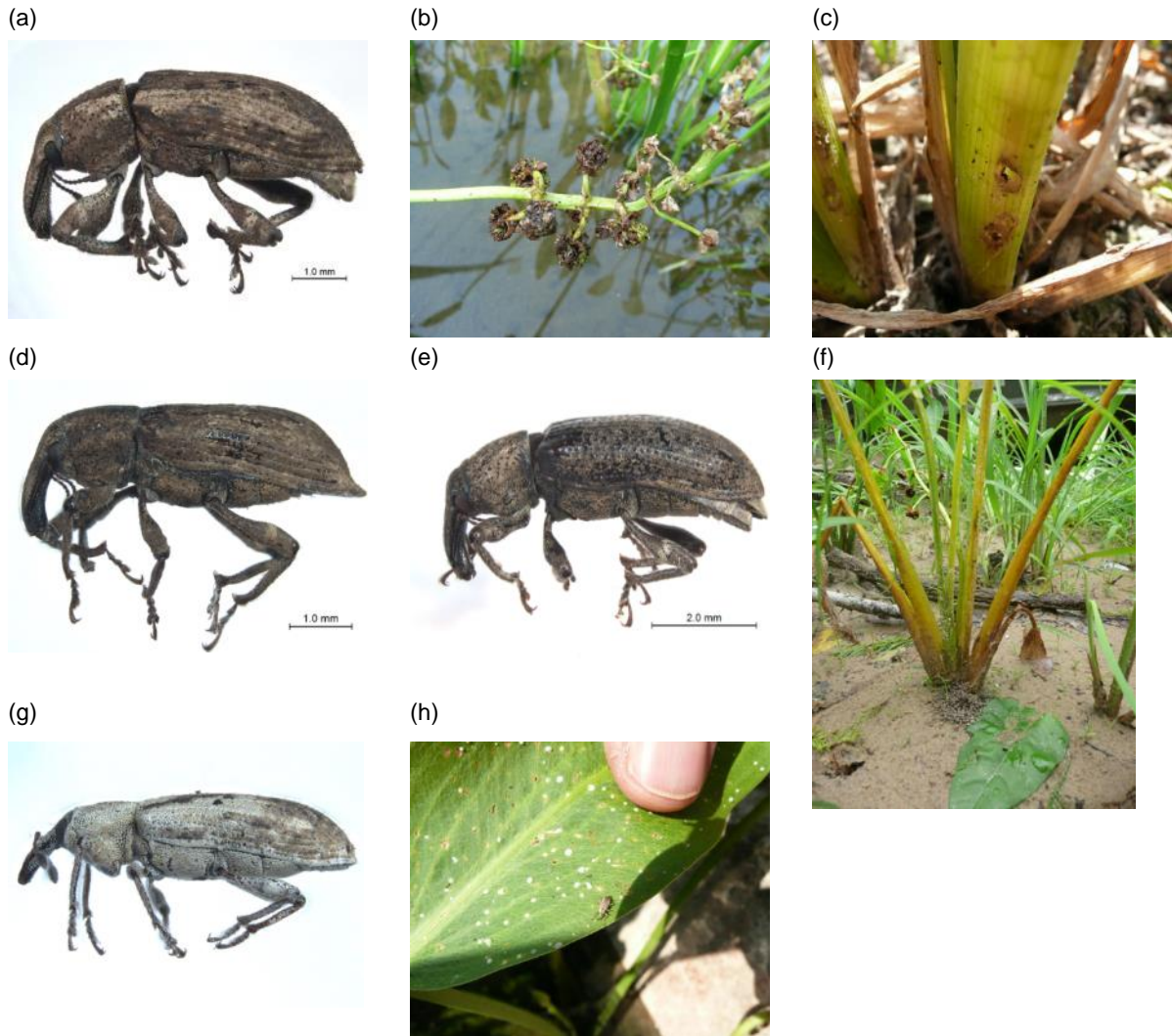
Figure 2.7 *Listronotus* weevil species associated with *Sagittaria platyphylla* in the native range of southern USA. (a) *L. appendiculatus*, (b) *L. appendiculatus* larva-damaged fruiting heads, (c) *L. appendiculatus* larvae bore into the base of petioles where they pupate, (d) *L. sordidus*, (e) *L. frontalis*, (f) crown-damage caused by *L. sordidus*, (g) *L. lutulentus*, (h) small shot holes on a leaf made by *L. lutulentus* adults.

3   Information on the biological control agent

3.1   Taxonomy

Class:	Insecta
Order:	Coleoptera
Superfamily:	Curculionoidea
Family:	Curculionidae
Genus species	*Listronotus appendiculatus* LeConte J.L., 1876 (provisionally accepted name)
Common names:	Sagittaria fruit-feeding weevil

3.2   Biology and ecology of the species

Listronotus appendiculatus (Figure 3.1a) is a small, slender weevil, 4.2-6.5 mm long (Blatchley and Leng, 1916). The biology of *L. appendiculatus* on *Sagttaria latifolia* was studied by Muenchow and Delesalle (1992). On *S. platyphylla* adult *L. appendiculatus* appear in spring (around May in southern USA) when plants come into bloom (Nathan Harms personal observation). Unlike many other *Listronotus* species, *L. appendiculatus* adults are diurnal and are often seen during the day congregating and feeding on male flowers (Kwong personal observation). At night, and during the heat of the day, adults shelter between the petioles towards the base of the plant. Eggs are laid among flower buds, underneath the nodal bracts or deposited underneath the sepals on fruiting heads in small clusters of up to four eggs (Kwong personal observation). Upon hatching, larvae tunnel through the carpels and into the receptacle (Figure 3.1a).

(a)   (b)

Figure 3.1. *Listronotus appendiculatus* (a) adult, and (b) a larva burrowing into the receptacle of the fruiting head.

Larvae pupate in the base of inflorescence stalks or leaf petioles and after eclosion, adults chew a small exit hole and emerge from the petiole (Figure 3.2).

Laboratory studies have shown that at a constant temperature of 25 °C, *L. appendiculatus* eggs hatch within four days, and take a further 20 days for the larvae to reach the adult stage (unpublished data). In the southern USA, at least two generations were observed over summer-autumn (June to September), with oviposition ceasing by October (Kwong et al. 2018). During winter when *Sagittaria* fruits are not present, adults enter into a state of quiescence (dormancy) and seek sheltered places such as leaf litter, to diapause (hibernate). There are no records of *L. appendiculatus* utilising alternative food sources during winter when *Sagittaria* are not flowering.

(a)   (b)

Figure 3.2 *Listronotus appendiculatus* (a) damaged receptacles of the fruiting heads caused by larvae tunnelling through the receptacle tissue, and (b) holes bored into the base of leaf petioles where adults have emerged from inside the stem. Arrows show damage to plant tissue caused by weevil larvae (a) and adults (b).

3.3 Native range of the agent

Listronotus appendiculatus is widely distributed within its native range of north and central America. In the literature, it has been recorded from a number of *Sagittaria* species including *S. australis*, *S. engelmanniana*, *S. latifolia*, *S. lancifolia*, *S. graminea, S. platyphylla* and *S. calycina* (Table 4.1), with a distribution ranging from Canada (from Quebec to British Columbia), the United States (all eastern and central states, California Oregon and Wyoming) and northern Mexico and Honduras (O'Brien, 1977).

3.4 Related species and a summary of their host range

The genus *Listronotus* Jekel, 1865 are widespread in the Americas, from Canada to Argentina and Chile (Maes and O'Brien, 1990; O'Brien, 1977; O'Brien, 1981; O'Brien and Wibmer, 1982; Wibmer and O'Brien, 1986). This distribution corresponds to the Nearctic, Neotropical and Andean regions, as well as the South American and Mexican Transition Zones. In North America, around 20 *Listronotus* species are associated with different *Sagittaria* species (Table 3.1).

In Australia, there are no native *Listronotus* (Morrone, 2013), although two exotic species are present (Atlas of Living Australia); *Listronotus bonariensis* (Kuschel) and *Listronotus setosipennis* (Hustache). *L. bonariensis*, the Argentine stem weevil, was introduced into Australia as adults in ryegrass seed and is a serious pest of pastures in New Zealand (http://www.padil.gov.au/pests-and-diseases/pest/othernames/135862). The stem-boring weevil, *Listronotus setosipennis* was deliberately introduced from South America into Australia in 1982 as a biological control agent for *Parthenium hysterophorus* L (Dhileepan, 2003; Wild et al., 1992).

Table 3.1 Host records *of Listronotus* species associated with Alismataceae in North America. Shown for each species are: the primary feeding niche, extent of host range, and associations with other species within Sagittaria. [a]F = foliage (leaves and petioles); S = sexual reproductive plant parts (flowers and fruit); R = roots and crowns; T = tubers. [b]M = monophagous (restricted to Sagittaria); O = oligophagous (restricted to Alismataceae); P = polyphagous (feeds on several plant families); ? = no indication of host range given in records. [A] *S. variabilis* has been used as a synonym for *S. engelmanniana* and *S. latifolia*, hence the exact host for organisms recorded from *S. variablilis* is unknown. [c] Host records for plants from other families are bracketed.

Order: Family: Genus species	Primary feeding niche[a]	Host range[b]	Hosts within Alismataceae [other hosts][c]	Reference
Coleoptera: Curculionidae				
Listronotus appendiculatus (Boheman) Syn. *Listronotus floridensis* Blatchley	F, S	M	*S. australis* (J. G. Smith) Small, *S. engelmanniana* J.G. Smith, *S. latifolia* Willendow, *S. lancifolia* Linnaeus, *S. graminea* Michaux*, S. platyphylla* (Engelmann) J.G. Smith*, S. calycina* Engelmann	Blatchley, 1920; Ciegler and Wheeler, 2010; Haller, 1993; Harms and Grodowitz, 2009; Henderson, 1940; Kwong et al., 2014; McGaha, 1952; Muenchow and Delesalle, 1992; O'Brien, 1981; O'Brien, 1997
Listronotus caudatus (Say)	F, S, T	P	*S. australis*, *S. latifolia*	Beutenmuller, 1893; Blatchley and Leng, 1916; Ciegler and Wheeler, 2010; Henderson, 1940; Muenchow and Delesalle, 1992
Listronotus (= *Hyperodes*) *crytops* (Dietz)	F, S	O	*S. lancifolia*, *S. graminea*, *Sagittaria* sp.	Blatchley 1920; Blatchley and Leng 1916; Center et al. 1999; Ciegler and Wheeler 2010; Haller 1993; Muenchow 1998; O'Brien 1997
Listronotus delumbis (Gyllenhal) Syn. *Listroderes (= Hyperodes) solutus* Boheman	F	M	*S. latifolia*, *Sagittaria* sp.	Blatchley and Leng 1916; Henderson 1940; Leng 1913; O'Brien 1981; O'Brien 1997
Listronotus echinodori O'Brien	F, S	O	Within Alismataceae: *S. australis*, *S. latifolia*, *Echinodorus cordifolius* (L.) Griseb.	Muenchow and Delesalle 1992; O'Brien 1981; O'Brien 1997)
Listronotus frontalis LeConte	F, S	M	*S. graminea*	Center et al. 1999; O'Brien 1981
Listronotus insignis Henderson	F	M	*S. graminea*, *S. filiformis* J. G. Smith	O'Brien 1981
Listronotuss lutulentus (Boheman) Syns. *Anchodemus angustus* LeConte, *Lixellus lutulentus* (Boheman)	F	M	*S. latifolia*, *Sagittaria* sp. *S. platyphylla*	Beutenmuller 1893; Blatchley and Leng 1916; Kwong et al., 2014; McGaha 1952; Morrone 2013
Listronotus manifestus Henderson	?	M	*S. longiloba* Engelmann, *Sagittaria* sp.	O'Brien 1981; O'Brien 1997

Order: Family: Genus species	Primary feeding niche[a]	Host range[b]	Hosts within Alismataceae [other hosts][c]	Reference
Listronotus nebulosus LeConte	?	?	*Sagittaria* sp.	Beutenmuller 1893; Henderson 1940; O'Brien 1997
Listronotus neocallosus O'Brien	F, S, R	M	*S. engelmanniana, S. graminea, S. filiformis*	Center et al. 1999; O'Brien 1981
Listronotus plumosiventris O'Brien	?	M	*S. latifolia*	O'Brien 1997
Listronotus rubtzoffi O'Brien	F, S	M	*S. cuneate* E.Sheld.	Center et al. 1999; O'Brien 1981
Listronotus scapularis Casey	?	M	*S. longiloba, Sagittaria* sp.	O'Brien1997, O'Brien 1981
Listronotus setosus LeConte	S	M	*Sagittaria* sp.	Blatchley and Leng 1916, O'Brien 1997
Listronotus sordidus (Gyllenhal)	?	M	*S. engelmanniana, S. filiformis*	O'Brien 1981, Henderson 1940)
Listronotus (= Macrops) sparsus Say	F, S	M	*S. latifolia, S. variabilis* [A]	Blatchley and Leng 1916, Beutenmuller 1894, Leng 1913
Syn Listroderes latiusculus Boheman				
Listronotus squamiger (Say)	F	P	*S. latifolia, S. variabilis* [A], [*Schoenoplectus tabernaemontani* (C.C.Gmel.) Palla (Cyperaceae)]	Beutenmuller(1894, Blatchley and Leng 1916, Henderson 1940, O'Brien 1997
Listronotus tuberosus LeConte	?	M	*Sagittaria* sp., *S. latifolia*	Beutenmuller 1893; Blatchley and Leng 1916; Ciegler and Wheeler 2010; Henderson 1940; O'Brien 1997
Listronotus turbatus O'Brien	F, S, R	O	*Sagittaria* spp, *S. engelmanniana*	Center 1999, O'Brien 1981

3.5 **Source of the agent**

Listronotus appendiculatus ex *Sagittaria platyphylla* were sourced from field locations in the southern USA using climate and plant genotype criteria to prioritise collection locations (Table 3.2).

3.5.1 **Climate compatibility**

Climate matching software (Climatch; http://data.daff.gov.au:8080/Climatch/climatch.jsp) was used to identify regions in the southern USA that most closely match the invaded climatic zones of Australia. Based on the Koppen-Geiger climate classification (Kottek et al., 2006), *S. platyphylla* and *S. calycina* infestations from the Riverina bioregion (cold-arid steppe climate represented in light blue in Figure 3.3a) matched similar climates in the United States across Georgia, Alabama, Mississippi, Tennessee, Arkansas and Louisiana (circled area in Figure 3.3b). Field trips to collect agents were focussed on these states. Note: the distribution of *S. platyphylla* in Australia (Figure 3.3a) is much greater than the Riverina with populations occurring along the east coast. However, the Riverina was chosen for climate matching purposes because this is where both *S. platyphylla* and *S. calycina* have reached greatest abundance and cause the greatest impact.

(a) Modified Koppen climate zones overlaid with *S. platyphylla* distribution points.	(b) Climate match between *S. platyphylla* locations in the Riverina bioregion to the USA.
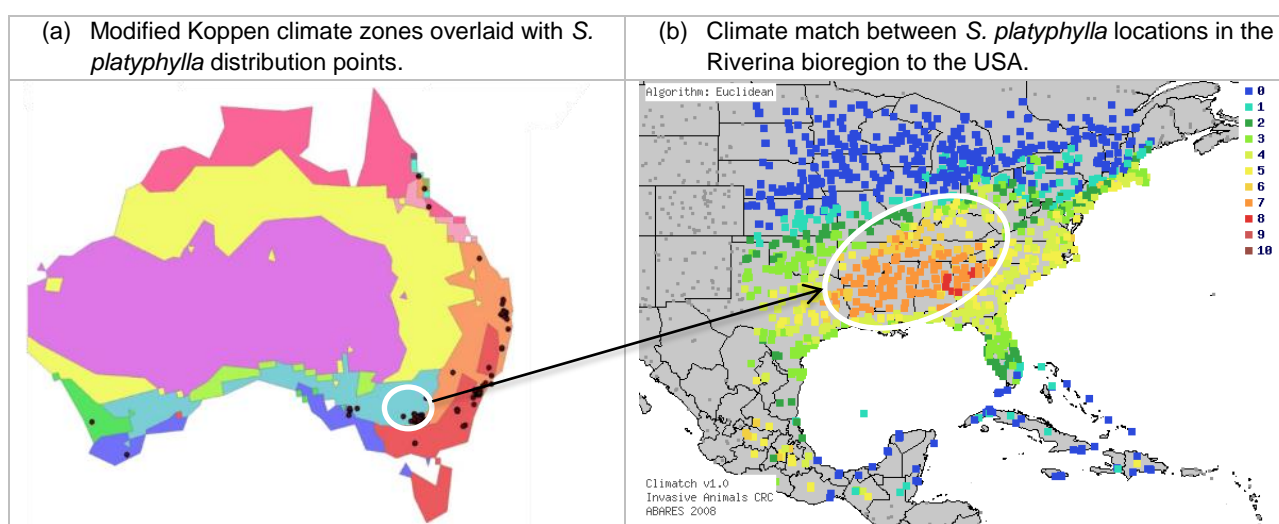
Figure 3.3 Climate match between (a) *Sagittaria platyphylla* in the Riverina bioregion (circled) to, (b) southern USA using Climatch. The highest climate matches are represented by red and orange squares and the lowest by green and blue ones.

3.5.2 **Genetic compatibility**

Levels of genetic diversity in weed populations and compatibility of biocontrol agents to invasive genotypes are two factors critical to the success of biocontrol. Hence, we used the results of our population genetic study to identify USA populations with the closest genetic match to Australian populations (Kwong et al., 2017a). The study identified three main genetic groups (denoted as red, blue and green in Figure 3.4) occurring in the USA. The same three groups are also found in Australia, suggesting that introduced populations may have been founded by multiple sources from the USA. However, in Australia the red genotype is the most widespread (the blue is under eradication efforts) and therefore we focussed our collections of weevils from the red populations in the USA. particularly those in western Tennessee and northern Texas (Figure 3.4b).

(a) Australian genotype locations	(b) USA genotype locations
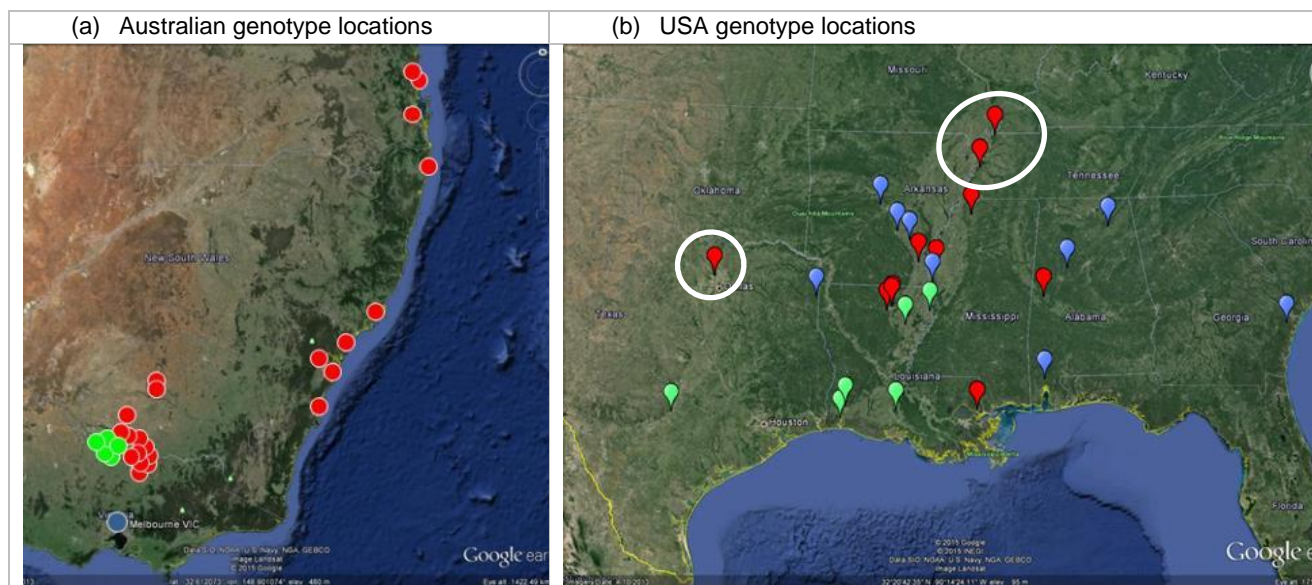
Figure 3.4 Location of *Sagittaria platyphylla* genotypes in (a) Australia, and (b) the USA based on a genetic study using AFLP markers. Collection locations for Listronotus appendiculatus are circled.

Table 3.2 *Listronotus appendiculat*us ex *Sagittaria platyphylla* collection locations in the southern USA.

Region	Location	Importation dates
Western Tennessee	Reelfoot Lake (36.4672, -89.31911) and Sunk Lake (35.7096, -89.73801)	5 October 2015 26 November 2015 2 October 2016
Northern Texas	Lewisville Aquatic Ecosystem Research Facility (33.0695, -96.95852).	5 October 2015 26 November 2015 2 October 2016

A genetic analysis of *L. appendiculatus* populations from these and other locations across the southern USA showed no obvious genetic difference between populations (see Section 4.3 Results – native range studies). Therefore, the testing utilised insects from both the Tennessee and Texas populations, as well as a mixed "Tennessee-Texas" laboratory population.

3.6 **Agent's potential for control of the target(s)**

Listronotus appendiculatus is predominantly a pre-dispersal seed predator. It reduces sexual reproductive capacity of its host plants by feeding on fruiting structures resulting in reduced seed production (Kwong et al. in press). At the Lewisville Aquatic Ecosystem Research Facility in north-eastern Texas, adults were observed in late May (spring) and oviposition occurred shortly after. Larvae were observed in the carpellate fruiting heads from June through to late September (autumn). Two peaks in the mean percentage of carpellate fruit attacked by larvae were observed: July (36.1 ± 38.8 SD) and September (61.5 ± 20.9 SD), suggesting two generations were completed across the season (Kwong et al. in press). During fieldsurveys conducted in 2011 and 2012, the numbers of achenes produced in attacked and non-attacked carpellate fruit were compared. Weevil-attacked fruiting heads produced significantly less achenes compared to undamaged heads and this was consistent across both years (61% less achenes in 2011 and 60% less achenes in 2012) (Kwong, 2016).

In a biogeographical study comparing plant and population parameters between the native (USA) and introduced (Australia and South Africa) ranges, the most striking difference between the ranges was sexual reproductive output. Plants from the introduced ranges yielded an average of 700 achenes per fruiting head, while in the native range, achene production was greatly affected by the damaged caused by *L. appendiculatus*. Where *L. appendiculatus* was absent or in low densities, achene production averaged 500 achenes per head but was reduced to as low as 20 achenes per head at high weevil densities (Figure 3.5). In addition, achenes from introduced plants were 50% heavier than USA plants (Kwong et al., 2017b).
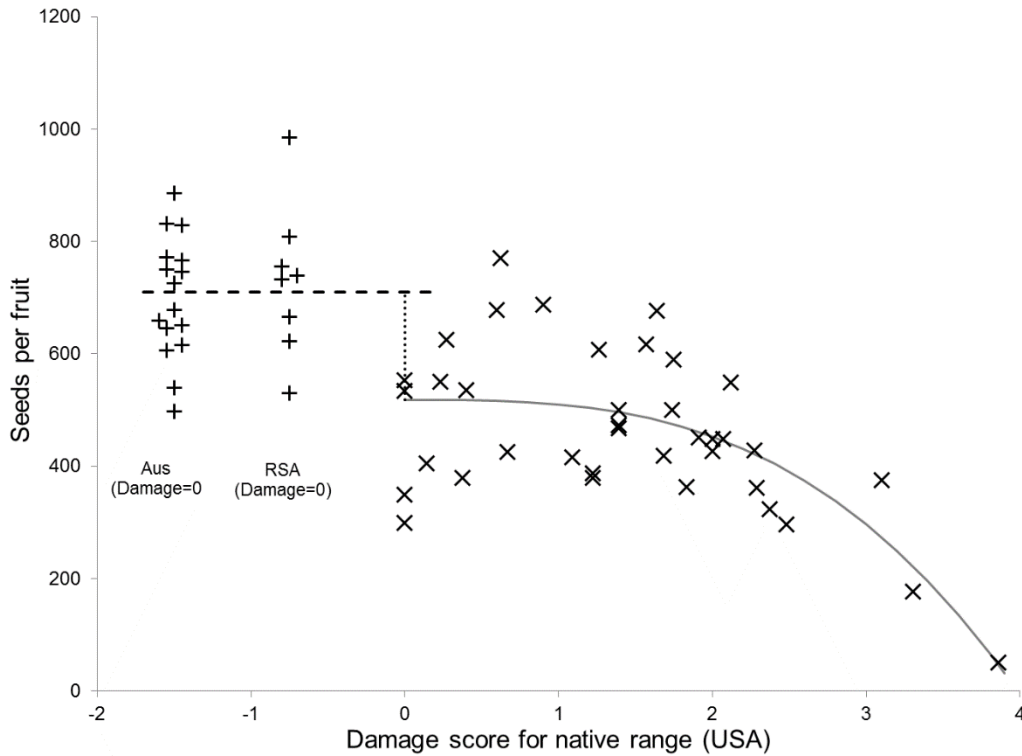
Figure 3.5 The relationship between achenes (seeds) per fruiting head and damage score for fruiting head from native (×) and introduced (+) range populations. Solid line is the fitted curves for the native range. Dashed line indicates average value for introduced ranges, that all have a damage score equal to 0. The dotted vertical line represents the predicted difference between introduced and native ranges, when damage score equals 0. Damage values are: 0 = 0% achenes damaged, 1 = 1 - 20% damaged, 2 = 21 - 50%, 3 = 51 - 80% damaged, 4 = > 80%. (from Kwong et al. 2017b).

3.7 Possible interactions, including conflicts-of-interest with existing biological control programs

Listronotus appendiculatus, if approved for release, will be the first biocontrol agent released against *Sagittaria platyphylla* and *S. calycina* in Australia. Therefore, no negative interactions with existing biological control agents for these weeds are anticipated. Two further candidate agents are also currently undergoing host specificity testing in the AgriBio quarantine facilities in Melbourne. These agents target different plant structures; *Listronotus sordidus* attacks the plant crown, and *Listronotus frontalis* consume the plant's tubers.

3.8 Information on where, when and how initial releases would be made

If approved for release, *L. appendiculatus* will be mass reared at one of DEDJTR's metropolitan (AgriBo) or regional research facilities (Tatura or Rutherglen). Initial releases onto *S. platyphylla* will occur in the Goulburn Murray and Riverina irrigation regions of northern Victoria and southern NSW respectively. Releases onto *S. calycina* will occur in the Riverina region of NSW, where *S. calycina* is widespread.

3.9 Information on whether this species has established invasive populations, and if so, where those populations are

The candidate agent, *Listronotus appendiculatus* has not been deliberately or accidentally introduced to areas outside of its native range. Hence, there are no records of this weevil species establishing invasive populations elsewhere.

3.10 Information on the results of any other environmental risk assessments undertaken on the species in Australia and overseas

This is the first time that *L. appendiculatus* has been investigated for the biological control of *S. platyphylla* and *S. calycina* in Australia. However, the species is under consideration for the biocontrol of *S. platyphylla* in South Africa but results of host testing are not yet available.

3.11 Details on the quarantine facility and methods on containment

Imported insect colonies are being held within DEDJTR's quarantine insectary based at the AgriBio centre, 5 Ring Road, Bundoora, Victoria. This is a DAWR approved facility: (#V2276) Class 7.2.

Containment and handling of all imported insects, including killing of required specimens, will be done according to DAWR quarantine approved directives. All staff involved in the project are accredited and experienced quarantine operators.

If approval to release is subsequently granted *L. appendiculatus* will be mass-reared for release at selected sites. Studies to determine release rates, spatial distribution, delivery systems, and impact of the agent will be conducted.

4 Information on non-target organisms at risk from the agent

4.1 Test list for determining the host-specificity of *Listronotus appendiculatus*

Test plant species were selected based on the modernised approach of the centrifugal phylogenetic method proposed by Briese (2005). This method is underpinned by evidence that specialist natural enemies such as plant pathogens and arthropod herbivores, are more likely to attack (infect or feed upon) plant species that are more closely-related to their natural host than evolutionarily-distant plant species (Gilbert et al. 2012). Within this phylogenetic/evolutionary framework, the traditional practice of testing "safeguard" species of distant phylogenetic relatedness has become redundant, as testing such species do not add to the statistical strength of the risk analysis (Briese and Walker 2002, Sheppard et al. 2005).

The test list for determining the host specificity of *Listronotus appendiculatus* included representative genera based on the molecular phylogeny of the Alismataceae family (Chen et al. 2012, 2013, see Figure 4.1), with an emphasis on Australian native species, species of economic importance and those that are likely to overlap biogeographically with the target weeds, *S. platyphylla* and *S. calycina*. Genera and/or species not present in Australia were omitted from testing (see Table 4.1). Alismataceae expert, Professor Brian Keener, University of West Alabama was also consulted to confirm the identity of the *Sagittaria* species naturalised in Australia.

Of the seven native Alismataceae, only four could be sourced. Two temperate species, *Alisma plantago-aquatica* and *Damasonium minus* commonly co-occur with *S. platyphylla* and *S. calycina.* Two of the three native *Caldesia* species (*Caldesia oligococca* and *C. acanthocarpa*) were sourced from the Darwin region (Northern Territory) and in the Lakefield area of Cape York Peninsula (Queensland). Seeds of *Astonia australiensis* were also sourced from the Lakefield area but were unable to be successfully germinated.

Achenes from *Astonia australiensis* were sourced from Cape York Peninsula but attempts to germinate the achenes were unsuccessful. Populations of *Butomopsis latifolia* around Darwin could not be found during collecting trips in 2014 and 2015. The species is also reported to occur in west Arnhem Land but these were too remote and inaccessible. *A. australiensis* and *B. latifolia* are ranked as lower priority species for testing as their native distributions do not overlap with the current distribution of *S. platyphylla* and *S. calycina.*

Four ornamental species were tested, including two *Sagittaria* (*S. latifolia*, *S. subulata*), *Echinodorus cordifolius* and *Hydrocelyes nymphoides*. The naturalised species, *Alisma lanceolatum* was added to the test list as it is closely-related to the native species, *A. plantago-aquatica*.

Two unrelated plant species were included. The native species, *Cycnogeton procerum* (R.Br.) Buchenau (Syn = *Triglochin procera*) (Juncaginaceae) was included because it commonly occurs in sagittaria-invaded habitats and has emergent inflorescences with fleshy fruit. The crop species, *Oryza sativa* (rice) (Poaceae) was included because *S. platyphylla* and *S. calycina* are common weeds of rice crops in New South Wales.

The final test list including details of where plants were sourced, is outlined in Table 4.2.
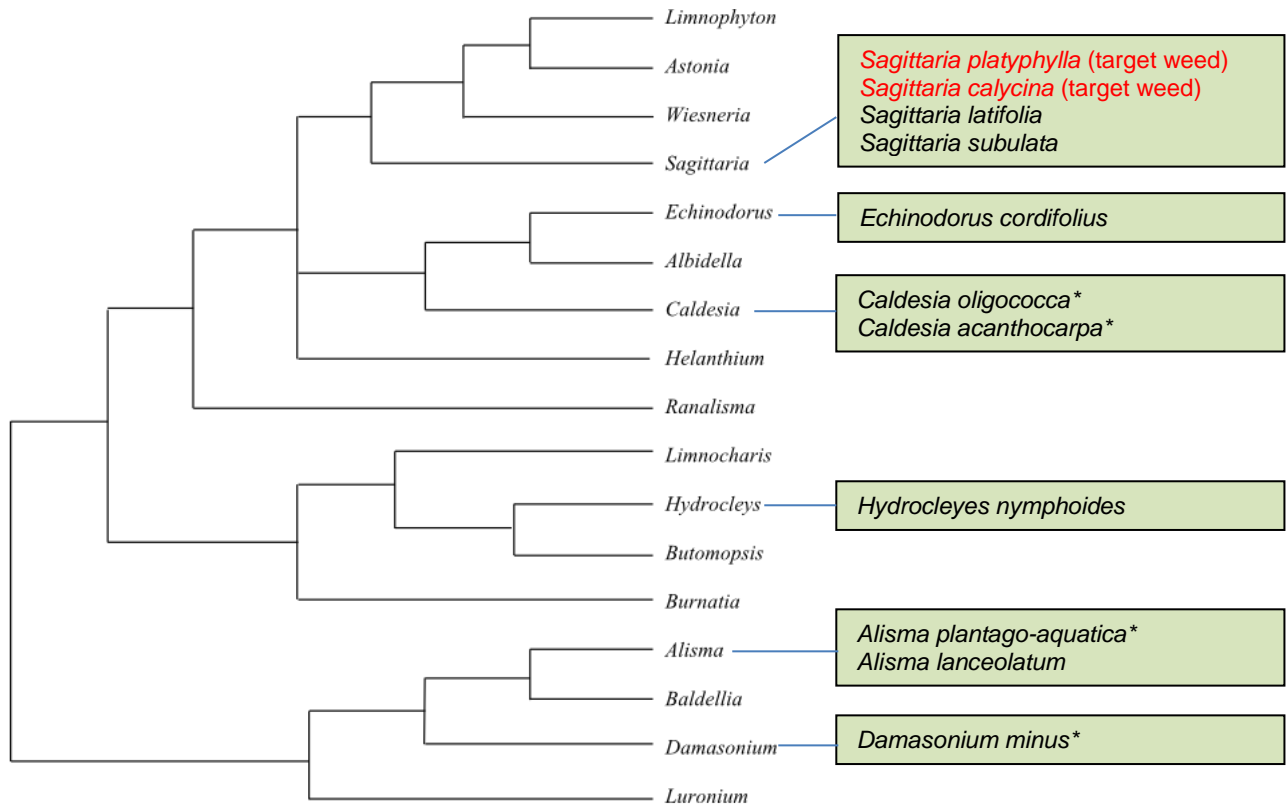
Figure 4.1 Molecular phylogeny of Alismataceae (Chen et al. 2012, 2013) with the taxonomic relationships between *Sagittaria* spp. and other plants used in the host range tests for *Listronotus appendiculatus* shown in boxes. Australian native species are indicated by an Asterix.

Table 4.1 Justification for selection of representative plant species for the host specificity of *Listronotus appendiculatus*.

Genus	Justification for testing	Species included in host specificity testing
Lymnophyton	There are no native or introduced members of this genus present in Australia.	Not included
Astonia	*Astonia australiensis* (Aston), could not be tested due to difficulties germinating the seeds.	Not included
Wiesneria	There are no native or introduced members of this genus present in Australia.	Not included
Sagittaria	There are no native *Sagittaria* species present in Australia. *Sagittaria* species that could be sourced from commercial suppliers were tested. *S. lancifolia* was not tested as its sale in Victoria become illegal in 2012 when it was declared a noxious weed. *S sagittifolia* was not tested as the form present in Australia is sterile and does not produce fruiting structures (https://greenharvest.com.au/Plants/Information/Arrowhead.html).	*S. platyphylla* (primary target) *S. calycina* (secondary target) *S. subulata* *S. latifolia*
Echinodorus	There are no native *Echinodorus* species present in Australia. Except for *E. cordifolius*, all other species are utilised in the aquarium trade as submerged aquatic plants that rarely produce flowers. *E. cordifolius* is grown in outdoor water gardens and produce emergent leaves and inflorescences.	*E. cordifolius*
Albidela	There are no native or exotic species present in Australia.	Not included
Caldesia	Two of the three native species were included in the test list.	*C. oligococca* F.Muell. *C. acanthocarpa* F.Muell.
Helanthium	There are no native species present in Australia. In Australia, *Helanthium* are used as submerged aquarium ornamentals.	Not included
Ranalisma	There are no native or exotic species present in Australia.	Not included
Limnocharis	No native species present in Australia. *L. flava* is a declared noxious weed in Queensland.	Not included
Hydrocleyes	No native species present in Australia. *H. nymhoides*, although naturalised in QLD, NSW and VIC, it is still sold as an aquatic ornamental, therefore it was included.	*Hydrocleyes nymphoides*
Butomopsis	This native species is difficult to propagate and occurs in remote locations in western Arnhem Land (NT). Plants could not be sourced from native populations.	Not included
Burnatia	There are no native or introduced members of this genus present in Australia.	Not included
Alisma	Only one *Alisma* species (*A. plantago-aquatica*) is native to Australia and this was included in all tests. Oviposition tests conducted on *A. lanceolatum*, but as this species has no importance in Australia, it	*Alisma plantago-aquatica* L. *Alisma lanceolatum* L.
Baldellia	There are no native or introduced members of this genus present in Australia.	Not included
Damasonium minus **(R.Br) Buchenau**	Temperate endemic species widespread in SE Australia.	Native
Luronium	There are no native or introduced members of this genus present in Australia.	Not included

Table 4.2 Test plant list used in the host specificity testing of *Listronotus appendiculatus*, a candidate biological control agent for *Sagittaria platyphylla* and *S. calycina*.

Genus	Species	Status (target weed, native, naturalised, ornamental or crop species). * = introduced	Source of test plants	Location(s)
Family: Alismataceae				
Sagittaria	*platyphylla*	Target*	Field	Numurkah, VIC (-36.106, 145.3635). Docklands, VIC (-37.818703, 144.946334). Barmah National Park VIC (-35.940510, 144.969987).
	calycina	Target*	Field	Coleambally NSW (-34.838682, 145.858734). Rosewood NSW (-34.766168, 145.977886).
	latifolia	Ornamental*	Nursery	Bells Watergardens, Newlyn, VIC (-37.39603, 143.99675).
	subulata	Ornamental*	Nursery	Bells Watergardens, Newlyn, VIC (-37.39603, 143.99675).
Echinodorus	*cordifolius*	Ornamental*	Nursery	Aquarium Gallery, Perth, WA (-31.791557, 115.823784).
Caldesia	*oligococca*	Native	Field	Knucky's Lagoon, NT (-12.429960, 130.946367). Howard Springs NT (-12.422226, 131.099571). Lakefield, QLD (seeds provided by Robin Adair).
	acanthocarpa	Native	Field	Lakefield, QLD (seeds provided by Robin Adair).
Hydrocleyes	*nymphoides*	Ornamental*	Nursery	Bells Watergardens, Newlyn, VIC (-37.39603, 143.99675). Gardenworld, Braeside VIC (-38.000312, 145.116181).
Alisma	*plantago-aquatica*	Native	Field	Dandenong wetlands, VIC (-38.017975, 145.238988). SE Water nursery (-38.068231, 145.170371). Karingal VIC (-38.140782, 145.171247). Nagambie VIC (-36.850751, 145.068270).
	lanceolatum	Naturalised*	Field	Paterson Lakes (-38.066586, 145.153794).
Damasonium	*minus*	Native	Field	Karingal VIC (-38.140782, 145.171247).
				Rosewood NSW (-34.766168, 145.977886).
				Coleambally NSW (-34.838682, 145.858734). Mincha, VIC (-35.963340, 144.097338).
Family: Juncaginaceae				
Cycnogeton	*procerum*	Native	Field	Langwarrin South VIC (-38.185669, 145.172089)
			Nursery	SE Water nursery (-38.068231, 145.170371).
Family: Poaceae				
Oryza	*sativa* L.	Crop*	Supplied by farmer	Finley, NSW (seeds provided by rice farmer - 35.550617, 145.667029)

4.2 **Materials and Methods**

The specificity of *L. appendiculatus* followed internationally-accepted protocols as outlined by Sheppard et al. (2005), using a three-stage process. While both *S. platyphylla* and *S. calycina* were nominated as "targets" for biocontrol by *L. appendiculatus*, for ease of testing only *Sagittaria platyphylla* was used as the designated "control" species. Hence, *S. calycina* was treated as a test species for adult oviposition and larval development trials.

5. Container trial: In these trials, adults were placed in a testing arena (plastic container) with only one (**no-choice single species)** or two test species (**choice-minus-target**), but not with the target. The target species (i.e. *S. platyphylla*) was offered to adults in a separate container. For both no-choice and choice-minus-target trials, bouquets of cut foliage and flowers were presented to adults and assessed for oviposition as well as levels of foliage and fruit herbivory.
6. **No-choice whole plant oviposition trials:** In these trials, Australian native plant species for which some oviposition and egg hatch had occurred in the container trials, were subjected to no-choice whole plant oviposition trials on potted plants contained within gauze sleeves. In these trials, oviposition (egg laying) and survival to adults was assessed.
7. **Whole plant larval development trials:** In these trials, a set number of mature eggs were placed on whole plants to assess the survival rate from egg to adult as well as to assess the damage caused by larval feeding on leaf petioles and flowering stems.
8. **Continuation trial:** As adults had emerged from the native species, *Damasonium minus* in the larval development trials a continuation trial was conducted to assess the reproductive performance of these F_1 adults and hence, the ability of *L. appendiculatus* to maintain a viable population on *D. minus*.

Data collected from these three trials were then used to evaluate two key biocontrol agent risk factors:1) the ability of *L. appendiculatus* to oviposit, survive and maintain viable populations on non-target plant species, and 2) the damage caused by adult and larval feeding on non-target species.

Trial 1. Adult no-choice and choice-minus-target oviposition trials

During the 2015/16 and 2016/2017 summer period, adult feeding and oviposition trials were conducted on ten test species, with *S. platyphylla* as the control. Most trials were conducted using the **choice-minus-target** design. For each trial, two separate test plant species were added to one rectangular plastic container (21 x 24 x 10 cm, width x height x depth), with a separate "control" container of only *S. platyphylla* being placed alongside. Each test species and control consisted of a bouquet of two flowering inflorescences and four leaves with the base wrapped in moistened paper towel and placed inside a plastic specimen jar (Figure 4.2a). To ensure true experimental replication, a different "mother" plant was used for each replicate test plant and control.

To each container, 10 *L. appendiculatus* weevils were added (5 females and 5 males), with the sex of each weevil determined based on its size (females are larger than males). The weevils were observed for oviposition prior to being used in the trial, to ensure that only sexually mature, gravid females were used during the oviposition trials. Sexually mature, as opposed to newly-emerged females were used to ensure that oviposition coincided with the availability of suitable test plant material at the right stage for oviposition (i.e. fresh flowers and immature fruits). The advantage of using sexually mature females was that if no oviposition occurred on a test species, it was most likely due to the unacceptance of the plant rather than the female being immature.

The paired species test followed a concurrence matrix design where different species were paired across the duration of the trial, mostly dependent on the timing and availability of flowering plants.

Some of the test species came into flower much later than the other test species and therefore could not be included in the paired species trials, hence these were tested separately using **a no-choice single species** method. In such cases, one bouquet of the test species was placed singly in a rectangular plastic container

with five pairs of *L. appendiculatus* adults (Figure 4.2b). At least one *S. platyphylla* container was set up for each series of trials. The number of species pairs tested per date was based on the availability of sexually-mature weevils. New adults were used for each series of trials.

Trials were conducted in a controlled environment room (CER) at 24°C, 16hr light, as these conditions had been proven as optimal for mating, oviposition and insect development (Kwong personal observations). Adults were removed after four days to prevent too many eggs from being laid, as well as to enable the eggs to be counted prior to hatching (eggs hatch in four days at 25°C). For each test species, the number of eggs laid on inflorescences, and the number of fruit damaged by weevil adult and larval feeding were recorded. The leaves of each test species and control were dried in a plant press and then scanned. Image J (Schneider et al., 2012) was then used to calculate the area of each damaged and whole leaf.

(a) (b)
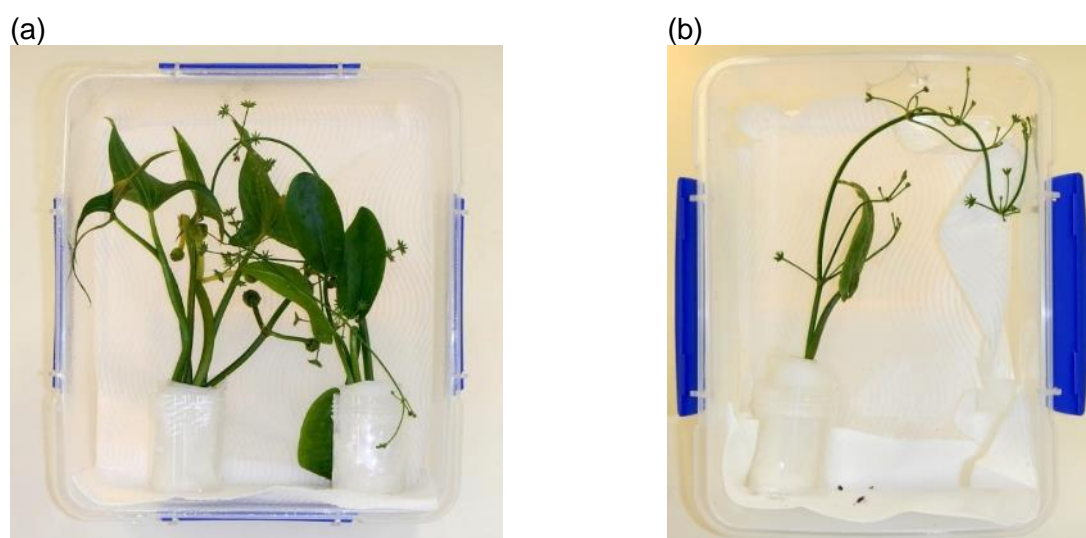
Figure 4.2 Container designs: (a) choice-minus-target *Sagittaria calycina* paired with *Damasonium minus*, and (2) single species trial with only *D. minus* present. For each trial, a separate control container (not shown) with a bouquet of *Sagittaria platyphylla* was set up.

Trial 2. No-choice whole-plant adult oviposition and larval survival trials on native species

An additional oviposition trial was conducted focusing primarily on native species, to determine if *L. appendiculatus* would oviposit on whole plants in a no-choice situation, and if so, whether larvae could complete development to adult stage.

Between March to June 2016, no-choice adult oviposition and larval development trials were conducted on native species that had shown some level of oviposition and larval survival during the container trials (i.e. *Damasonium minus, Alisma plantago-aquatica* and *Caldesia oligococca). Caldesia oligococca* could not be tested however, as no fruiting plants were available. Instead, another closely-related species (*C. acanthocarpa*) was tested in its place (see below). *Cycnogeton procerum* was excluded from this and all further tests because oviposition and adult feeding results from Trial 1 indicated that this species was not an acceptable host.

Although the focus of this trial was on Australian native species, the non-native species, rice (*Oryza sativa*) was included as it was not available during Trial 1.

For each replicate series, at least the same number of *S. platyphylla* were included as controls. Ten sexually mature adults (5 large and 5 small) were released onto individually potted, gauze-covered flowering plants of each test species (Figure 4.3). For each time series, two plants of each species were set up; one for adult oviposition and one for larval development. Pots were placed randomly into rectangular aquatic tubs held in the quarantine glasshouse at around 24°C. The natural light was augmented with florescent lights in the morning and evening to maintain a daylength of 16 hr light. Trials were replicated in time (referred to as "series" in the data files), based on the availability of flowering plants and reproductive adults. After four

days, the adults were retrieved from each plant. For the adult oviposition trials, the inflorescences from each test and control plant were excised from the plant and the number of eggs counted. For the larval development trials, the plants were re-covered with their gauze bags following adult removal and kept in the quarantine glasshouse at 24°C, with natural light augmented with additional lighting to maintain a daylength of 16 hrs. After five to six weeks, plants were inspected for adult emergence, and dissected and checked for larvae and pupae.

In April 2017 and April 2018, *Caldesia acanthocarpa* was tested as this species was not available in 2016 when the other species were tested. A total of six replicates were set up using the same method described above except, due to the limited number of plants available, the larval development tests were not set up concurrently as they were in 2016.

(a)   (b)
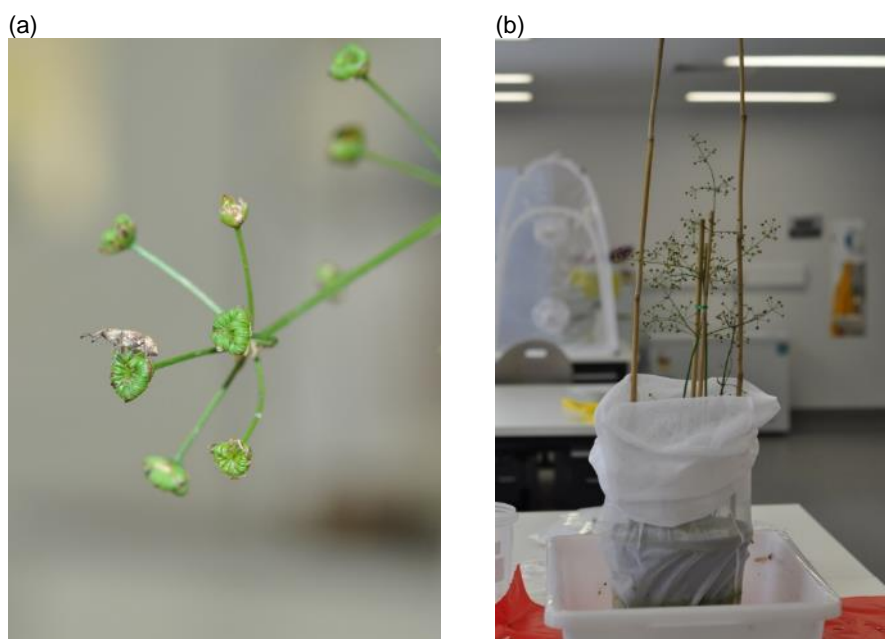
Figure 4.3 *Listronotus appendiculatus* (a) adult sitting on the fruiting inflorescence of *Alisma plantago-aquatica*. (b) A gauze sleeve used to confine *L. appendiculatus* weevils onto potted test plants (*A. plantago-aquatica* pictured) in adult no-choice oviposition and larval development trials.

Trial 3. No-choice whole-plant larval development trial

Between February and April 2017, no-choice larval development trials were conducted on the following species: *S. calycina*, *D. minus*, *S. latifolia*, *A. plantago-aquatica* and *E. cordifolius*. This trial focused on species with emergent growth habits, as the larvae of *L. appendiculatus* do not feed within submerged petioles or fruiting heads. As such, *Sagittaria subulata* was excluded from the trial as the petioles and scapes (flowering stems) of this species are predominantly submersed.

Eggs were collected from adult oviposition containers using *S. platyphylla* as the host, and placed in petri-dishes at 24°C, 16 hr light for three to five days and inspected daily for egg maturation. A total of 20 fertilised eggs were used per replicate. Eggs were attached using cotton string to flowering inflorescences of potted test plants and a *S. platyphylla* (control). Plants were enclosed in gauze sleeves and placed randomly into rectangular aquatic tubs in the quarantine glasshouse under the same lighting conditions described above for the no-choice oviposition whole plant trials. Every week, pots were haphazardly relocated to other tubs on the bench. After eight weeks, plants were inspected for adult emergence. The number of leaves/petioles with larval tunnelling damaged was recorded. Trials were replicated in time based on the availability of flowering plants and reproductive adults. Across the duration of the trial, a total of ten replicates were set up for *D. minus,* nine each for *A. plantago-aquatica, S. latifolia* and *S. calycina* and six for *E. cordifolius.* A total of 11 replicates were set up for *S. platyphylla* controls.

In April 2017 and 2018, a no-choice larval development trial was conducted on *Caldesia acanthocarpa* using the same methods applied above. Due to the limited availability of flowering plants, only three replicates

were conducted in 2017 and a further two replicates in 2018. For each *C. acanthocarpa* replicate, a *S. platyphylla* control was also set up.

Trial 4 Continuation trial

First generation adults (F_1) that had emerged from the no-choice larval development trials were individually weighed and placed into plastic containers lined with moist paper towel into which bouquets of foliage and inflorescences from the host plant from which they emerged, were provided. Only *Damasonium minus*, *S. calycina* and *S. platyphylla* plants produced enough adults to enable the subsequent oviposition study to be conducted. Initially, the adults that had emerged from all replicates of *D. minus* were grouped together within a feeding container. This was also done for the *S. calycina* and *S. platyphylla* replicates. Fresh food was changed every three to four days and inflorescences inspected for oviposition. After oviposition was observed (on the 28 April 2017), four groups of adults consisting of five females and between four and five males (depending on the availability of males) were collected from each test plant container. Each group was set up into a new rectangular container in the same manner as for adult no-choice container trials, with a bouquet of test plant foliage and inflorescences added. Every three to four days, bouquets were inspected for eggs, and fresh ones added. As adults died, they were dissected to determine their sex. The trial was concluded on 3 June 2017, because fruit was becoming scarce due to the onset of winter. The adults were placed into hibernation in the CE room at 10°C, 10 hr light until 25 September 2017 and then inspected for survival.

Trial 5. Achene predation trial

As the larval starvation trial showed that *L. appendiculatus* larvae could feed on and develop on the native plant, *D. minus*, a feeding trial was set up to quantify the number of achenes each larva could consume. The trial was set up on 8 January 2018 using three-day old eggs collected from the oviposition containers. Plastic specimen jars were lined with filter paper and sprayed with reverse osmosis water to moisten. A fresh sprig of a fertilised inflorescence (approx. 5 cm long) was added to each jar. For *S. platyphylla*, one fruiting head was added, and for *D. minus* the terminal end including one complete whorl of fruit was used. To each jar, one *L. appendiculatus* egg was gently placed onto the fruit. The jars were sealed with a screw lid and placed into the CE Room at 23°C, 16 hr light. A total of ten replicates were set up for each plant species. Fresh fruit was added every 4 days and the filter paper re-moistened if necessary. On 31 January 2018 (23 days later), the trial was assessed. The number of *D. minus* achenes consumed by each larva was counted by visual inspection under a dissecting microscope. For *S. platyphylla* however, the fruit were often destroyed to the point that individual achenes could not be counted. Hence, the number of achenes destroyed were estimated based on the level of destruction of each fruiting head, assuming that each fruiting head produced an average of 700 achenes (based on achene production data for Australian *S. platyphylla* in Kwong et al., (2017)).

Native range studies

To gain a better understanding of the host utilisation of *L. appendiculatus* in its native range, field surveys were conducted in the southern USA over two years (August/September 2015 and 2016). The objectives of the study were to determine if host races (biotypes) exist in *L. appendiculatus* that are: (1) aligned to different genotypes of *S. platyphylla*, and/or (2) aligned to sympatric plant species (i.e. closely-related species growing in the same geographic areas).

Sampling primarily targeted locations where known *S. platyphylla* genotypes had previously been surveyed (see Kwong et al, 2017a). If plants of other Alismataceae species were found at the site, they were also sampled, except for Ghost River in Tennessee and Mingo National Park in Missouri, where *S.platyphylla* did not co-occur with the other species sampled. At each site, up to 20 plants were collected and dissected to search for larvae feeding within the fruiting heads, petioles and inflorescence stems. Larvae were extracted

and squashed onto filter paper cards (FTA® cards) to preserve the insect DNA. Adult specimens observed on plants were also collected and preserved in vials of 100% ethanol.

DNA was extracted from preserved adult and larval specimens using DNeasy Blood and Tissue extraction kit (Qiagen), following the manufacturers protocol. PCR amplification and sequencing of the mitochondrial cytochrome oxidase I (COI) gene followed standard protocols as outlined in Blacket et al. (2015), except that specific primers were developed for *Listronotus*. To identify the specimens, the DNA sequences were then compared to a reference library of known species extracted from the BOLD database (http://www.boldsystems.org). In addition, we included *L. appendiculatus* and *L. echinodori* adult specimens that had been identified by curculionid expert, Charles O'Brien, as well as specimens from our quarantine laboratory cultures used in the host specificity tests. The phylogenetic relationship between specimens was constructed using GENEIOUS (https://www.geneious.com/).

Statistical analyses

Trials 1 and 2: Adult no-choice and choice-minus-target container and whole-plant trials

For the container and whole plant trials, the square root of the number of eggs from each bouquet or plant was analysed as a general linear model with additive terms for Series (date or trial) and Species. In the container trial however, the analysis for *S. calycina* paired with another species, and when not paired (i.e. no-choice trial) were formally considered as two separate species, so that the experimental effect of pairing with another species could be examined. For both the container and whole-plant trials, a hypothesis test for the effect of species was based on the F statistic for Species adjusted for Series, and the P value calculated by comparing the F statistic to the F distribution. This was based on 4, 19 degrees of freedom for the container trials, and 3, 24 degrees of freedom for the whole-plant trial. *Caldesia acanthocarpa* was omitted from the analysis of the whole-plant trials as this was conducted in a separate year to the other species, and, no oviposition was recorded.

Trial 3: No-choice whole plant larval development trial

The number of adults emerging from 20 eggs placed on each test species and *S. platyphylla* control was analysed as a logistic regression with binomial errors with estimated overdispersion. The overdispersion parameter and overdispersion degrees of freedom were obtained using only data from *S. platyphylla*, *S. calycina* and *D. minus* to avoid using species with mostly zero adult counts, which would bias the estimates of the dispersion downwards. *C. acanthocarpa* was excluded from the statistical analysis as explained in the previous section. T-tests were used to compare the average weight of *L. appendiculatus* adults emerging from test plants.

Trial 4. Continuation trial

The cube root total number of eggs laid throughout the duration of the trial was analysed as a one-way analysis of variance.

Overall assessment of the reproductive rate of *L. appendiculatus* on single plant species over multiple generations

A detailed analysis of the reproductive rate of *L. appendiculatus* was conducted to determine if viable populations could be maintained on different test plant species over two generations. The analysis only included oviposition data obtained from no-choice container and pot trials. This was to reduce any possible biases in the oviposition data from choice-minus-target trials that might have be influenced by the presence of another test species in the testing arena.

Firstly, the number of eggs counted in the single species no-choice container trials (Trial 1), the no-choice whole plant trials (Trial 2) and continuation trial (Trial 4) were each separately analysed using a generalised linear model with Poisson distribution, over-dispersion parameter and logarithmic link, and with an effect of species in the model.

Secondly, the proportion of eggs that survived to adult in the no-choice larval development trial (Trial 3) was analysed using a generalised linear model with binomial distribution (n =20, the number of eggs added to each pot), over-dispersion parameter and logistic link, and with an effect of species in the model. Since *Alisma plantago-aquatica*, *Echinodorus cordifolius*, *Sagittaria latifolia* and *Caldesia acanthocarpa* produced very few adults, which can lead to severe under-estimation of the over-dispersion parameter, the over-dispersion parameter for the proportion of eggs that survived to adult was estimated using a prior analysis on a reduced data set that excluded plots with *A.plantago-aquatica*, *E. cordifolius*, *S. latifolia* and *C. acanthocarpa*. However, the estimation of species effects used the complete data set.

Except for the continuation trial (Trial 4), in which there was only one start date, an extra additive effect of start date (series) on the link scale was examined for all other trials using a deviance ratio F test. To avoid over-sensitivity due to under-estimation of the over-dispersion parameter for the proportion of eggs that survived to adult in the larval starvation trial (Trial 3), the *A.plantago-aquatica*, *E. cordifolius* and *S. latifolia* pots were excluded from this test. It was found that all these series effect tests were not statistically significant ($P > 0.05$), and thus the species estimates produced in these analyses were not adjusted for series.

From these individual analyses, for several species, we have obtained
  i. An estimate of the expected number of eggs ($\widehat{\mu_X}$) produced over 4 days by 10 adults from the first-generation oviposition trial and, in some cases from single plant species containers in the firs-generation oviposition container trial.
  ii. An estimate of the expected number of adults per egg ($\widehat{\mu_Y}$), from the first-generation component of the starvation trial, and
  iii. An estimate of the expected number of eggs produced over 33 days by 10 adults from the second generation ($\widehat{\mu_Z}$), in the oviposition component of the starvation trial.

If we make the extra assumptions that:

  a) there is a 50:50 male/female reproductive split,
  b) all adults are reproductive for 90 days, and reproduction is consistent over the 90-day period,
  c) the number of adults obtained per egg in the second generation is the same as was obtained in the first generation, for each species, and
  d) the 3 component experiments were carried out independently,

then the expected first-generation reproductive rate (number of adult female second generation progeny produced from each adult female (Nf)) can be calculated as:

$\mu_{Nf} = (90/(4\times5))\mu_X \times \mu_Y/2 = 2.25 \times \mu_X \times \mu_Y.$

and estimated as

$\widehat{\mu_{Nf1}} = 2.25 \times \widehat{\mu_X} \times \widehat{\mu_Y}$

This estimate was calculated for a number of species.

Then the expected second-generation reproductive rate (number of adult female second generation progeny produced from each adult female (Nf)) can be calculated as

$\mu_{Nf} = (90/(4\times5))\mu_X \times (\mu_Y/2)2 \times (90/(33\times5))\mu_Z = (27/44)\times \mu_X \times (\mu_Y)2 \times \mu_Z.$

and estimated as

$\widehat{\mu_{Nf2}} = (27/44) \times \widehat{\mu_X} \times ((\widehat{\mu_Y})^2 + (se(\widehat{\mu_Y}))^2) \times \widehat{\mu_Z}$; where se denotes standard error.

This estimate was calculated for a number of species.

Predicted Reproductive Risk

The predicted reproductive risk for each species was then calculated from the individual analyses of reproductive rate to provide a combined risk score relative to that of the target weed, either *S. platyphylla* or *S. calycina.* This was conducted separately for both whole-plant and container trials.

Hence, the combined risk score for a species, compared to a standard (target) species, was calculated using the first-generation reproductive rate, as:

$$\text{CRS1}(species) = \widehat{\mu_{Nf1}}(species) / \widehat{\mu_{Nf1}}(standard)$$

A combined risk score across two generations for a species, compared to a standard species, was calculated using the second-generation reproductive rate, as:

$$\text{CRS2}(species) = \sqrt{\widehat{\mu_{Nf2}}(species) / \widehat{\mu_{Nf2}}(standard)}$$

These risk scores are directly comparable to other combined risk scores that have been developed for predicting the risk of non-target attack in host specificity tests (see Paynter et al. 2015; Paynter et al. 2017).

All analyses used a single pot or container as the unit of analysis. All analyses were carried out using the generalised linear model facilities in GenStat 18 (VSN International (2015)).

Overall assessment of plant damage

Leaf damage by adult weevils

The analysis of percentage leaf damage (LD%) was analysed separately for plants in no-choice and those in choice-minus-target container trials, since the experimental environment was different for these two situations. After a logit((LD%+0.1)) transformation (logit(X%) = log((X%/100)/(1-(X%/100)))) the plants in no-choice containers were analysed using a general linear model. After the same logit((LD%+0.1)) transformation, the plants in choice-minus-target containers were analysed using a restricted maximum likelihood (REML) mixed model with a random effect for the container the plant was included in. The random effect was allowed to have a 'negative variance', which created a negative correlation between the two plants in the same container. This negative correlation is biologically sensible because, at any specific time, if a weevil is eating one plant in a container it cannot be eating the other plant in a container. In both analyses, F tests were carried out to determine fixed effect terms in the model. These F tests examined effects related to species of plant, series and, in the case of the paired container analysis, the species of the other plant in the same container.

Fruit damage

The proportion of fruit damaged on a plant (p) was analysed using binomial logistic modelling with an estimated overdispersion parameter. The unit of analysis was an individual plant, and the binomial totals were the number of fruit on a plant. The *Sagittaria latifolia* plants were excluded from these analyses because these plants had no fruit (they bear only male flowers). A model was fitted that only had an effect of species, so that species could be compared. An F test was carried out to determine if an effect of series would improve the model. The proportions of fruit damaged were converted to percentages damaged by multiplying by 100. Analyses were carried out using the generalised linear model facilities in GenStat 18 (VSN International (2015)).

Stem (petiole and scape) damage

The proportion of stems damaged on whole plants during the larval development trial (Trial 3) was analysed using binomial logistic modelling with an estimated overdispersion parameter. The unit of analysis was an individual plant, and the binomial totals were the number of petioles on a plant. The *A. plantago-aquatica* and *S. latifolia* plants were excluded from these analyses because the total number of petioles on a plant were not recorded for all replicates, although the small number of stems damaged has still been reported. To avoid under-estimation of the overdispersion parameter from treatments with low damage, the overdispersion parameters, and the degrees of freedom for those overdispersion parameters, in each model examined came from a similar model (reduced model) that excluded species with no petioles damaged (i.e. from *E. cordifolius* and *Caldesia acanthocarpa*). However, the analyses reported included all the species; only the overdispersion parameter and overdispersion degrees of freedom came from the reduced analysis. F tests were carried out to determine fixed effect terms in the model. These F tests examined effects related to species of plant and series. The only fixed effect included was a term for the species of the plant as not

effect was found for series ($F_{7,25}$ = 0.92, P = 0.51). The proportions of stems damaged were converted to percentages damaged by multiplying by 100. Analyses were carried out using the generalised linear model facilities in GenStat 18 (VSN International (2015)).

Trial 5. Achene predation trial

A one-way analysis of variance was used to compare achene predation rates between *D. minus* and *S. platyphylla.*

4.3 **Results**

Trial 1. Adult no-choice and choice-minus-target oviposition container trials

Of the ten test plant species offered in adult oviposition trials, eight were accepted for oviposition to varying degrees (Table 4.3). Normal oviposition behaviour, i.e. eggs deposited in clusters underneath the nodal bracts or under the sepals on fruiting heads, only occurred on *Sagittaria* species (Figure 4.4). Of the *Sagittaria* species, the highest oviposition rates were observed on *S. calycina*, followed by *S. platyphylla,* while less than 20 eggs were laid on *S. latifolia* and *S. subulata* (Table 4.4).

On four test species (*Caldesia oligococca*, *A. plantago-aquatica*, *A. lanceolatum* and *Cynogeton procerum*) eggs were laid individually on the outside of plant material and not inserted beneath the sepals or bracts. These eggs either desiccated, or if they hatched, the larvae generally did not survive for more than a few days. As only none of the eggs laid on *C. procerum* hatched, this species was excluded from further testing.

Oviposition on *D. minus* was absent when paired with *S. calycina* and *H. nymphoides.* However, a total of sixteen eggs were laid into unopened flower buds on *D. minus* when paired with other test species (*C. oligococca*, *A. lanceolatum*, *A. plantago-aquatica* and *C. procerum*), although the average number per test species was only two eggs per bouquet (Table 4.3). While only 8 of the 16 eggs hatched, larvae survived between 9 to 15 days, but only three reached pupal stage.

(a)   (b)
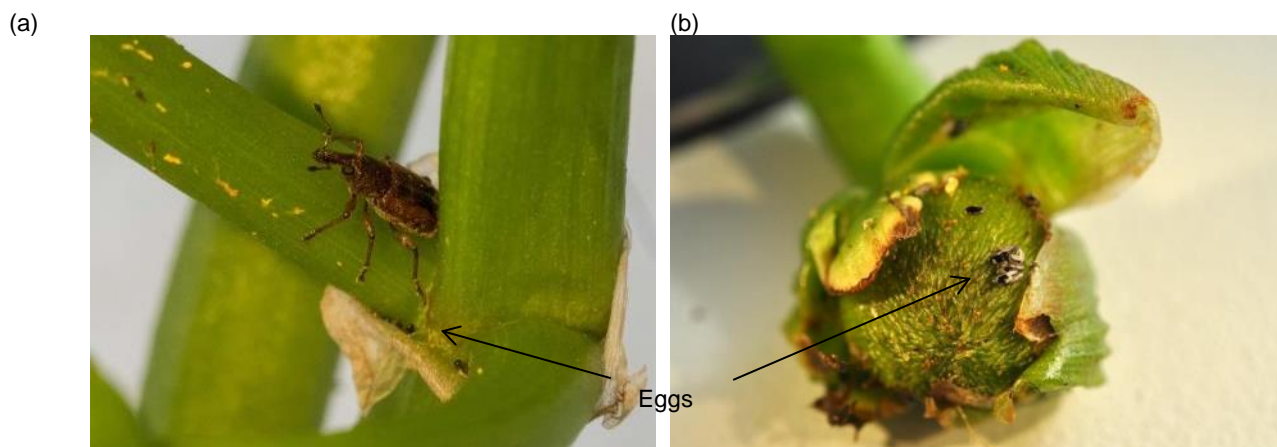
Figure 4.4 *Listronotus appendiculatus* eggs laid onto *Sagittaria calycina*; (a) deposited underneath the nodal bracts, (b) deposited underneath the sepals on fruiting heads.

Table 4.3 Summary of results of oviposition container trials (2016-2017). For **choice-minus control** trials, the number of eggs laid for each species are shown along the rows, with the corresponding paired species shown in the column heading. For *Sagittaria platyphylla* (control) and species tested without a pair (i.e. a no-choice trial) the data are highlighted in green. Species pairs were tested more than once, the values for number of eggs are shown as mean ± standard deviation with the number of replicates given in parentheses.

Number of eggs	Species paired with											Average number of eggs Mean ± SD (# replicates)	Egg hatch
	S.p	*S.c*	*S.l*	*S.s*	*E.c*	*C.o*	*H.n*	*A.p-a*	*A.l*	*D.m*	*C.p*		
***Sagittaria platyphylla* (control)**	75 ± 40 (19)											74.7 ± 40.1 (19)	Yes
Sagittaria calycina		232 ± 105.1 (3)	98 ± 28.6 (3)		88 ± 63.4 (3)		55	134	157	17		124.4 ± 86.6 (13)	Yes
Sagittaria latifolia		0.7 ± 1.1(3)	22 ± 18.3 (5)		11.3 ± 12.1 (3)							13.3 ± 15.9 (11)	Yes
Sagittaria subulata				2.0 ± 2.0 (5)								2.0 ± 2.0 (5)	Yes
Echinodorus cordifolius		0 (3)	0 (3)									0 (6)	
Caldesia oligococca									0	2.0 ± 2.8 (2)	1	1.3 ± 1.9 (4)	Yes
Hydrocleyes nymphoides		0						0		0	0	0 (4)	
Alisma plantago-aquatica		0					0		0	0	22	4.4 ± 9.8 (5)	Yes
Alisma lanceolatum		0				0		0		8 ± 1 (2)	3	3.2 ± 4.0 (6)	Yes
Damasonium minus		0				4 ± 2 (2)	0	1	3 ± 2 (2)		1	2.0 ± 2.3 (8)	Yes
Cycnogeton procerum						2	0	1	0	0		0.6 ± 0.9 (5)	No

Table 4.4 Statistical results for effect of species on the mean number of eggs for each of four *Sagittaria* species. In the analysis the *Sagittaria calycina* bouquets in pairs are considered as separate treatments to *Sagittaria calycina* alone. All results are adjusted for effect of series.

Test plant species	Square root transformed	Back transformed
Sagittaria platyphylla (control)	8.7	75
Sagittaria calycina (alone)	14.3	204
Sagittaria calycina (in pair)	10.4	109
Sagittaria subulata	1.1	1
Sagittaria latifolia	3.5	12
sed	1.69-2.81	
P value	**2.9×10^{-6}**	

Trial 2. No-choice whole-plant adult oviposition and larval survival trials

In the no-choice adult oviposition trial on potted plants, oviposition was greatest on *S. platyphylla* compared to the test species (P < 0.001, Table 4.5). On average, less than one egg was laid per plant on *A. plantago-aquatica* and less than four eggs on *D. minus*. No eggs were laid on rice (*O. sativa*) or *C. acanthocarpa*.

Table 4.5 Summary of results for no-choice whole-plant adult oviposition trial. Values for number of eggs are shown as mean ± standard deviation with the number of replicates given in parentheses.

Test plant species	Mean number of eggs Mean ± SD (# replicates)	Square root transformed	Back transformed
S. platyphylla	76.3 ± 37.4 (16)	8.2	68
A. plantago-aquatica	0.7 ± 1.6 (6)	.3	0
D. minus	3.4 ± 6.6 (7)	1.1	1
C. acanthocarpa	0 (6)	0	0
O. sativa	0 (5)	0	0
sed (range)		1.0 – 1.2	
P value		**9.0×10^{-9}**	

In the larval development tests conducted in 2016, no weevils emerged from *A. plantago-aquatica*. Adult emergence was low for both *S. platyphylla* (mean ± SD, 1.7 ± 1.6) and *D. minus* (1.0 ± 2.2). The low rate of adult emergence on *S. platyphylla* was most likely due to the starvation of larvae due to the overburdening of inflorescences with eggs (i.e. and average of 76 eggs were laid per plant).

Trial 3. No-choice whole-plant larval development trial

Larval development indicated that *S. platyphylla* and *S. calycina* were the superior hosts, with 43 and 45% of eggs developing through to adults respectively. Adult emergence, albeit at a significantly lower level, was recorded from *S. latifolia* and *D. minus* ($F_{5,27}$ = 19.53, *P* < 0.001) (Table 4.6). No adults emerged from *A. plantago-aquatica*, *C. acanthocarpa* or *E. cordifolius*. There was a significant difference between the average weight of emerged adults between *S. platyphylla, S. calycina* and *D. minus* with adults reared from *S. calycina* being the heaviest, and adults from *D. minus* the lightest ($F_{2,158}$ = 15.3, *P* = <0.001).

Table 4.6 Summary of results for no-choice whole-plant larval starvation trial adult oviposition trial. Values for number of eggs are shown as mean ± standard deviation with the number of replicates given in parentheses.

Test plant species	Mean number of adults (F_1) produced from 20 eggs Mean ± SD (# replicates)	% survival from eggs to adult	Mean weight of F_1 adults Mean ± SD (mg)
S. platyphylla	8.6 ± 5.5 (16)	43.1 ± 11.1	7.5 ± 1.7
S. calycina	9.0 ± 3.6 (9)	45.0 ±18.2	8.3 ± 1.3
S. latifolia	0.4 ± 0.9 (9)	2.2 ± 4.4	NA
A. plantago-aquatica	0 (9)	0	NA
D. minus	3.5 ± 3.9 (10)	17.5 ± 19.7	6.6 ± 1.3
C. acanthocarpa	0 (5)	0	NA
E. cordifolius	0 (5)	0	NA

Trial 4. Continuation trial

First generation (F_1) adults that had emerged from *S. platyphylla* and *S. calycina* from the no-choice larval starvation trials (Trial 3), produced significantly higher numbers of eggs over a four-week period compared to adults that emerged from and fed only *D. minus* (Table 4.7) and oviposition by *D. minus*-fed adults was delayed (Figure 4.5). After four weeks, there was no significant difference in the mean percentage survival of adults between the three species: *D. minus* (81.9%), *S. platyphylla* (67.5%) and *S. calycina* (67.5%) ($F_{2,9}$ = 0.6, *P* = 0.6), nor was there a significant difference in the survival of adults following diapause treatment from the start of the trial: *D. minus* (18.6% of F_1 adults still alive after diapause), *S. platyphylla* (27.5%) and *S. calycina* (17.5%) ($F_{2,9}$ = 1.3, *P* = 0.3). This level of mortality was anticipated, particularly for adults reared on *S. platyphylla* and *S. calycina*, as adults generally die following oviposition. For *D. minus* however, the high level of mortality following diapause suggests that the adults had not accumulated enough energy reserves to survive this period of dormancy. Due to the low survival of adults following diapause, insufficient numbers were available to assess reproduction into the second generation.

Table 4.7 Statistical results for no-choice whole-plant adult oviposition trial.

	Mean total number of eggs produced	
	Cube root transformed	Back transformed
S. platyphylla	7.5	430
S. calycina	10.1	1030
D. minus	2.3	10
sed	0.73	
P value	**1.7×10^{-6}**	
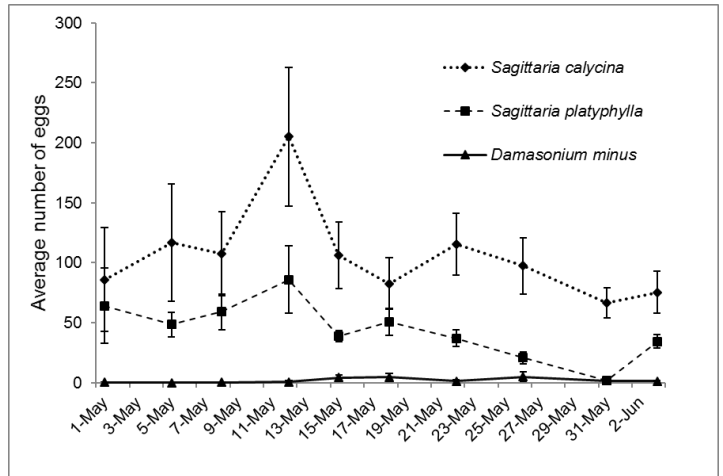
Figure 4.5 *Listronotus appendiculatus* oviposition rate of first generation (F1) adults that emerged from no-choice whole plant larval starvation trials (Trial 3) conducted in 2017. Each sampling date represents the average number of eggs (mean ± standard error) counted on fruiting bouquets per replicate.

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Overall assessment of the reproductive rate of *L. appendiculatus* on single plant over multiple generations

The overall assessment of the reproductive performance of *L. appendiculatus* showed that in the first generation, oviposition and larval survival to adult was greatest on *S. calycina* and *S. platyphylla* and low on *S. latifolia*, *S. subulata*, *D. minus* and *A. plantago aquatica* (Table 4.8; Supplementary Material Table S2). No oviposition occurred on *O. sativa* or *C. acanthocarpa*. The only non-target species to support oviposition in the second generation was *D. minus*, but this was significantly lower compared to the target species, *S. platyphylla* and *S. calycina*. The estimated rate of increase, i.e. the number of adults produced in the second generation per first-generation adult was greatest for *S. calycina* and *S. platyphylla*, compared to *S. latifolia* and *D. minus*, where the rate of increase was only marginally greater than 1.

Predicted reproductive risk. The overall reproductive risk scores to non-target species relative to *S. platyphylla* and *S. calycina* are presented in Table 4.9. Irrespective of which data were used for the oviposition trials (i.e. container or whole plant trials), the combined risk scores for all non-target species relative to *S. platyphylla* were extremely low (less than 0.023 for both first and second-generation assessments) for *S. latifolia* and *D. minus* and zero for *A. plantago-aquatica*, *E. cordifolius* and *O. sativa*. As the reproductive performance of *L. appendiculatus* was even greater on *S. calycina* the combined risk scores for all non-target species were even lower, than those estimated for *S. platyphylla*.

Table 4.8 Overall assessment of the reproductive rate of *Listronotus appendiculatus* on single plant species over multiple generations.

Description Estimate Generation Species	No eggs per 10 adults in 4 days (from Trials 1 & 2) $\widehat{\mu_X}$ First generation	se($\widehat{\mu_X}$)	Number of adults per egg (from Trial 3) $\widehat{\mu_Y}$ First generation	se($\widehat{\mu_Y}$)	No. eggs per 10 adults in 33 days (from Trial 4) $\widehat{\mu_Z}$ Second generation	se($\widehat{\mu_Z}$)	No. of adults per original adult $\widehat{\mu_{Nf}}$ First	Second
S. platyphylla								
$\widehat{\mu_X}$ from whole-plant trial	76.3[a]	7.17					74	3888
$\widehat{\mu_X}$ from container trial	90.2[b]	14.68	0.43	0.046	442[c]	80.8	87	4594
S. calycina	232.0[b]	33.29	0.45	0.061	1060[c]	125.2	235	31108
S. latifolia	22.0[b]	7.94	0.02	0.018	na	na	1.10	2.13[c]
S. subulata	2.0[b]	2.30	na	na	na	na	-	-
D. minus	3.4[a]	2.62	0.18	0.044	19	16.9	1.35	1.32
A. plantago-aquatica	0.7[a]	1.09	0	-	na	na	0	0
E. cordifolius	na	na	0	-	na	na	0	0
O. sativa	0[a]	-	na	na	na	na	0	0
C. acanthocarpa	0[a]	-	0	-	na	na	0	0

[a] Calculated using pot trial
[b] Calculated using container trial
[c] Calculated assuming $\widehat{\mu_Z} = (33/4) * \widehat{\mu_X}$

Notes:
A. plantago-aquatica: No adults produced from 180 eggs
E. cordifolius: No adults produced from 120 eggs.
O. sativa (rice): No eggs produced from 50 adults (25 females) in no-choice adult oviposition whole-plant trial (Trial 2)
C. acanthocarpa: No eggs produced from 60 adults (30 females) in no-choice adult oviposition whole-plant trial and no adults produced from 120 eggs in the larval starvation trial (Trial 3).

Table 4.9 Relative reproductive risks scores on the reproductive performance of *Listronotus appendiculatus* on non-target plant species relative to that on *Sagittaria platyphylla* and *S. calycina*.

Standard plant Generations used	cf *S. platyphylla* ($\widehat{\mu_X}$ from whole-plant trial)		cf *S. platyphylla* ($\widehat{\mu_X}$ from container trial)		Cf. *S. calycina* ($\widehat{\mu_X}$ from container trial)	
	First generation	Two generations combined	First generation	Two generations combined	First generation	Two generations combined
S. platyphylla $\widehat{\mu_X}$ from whole-plant trial	1	1	0.85	0.92	0.31	0.35
$\widehat{\mu_X}$ from container trial	1.18	1.09	1	1	0.37	0.38
S. calycina	3.20	2.83	2.71	2.60	1	1
S. latifolia	0.015	0.023	0.013	0.023	0.005	0.008
D. minus	0.018	0.018	0.016	0.018	0.006	0.007
A. plantago-aquatica	0	0	0	0	0	0
E. cordifolius	0	0	0	0	0	0
O. sativa	0	0	0	0	0	0

Overall assessment of plant damage

Leaf damage by adult weevils

Foliage consumption by adult weevils differed greatly between species in both the no-choice and choice-minus-target container trials (Table 4.10). The highest feeding damage observed was on *S. subulata* (33% loss), as this species has very small leaves, while minimal feeding occurred on the remaining test species with less than 2% loss of foliage consumed. No damage occurred on *C. oligococca* or *E. cordifolius*. In the no-choice whole plant trials, no leaf damage was observed on *O. sativa* or *C. acanthocarpa*.

Table 4.10 Percentage of leaf damaged (LD%) by ten *Listronotus appendiculatus* adults in no-choice and choice-minus-target container trials.

Species	Plants in unpaired containers		Plants in paired containers	
	Logit((LD%+0.1)/100) transformed	Back transformed (LD%)	Logit((LD%+0.1)/100) transformed	Back transformed (LD%)
S. platyphylla	-6.2	0.1		
S. calycina	-5.6	0.3	-3.6	2.6
S. latifolia	-4.6	0.9		
S. subulata	-0.7	32.6		
A. lanceolatum			-6.0	0.1
A. plantago-aquatica			-5.6	0.3
C. oligococca			-7.1	0.0
D. minus			-4.3	1.2
H. nymphoides			-5.9	0.2
C. procerum			-6.4	0.1
sed (range)				
Comparisons involving *S.platyphylla*	0.49 – 0.60		na	
Other comparisons	0.60 – 0.70		0.39 – 0.55	
Degrees of freedom	3, 24		6, 25	
F value	41.30		5.68	
P value	**1.2×10^{-9}**		**0.00078**	

Fruit damage

A summary of the results for the percentage of fruit damaged by *L. appendiculatus* adults in the choice-minus-target container trial (Trial 1) is provided in Table 4.11. The results for each species when paired with other test species is also shown in a concurrence matrix form in Table 4.13. For the *S. platyphylla* control, 19 replicates were set up over the trial period. In these containers the mean percentage of fruit damaged by 10 adults was (mean ± SD) 58 ± 33, and the mean number of fruit was 19 ± 7. Adults also preferred *S. calycina* fruit, while few fruit were damaged for the remaining test species. There is no fruit damage data provided for *S. latifolia* as the plants grown in Australia are a non-fruit-bearing form. For the no-choice container trials there was no difference in the percentage of fruit damaged by 10 adults between *S. platyphylla*, *S. calycina* or *S. subulata* (Table 4.12).

Table 4.11 Summary table for percentage of fruit damaged by 10 *Listronotus appendiculatus* adults in choice-minus-target trials.

	Number of Pairings	Number of fruit	Average (over pairings) % damage	% of fruit examined that was damaged
S. calycina	10	129	48	47
A. lanceolatum	6	207	1	1
A. plantago-aquatica	5	254	2	2
Caldesia oligococca	4	267	2	0
D. minus	7	447	14	13
H. nymphoides	4	11	0	0
C. procerum	5	717	0	0
E. cordifolius	6	177	0	0

Table 4.12 Percentage of fruit damaged for Sagittaria species in no-choice container trials.

	Logit(p)	% damaged
Sag platyphylla (a)	0.0	49
Sag calycina (a)	0.3	58
S. subulata (b)	2.0	88
sed (range)		
(a) vs (a)	0.74	
(a) vs (b)	1.68-1.77	
Degrees of freedom	2, 24	
F value	1.08	
P value	0.36	

Table 4.13 Percent of fruit damaged by 10 adults on species in paired cut flower trial (Trial 1). Two or three values in a cell indicates that the 2 species were paired twice or thrice, and each value represents the percentage in a particular pair. *Sagittaria latifolia* is not included in the first column, as there was no fruiting.

Percentage of fruit damaged (Number of fruit)	Species paired with								
	S. calycina	*A. lanceolatum*	*A. plantago-aquatica*	*C. oligococca*	*D. minus*	*H. nymphoides*	*C. procerum*	*E. cordifolius*	*S. latifolia*
S. calycina	-	21(14)	0(5)		0(15)	0(11)		33(12),100(10), 83(18)	100(9),100(9), 38(26)
A. lanceolatum	0(32)	-	0(107)	0(54)	0(26), 3(32)		3(31)		
A. plantago-aquatica	0(50)	0(53)	-		0(59)	11(46)	0(46)		
C. oligococca		0(107)		-	0(59), 9(11)		0(90)		
D. minus	13(53)	8(108), 26(39)	21(63)	4(52), 25(48)	-	4(55)	14(29)		
H. nymphoides	0(2)		0(3)			-	0(1)		
C. procerum		0(187)	0(182)	0(125)	1(119)	0(104)	-		
E. cordifolius	0(30),0(23), 0(39)							-	0(30), 0(25), 0(30)

Stem (petiole and scape) damage

Larval damage to the stems (including leaf petioles and scapes of fruiting stems) of plants was greatest on *S. calycina* plants with an average of 75% of stems damaged, followed by *S. platyphylla* (44%), *S. latifolia* (17%) and *D. minus* (12%) (Table 4.14). No stem damage was recorded on *Alisma plantago-aquatica*, *Caldesia acanthocarpa* or *E. cordifolius*. Except for *S. calycina* plants of which 89% of plants were dead at the completion of the trial (because of the damage caused by larvae burrowing into petioles and crowns), larval damage did not impact upon the survival of individual plants of the other species, which were able to sprout new foliage and flowers after the completion of the trial.

Table 4.14 Percentage of stems (petioles and scapes) that were damaged from *Listronotus appendiculatus* larvae in whole-plant larval development trials (Trial 3).

	Number of plants	Logit(p)	% damaged stems	% of plants dead
S. platyphylla (a)	11	-0.3	44	0
S. calycina (a)		1.1	75	88.9
D. minus (a)	10	-2.0	12	0
E. cordifolius (b)	6	-∞	0	0
Caldesia acanthocarpa (b)	5	-∞	0	0
A. plantago-aquatica	9	-	0	0
S. latifolia	9	-	17	0
sed (range)				
(a) vs (a)		0.37-0.51		
(a)or(b) vs (b)		Not defined		
P value		**4.3 × 10⁻⁹**		

Trial 5. Achene predation trial

Achene predation by *Listronotus appendiculatus* larvae was 24 times greater on *S. platyphylla* (mean ± SD, 1443.7 ± 436.2) compared to *D. minus* (58.1 ± 17.3) ($F_{1,14}$ = 80.4, $P < 0.001$). While the survival of larvae reared on *D. minus* remained relatively high (87.5% survived to pupation), none of the pupa emerged into adults, whereas 100% of the larvae reared on *S. platyphylla* survived to the adult stage.

Native range studies

1. Assessment of genetic variation of *L. appendiculatus* on *S. platyphylla*. The COI sequences from larvae collected from *S. platyphylla* in the USA, as well as those from the quarantine laboratory culture (i.e. originating from Sunk Lake and Reelfoot Lake (TN) and LEARF (TX) aligned with the *L. appendiculatus* adult specimen identified by Charles O'Brien. The phylogenetic tree revealed that there was little genetic variation between larval samples collected from the three *S. platyphylla* genotypes (red, green, blue) across the native range (Figure 4.6).

Consensus sequences alignment|

```
5704a_Splaty_Sunk_TN_red|
5784a_Splaty_Caddo_TX_blue|
5763a_Splaty_Martin_LA_green|
5721a_Splaty_Caddo_TX_blue|
5763b_Splaty_Martin_LA_green|
5704b_Splaty_Sunk_TN_red|
5653c_Splaty_Sunk_TN_red|
5754a_Splaty_Sunk_TN_red|
5753b_Splaty_Sunk_TN_red|
5705b_Splaty_Reel_TN_red|
5785b_Splaty_Caddo_TX_blue|
5785a_Splaty_Caddo_TX_blue|
5753a_Splaty_Sunk_TN_red|
5763c_Splaty_Martin_LA_green|
5773a_Splaty_Baton_LA_green|
5774a_Splaty_Baton_LA_green|
5773b|Splaty|Baton|LA|green|
5701b_Splaty_LEARF_TX_red|
5796a_Splaty_Pines_TX_blue|
5701a_Splaty_LEARF_TX_red|
5704c_Splaty_Sunk_TN_red|
5702a_Splaty_LEARF_TX_red|
5657c_Splaty_Reel_TN_red|
5795a_Splaty_Pines_TX_blue|
5795b_Splaty_Pines_TX_blue|
```

0.2

Figure 4.6 Phylogenetic relationships between *Listronotus appendiculatus* larvae collected from three genotypes (red, green, blue) of *Sagitaria platyphylla* in the southern USA. Codes: Sunk (Sunk Lake, Tennessee), Caddo_TX (Caddo Lake, Texas, Martin (Lake Martin, Louisiana), Reel_TN (Reelfoot Lake, Tennessee), Baton_LA (Baton Rouge, Louisiana), LEARF_TX (Lewisville Aquatic Ecosystem Research Facility, Texas), Pine (Lake O'the Pines, Texas), red (red genotype), blue (blue genotpe), green (green genotype).

2. Assessment of host utilisation in the native range. During the field surveys in the USA, five Alismataceae species were found growing in proximity to *S. platyphylla*: *S. calycina*, *Sagittaria latifolia*, *Echinodorus cordifolius* and *E. berteroi.* The molecular data from field host range sampling showed that *L. appendiculatus* larvae were only found feeding within *S. platyphylla* and *S. calycina* (Figure 4.7). Furthermore, there was no evidence of biotype differences between *L. appendiculatus* collected from either species. These samples formed a clade (Clade 1) that was separate to all samples collected from other plant hosts, including *Sagittaria latifolia*, *Echinodorus cordifolius* and *E. berteroi.* For larvae collected from *S. latifolia* (Clade 2), this separation was supported by morphological examination of adults collected from the Lake Martin population which showed slight differences in size, colouration and shape of elytra. No reference specimens in BOLD were able to identify these specimens, hence the identity of the *S. latifolia* specimens remains uncertain but are most likely a different species to *L. appendiculatus*. Similarly, larvae collected from the *Echinodorus* species, *E. cordifolius* and *E. berteroi* (Clade 3) did not align with *L. appendiculatus* or other DNA barcodes of known species including *L. echinodori*.

In summary, we are confident that the clade that corresponds to the *L. appendiculatus* samples is taxonomically distinct from larvae sampled from non-target species within the Alismataceae.
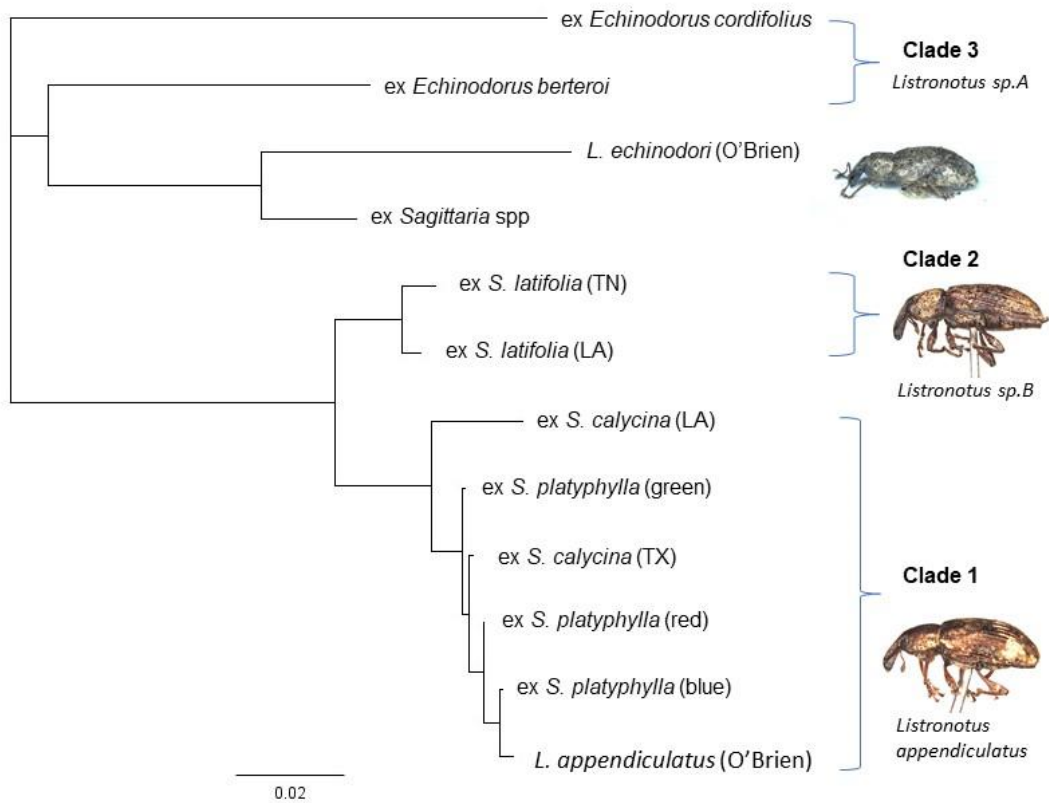
Figure 4.7 Phylogenetic tree showing relationship among Listronotus weevil samples collected from *Sagittaria platyphylla* (red, green and blue genotypes), *S. calycina*, *S. latifolia*, *Echinodorus cordifolius* and *E. berteroi* in the USA. Two adult specimens identified by curculionid expert, C. O'Brien are shown as: *L. appendiculatus* (O'Brien) and *L. echinodori* (O'Brien).

5   Discussion

Based on literature records, *L. appendiculatus* has a narrow host range that is confined to the genus *Sagittaria* (Table 3.1 and references therein). From an Australian perspective, there are few species that are potentially at risk because there are no native *Sagittaria* species and only seven native species within the Alismataceae family.

The laboratory host specificity testing results presented in this report show that *L. appendiculatus* is highly host specific to the two target weeds, *S. platyphylla* and *S. calycina.* High rates of oviposition in both the container and whole-plant pot trials strongly suggest that *S. platyphylla* and *S. calycina* are highly attractive to *L. appendiculatus,* with *S. calycina* likely to be a more superior host. This may be due to the much larger fruiting heads and thicker, fleshier petioles on *S. calycina*, enabling these plants to support more *L. appendiculatus* than on *S. platyphylla*. In addition to laying more eggs, *L. appendiculatus* adults consumed more leaf material and larvae damaged more petioles when reared on *S. calycina* compared to *S. platyphylla*. At low water levels, larval damage to the crown of *S. calycina* often resulted in plant death, while on *S. platyphylla*, plants were rarely killed but were able to re-sprout from the crown or tubers. As *S. calycina* lacks this ability to re-sprout, and does not produce tubers, the impact of *L. appendiculatus* on *S. calycina* populations is likely to be greater than on *S. platyphylla*.

Risks to native species

Eggs were laid, damage was done, and larvae developed on the non-target species, *D. minus*, but at rates much lower than *S. platyphylla* and *S. calycina*. Despite some acceptance of *D. minus* under laboratory host specificity testing, we don't expect this species to be at risk of attack from *L. appendiculatus* in the field. Firstly, in both container and whole plant oviposition trials, *L. appendiculatus* laid few eggs on *D. minus* suggesting that adults do not recognise *D. minus* as a suitable host for oviposition. Secondly, the fecundity of adults reared from larvae on *D. minus* was extremely low, suggesting that *D. minus* is nutritionally a poor host. Finally, it is unlikely that *D. minus* would be able to support successive generations of *L. appendiculatus,* particularly from one season to the next, because the survival of F_1 adults following winter diapause conditions was extremely low. The damaged caused by the few developing larvae on potted plants was minimal and did not inhibit the plant's ability to re-sprout new leaves and flowering stems. Furthermore, the number of fruit consumed by larvae was minor (less than 59 achenes per larva). As *D. minus* produces prolific seeds throughout the year with seedling densities of up to 2000 seedlings/m^2 reported (Fox et al 1999), damaged caused by a few *L. appendiculatus* larvae would be unlikely to impact on the population dynamics of this species. It is worth noting that in south-eastern Australia, *D. minus* is regarded as a serious weed of rice crops (McIntyre et al. 1991). The risk of "spill-over" damage caused by larvae migrating from *S. platyphylla* or *S. calycina* to *D. minus* would be minimal and only likely to occur where plants are in direct contact with each other. This is because larvae are unable to swim from plant to plant.

Of the other native Alismataceae species tested, few eggs were laid onto *Alisma plantago-aquatica* in container and whole-plant pot trials, however larval development could not be supported in larval starvation trials. The two tropical species, *Caldesia oligococca* and *C. acanthocarpa* were found to be unsuitable hosts for adult feeding, oviposition and larval development.

Risks to ornamental species

Of the four ornamental species tested, oviposition was greatest on *S. latifolia* however the survival rate of larvae was very low. This may be due to the lack of fruit on this sterile form, resulting in insufficient nutrients being available to enable larvae to complete development. In Australia, should the fertile form become more widely available through the ornamental trade, it might be at risk of attack. *Sagittaria subulata, Hydrocleyes. nymphoides* and *Echinodorus cordifolius* are unlikely to be suitable hosts as few eggs were laid on the inflorescences of these species. No larval development occurred on *E. cordifolius* in the larval development trials. The only risk to *S. subulata* however, would be from adult feeding but this would require plants to be

growing in proximity to *S. platyphylla* or *S. calycina,* as *S. subulata* alone is unlikely to support the maintenance of viable *L. appendiculatus* populations. Hence, while there is a minor risk that *L. appendiculatus* could damage ornamental *Sagittaria* species, the impact to the horticultural industry would be low as the genus is of minor importance. In Victoria, the sale of all *Sagittaria* species is prohibited as the entire genus is declared (see Section 2.8 Legislation).

An interesting result of the host specificity testing was that *D. minus*, the most distantly-related member of Alismataceae species tested, was able to support the development of *L. appendiculatus* larvae, when other more closely-related species did not. A likely explanation may be due to the structure of the test plants, such as the thickness and toughness of epidermal tissue. For instance, *A. plantago-aquatica, A. lanceolatum* and *E. cordifolius* all have very tough, woody stems, which may prevent neonate larvae from burrowing into the tissue. The structure of the inflorescences may also influence the acceptance of the plant species for oviposition. Adult *L. appendiculatus* selectively lay their eggs underneath the nodal bracts on flowering stems and under the petals enclosing developing fruits (Figure 4.4). Oviposition was negligible on plant species that lacked suitable oviposition sites, such as *Caldesia oligococca*, *C. acanthocarpa*, *A. plantago-aquatica*, *A. lanceolatum* and *D. minus*. Finally, the growth habit (submerged, floating or emergent foliage) is also a key factor in the degree of risk of *L. appendiculatus* to non-target native and ornamental species. Submerged species that are often used in aquaria, such as *Helanthium* spp. and *Echinodorus* would not be at risk from adult feeding or oviposition (Fig. 5.1a). Similarly, plant species with floating leaves such as *S. subulata* and the native *Caldesia* species are unlikely to support the development of *L. appendiculatus* larvae as larvae do not feed portions of petioles that are underwater (Fig 5.1b, c).

(a) *Helanthium tenellum* (b) *Sagittaria subulata* (c) *Caldesia acanthocarpa*

Figure 5.1 Alismataceae species with (a) submerged or (b), (c) floating leaves are at low risk of attack by the sagittaria fruit-feeding weevil, *Listronotus appendiculatus*.

Predicted risk

We utilised a predicted risk analysis following the approach developed by Paynter et al. (2015) and which has been adopted by New Zealand regulatory authorities (Paynter et al. 2017). The combined risk scores for non-target species relative to *S. platyphylla* and *S. calycina* using no-choice oviposition and larval survival data ranged from 0 – 0.023 for both first and second-generation assessments. These threshold scores were much lower than the 0.33 threshold score proposed by Paynter 2015.

Native range studies

A common artefact of no-choice laboratory testing is the acceptance of non-target species for oviposition or larval development that would not normally be attacked under natural field conditions (Paynter et al. 2015). Insects typically follow a sequence of behavioural steps in host utilisation, using a wide array or sensory stimuli in the process (Bernays and Chapman 1994). When these conditions are not provided or are masked by volatile plant chemicals from nearby preferred host plants, acceptance of non-hosts may occur (Heard 2000). Hence, while a non-target plant species might meet the physiological requirements for larval

development, this species may not necessarily be at risk of attack if adults do not perceive the plant as acceptable for oviposition. Field trials carried out in the native range provide the opportunity to study the natural host-selection behaviour within natural or augmented field populations of the agent (Sheppard et al. 2005). In our study, we utilised molecular genetic tools to improve our understanding of the ecological host range of *L. appendiculatus* in the southern USA.

Mitochondrial DNA extracted from field-collected weevil larvae confirmed our laboratory predictions that the preferred hosts of *L. appendiculatus* were *S. platyphylla* and *S. calycina*. The phylogenetic analysis grouped the samples into three major clades according to their host affiliations. All samples collected from *S. platyphylla* and *S. calycina* were clustered into one clade that aligned with the *L. appendiculatus* adult specimen identified by *Listronotus* expert, Charles O'Brien. Larvae collected from *S. latifolia* and *Echinodorus* species did not align with *L. appendiculatus* but rather, are likely to be different species altogether (Figure 4.7). Hence, our studies revealed that in the native range, *L. appendiculatus* appear to rarely utilise other closely-related species. While it's possible that this may be due to the niche being already occupied by other herbivores, we observed instances where sympatric plant species were free from attack by other weevil species despite high levels of attack by *L. appendiculatus* on *S. platyphylla* plants nearby.

Intraspecific variation in the candidate agent

Intraspecific variation within the agent species can have important implications for determining the host specificity of an agent species. Disparate populations of the same agent species, known as biotyes or host races, can show high specificity to different host species or to different genotypes of the preferred host. Our molecular studies suggested that neither of these cases applied to *L. appendiculatus*. The phylogenetic analysis showed that there were no discernible genetic differences in populations collected from *S. platyphylla* compared to *S. calycina* (Figure 4.7), nor between different genotypes of *S. platyphylla* (Figure 4.6). Hence, for the two *L. appendiculatus* populations imported into AgriBio's quarantine facility, (i.e. the Tennessee population sourced from Sunk/Reelfoot Lakes and Texas population sourced from the Lewisville Aquatic Ecosystem Research Facility), no genetic difference was found between these populations used in the in the laboratory host specificity testing. Furthermore, if approved for release it is unlikely that incompatibility between agent and host biotypes is likely to impact on the success of *L. appendiculatus* for the biocontrol of *S. platyphylla* or *S. calycina* in Australia.

Potential for asynchrony between the lifecycle of the candidate agent and the phenology of the target plant

For seed-feeding insects, there is a potential risk that the candidate agent might attack non-target species particularly if the flowering period of the host is discrete, and if the phenology of the target weed is different to that in the native range (Fowler 2003). For instance, the seed-feeding beetle, *Bruchidius villosus* introduced into New Zealand against English broom (*Cytisus scoparius*), was found to attack the seed pods of the exotic plant, tagasaste (*Chamaecytisis palmensis*) because in Australia, tagasaste plants flower much earlier in spring than broom (Fowler et al. 2000).

Should *L. appendiculatus* be released into Australia, it is unlikely that there would be an asynchrony between the lifecycle of the weevil and its hosts, because the flowering period of *S. platyphylla* and *S. calycina* is very long (September to June) and comparable in duration to that of the native range. In Texas, *L. appendiculatus* had finished ovipositing on *S. platyphylla* by October, one month before the *S. platyphylla* ceased fruiting Kwong et al (2018).

Predicted risk based on climate suitability

In Australia, the current distribution of *Sagittaria platyphylla* occurs in climates similar to that of the native range in the south-central USA. Based on Koppen-Geiger Climate Classification, *S. platyphylla* in the USA is restricted to the warm temperate climates (Cfa) and does not occur in the tropical equatorial climate (Aw) of southern Florida (Figure 5.2a). Hence, it is unlikely that *S. platyphylla* would invade the equatorial climates of far northern Australia, where the native tropical Alismataceae (*Astonia* and *Caldesia*) occur (Figure 5.2b). There has only been one population of *S. platyphylla* found in the Northern Territory, which was in an

ornamental pond in Darwin. No naturalised populations have been detected despite the species being present in in the vicinity for at least ten years.

(a) *Sagittaria platyphylla* locations in the USA (native range)
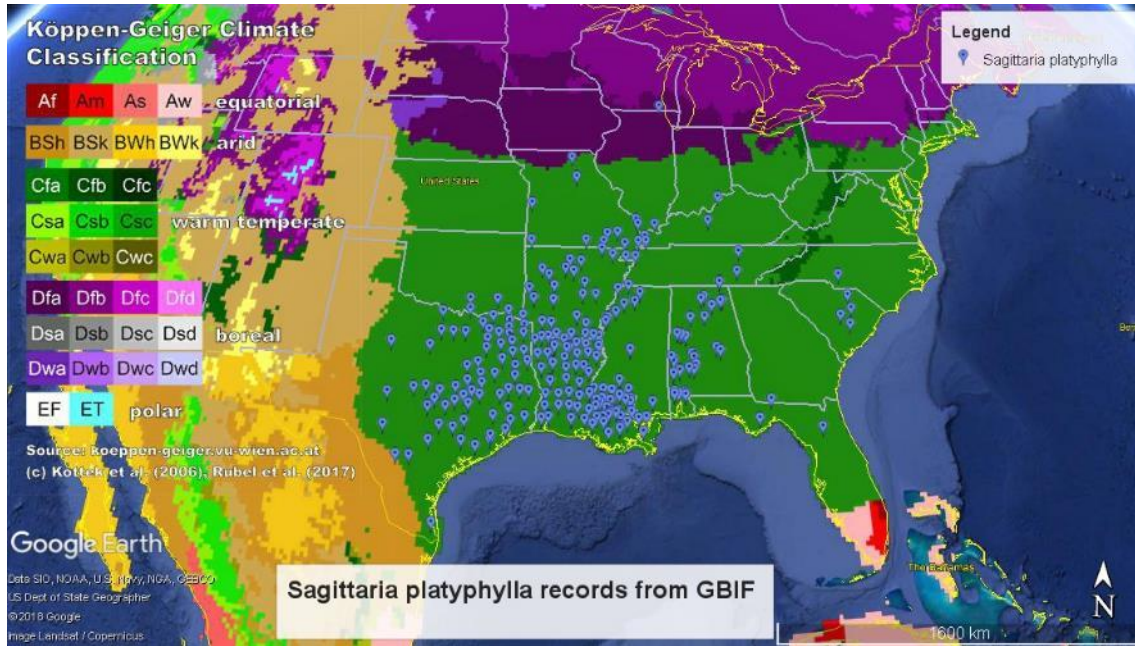
(b) *Sagittaria platyphylla* locations in Australia (invaded range)
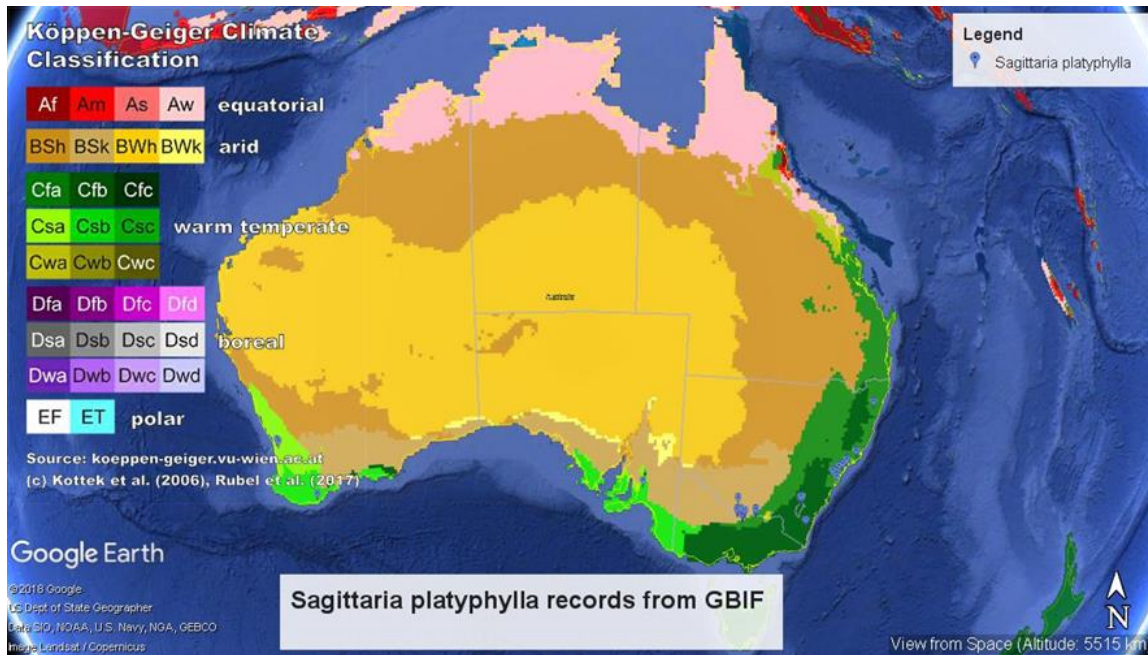
Figure 5.2 Koppen-Geiger Climate maps overlayed with occurrence records of *Sagittaria platyphylla* in (a) the USA and (b) Australia retrieved from GBIF.org.

Environmental consequences of release

There is little risk of hybridisation of *L. appendiculatus* with native curculionid species as there are no native *Listronotus* species in Australia. There are only two introduced species: *L. bonariensis* (a pest of ryegrass pastures) and *L. setosipennis* (an introduced biocontrol agent for parthenium weed). No evidence of hybridisation within the genus has been recorded.

6   Conclusion

There are at present no satisfactory means of effective long-term control of *S. platyphylla* or *S. calycina,* particularly in sensitive aquatic habitats and difficult to access locations such as wetlands. The measures presently in use are expensive, non-selective and not always effective. In a biogeographic study comparing native populations of *S. platyphylla* with invasive populations in Australia and South Africa, the key difference between native and invasive plants was their sexual reproductive capacity; invasive plants produce 40% more achenes per fruit and individual achenes are 50% heavier (Kwong et al. 2017). The release and establishment of the pre-dispersal seed predator, *L. appendiculatus* in Australia would assist in reducing the prolific seed production of these weeds, thereby reducing their rate of spread and re-invasion back into previously-treated areas.

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8   Supplementary Material

Appendix 1: Statistical analyses for Reproductive Cycle Assessment.

Appendix 1: Reproductive Cycle Assessment

Statistical analysis conducted by Kym Butler, Senior Research Scientist – Biometrics, DEDJTR

Preliminary Screening for Egg Deposition – Paired Containers

Table S1. Number of eggs on species in paired cut flower trial. Comparisons involving *Sagittaria calycina* are excluded since very heavy egg laying occurred on *S. calycina* plants, and it was rare for an egg to be laid on a species paired with *S. calycina* (only 2 eggs with *S. latifolia* on one of three occasions). Two or three values in a cell indicates that the 2 species were paired twice or thrice, and each value represents number of eggs observed in a particular pair.

Number of eggs	Species paired with							
	Hydrocleyes nymphoides	*Cynogeton procerum*	*Caldesia oligococca*	*Damasonium minus*	*Alisma lanceolatum*	*Alisma plantago-aquatica*	*Echinodrus cordifolius*	*Sagittaria latifolia*
Hydrocleyes nymphoides	-	0		0		0		
Cynogeton procerum	0	-	2	0	0	1		
Caldesia oligococca		0	-	0, 4	0			
Damasonium minus	0	1	2, 6	-	5, 1	0		
Alisma lanceolatum		3	0	7, 9	-	0		
Alisma plantago-aquatica	0	22		0	0	-		
Echinodrus cordifolius							-	0, 0, 0
Sagittaria latifolia							0, 0, 10	-

S. calycina was paired with one of the above species in 10 containers. In these containers the mean egg count for *S. calycina* was 92 with a standard deviation of 50. The range was 17 to 157.

S. platyphylla was in 19 single species (not paired) containers over the trial period. In these containers the mean egg count for *S. platyphylla* was 75 with a standard deviation of 40. The range was 10 to 166.

Appendix 1. Reproductive cycle assessment

Formal Assessment – Single species pots/containers

The number of eggs counted in the single species containers set up from 29 January 2016 in the first-generation oviposition container trial, the pots in the first-generation oviposition pot trial and the container in the oviposition component of the second-generation trial were each (separately) analysed using a generalised linear model with Poisson distribution, over-dispersion parameter and logarithmic link, and with an effect of species in the model. The proportion of eggs that survived to adult in the first component of the starvation trial was analysed using a generalised linear model with binomial distribution (n =20, the number of eggs in each pot), over-dispersion parameter and logistic link, and with an effect of species in the model. Since *Alisma plantago-aquatica*, *Echinodorus cordifolius*, *Sagittaria latifolia* and *Caldesia acanthocarpa* produced very few adults, which can lead to severe under-estimation of the over-dispersion parameter, the over-dispersion parameter for the proportion of eggs that survived to adult was estimated using a prior analysis on a reduced data set that excluded plots with *A.plantago-aquatica*, *E. cordifolius*, *S. latifolia* and *C. acanthocarpa*. However, the estimation of species effects used the complete data set.

With the exception of the oviposition component of the second-generation trial, with each measurement, an extra additive effect of start date (series) on the link scale was examined using a deviance ratio F test. To avoid over-sensitivity due to under-estimation of the over-dispersion parameter, for the proportion of eggs that survived to adult in the first component of the starvation trial, the *A.plantago-aquatica*, *E. cordifolius* and *S. latifolia* pots were excluded from this test. It was found that all these series effect tests were not statistically significant ($P > 0.05$), and thus the species estimates produced in these analyses were not adjusted for series. There was only one start date in the oviposition component of the second-generation trial.

All analyses used a single pot or container, containing a single species, as the unit of analysis. All analyses were carried out using the generalised linear model facilities in GenStat 18 (VSN International (2015)).

From these individual analyses, for several species, we have obtained
  iv.   An estimate of the expected number of eggs ($\widehat{\mu_X}$) produced over 4 days by 10 adults from the first-generation oviposition trial and, in some cases from single plant species containers in the firs- generation oviposition container trial.
  v.   An estimate of the expected number of adults per egg ($\widehat{\mu_Y}$), from the first-generation component of the starvation trial, and
  vi.   An estimate of the expected number of eggs produced over 33 days by 10 adults from the second generation ($\widehat{\mu_Z}$), in the oviposition component of the starvation trial.

If we make the extra assumptions that
  e)   There is a 50:50 male/female reproductive split
  f)   All adults are reproductive for 90 days, and reproduction is consistent over the 90-day period
  g)   The number of adults obtained per egg in the second generation is the same as was obtained in the first generation, for each species
  h)   The 3 component experiments were carried out independently

Then the expected first-generation reproductive rate (number of adult female second generation progeny produced from each adult female (Nf)) can be calculated as
  $\mu_{Nf} = (90/(4\times5))\mu_X \times \mu_Y/2 = 2.25 \times \mu_x \times \mu_Y.$
and estimated as
  $\widehat{\mu_{Nf1}} = 2.25 \times \widehat{\mu_X} \times \widehat{\mu_Y}$
This estimate was calculated for a number of species.

Then the expected second-generation reproductive rate (number of adult female second generation progeny produced from each adult female (Nf)) can be calculated as
  $\mu_{Nf} = (90/(4\times5))\mu_X \times (\mu_Y/2)2 \times (90/(33\times5))\mu_Z = (27/44)\times \mu_x \times (\mu_Y)2 \times \mu_Z.$
and estimated as
  $\widehat{\mu_{Nf2}} = (27/44) \times \widehat{\mu_X} \times ((\widehat{\mu_Y})^2 + (se(\widehat{\mu_Y}))^2) \times \widehat{\mu_Z}$; where se denotes standard error.
This estimate was calculated for a number of species.

Appendix 1. Reproductive cycle assessment

A combined risk score for a species, compared to a standard species, can be calculated using the first-generation reproductive rate, as

CRS1(*species*)= $\widehat{\mu_{Nf1}}(species)/\widehat{\mu_{Nf1}}(standard)$

A combined risk score across 2 generations for a species, compared to a standard species, can be calculated using the second-generation reproductive rate, as

CRS2(*species*)= $\sqrt{\widehat{\mu_{Nf2}}(species)/\widehat{\mu_{Nf2}}(standard)}$

These risk scores are directly comparable to other combined risk scores in the literature.

VSN International (2015). The Guide to the Genstat Command Language (Release 18), Part 2 Statistics. VSN International, Hemel Hempstead, UK.

Appendix 1. Reproductive cycle assessment

Table S2. Representation of species in each trial analysis.

	First Generation oviposition pot trial		First Generation oviposition container trial (not paired, from 29 Jan 2016)		First generation component of starvation trial		Oviposition component of starvation trial	
	Number of pots	Number of series[a]	Number of containers	Number of series[a]	Number of pots	Number of series[a]	Number of pots	Number of series[a]
S. platyphylla	16	6	6	3	16	7	4	1
S. calicyna	-	-	3	1	9	5	4	1
S. latifolia	-	-	5	2	9	4	-	-
S. subulata	-	-	5	2	11	6	-	-
D. minus	7	4	-	-	10	4	4	1
A. plantago-aquatica	6	3	-	-	9	5	-	-
E. cordifolius	-	-	-	-	6	1	-	-
Rice	5	2	-	-	-	-	-	-
C. acanthocarpa	6	2	-	-	5	2	--	-
Number of series in trial	6		3		8		1	
Test of species difference (unadjusted)								
F value	47.43		36.32		23.13		44.46	
Degrees of freedom	4, 35		3, 15		5, 32		2, 9	
P value	1.2×10^{-16}		4.0×10^{-7}		7.4×10^{-10}		2.2×10^{-5}	
Test of series difference (adjusted for species)								
F value	1.75		0.19		1.91		na	
Degrees of freedom	5, 30		2, 13		7, 25		na	
P value	0.15		0.83		0.19		na	

[a] The number of separate starting dates that species has representation

Appendix 1. Reproductive cycle assessment

Table S3. Overall assessment of the reproductive rate of *Listronotus appendiculatus* on single plant species over multiple generations.

Description	No eggs per 10 adults in 4 days		Number of adults per egg		No eggs per 10 adults in 33 days		No of adults per original adult	
Estimate	$\hat{\mu}_X$	se($\hat{\mu}_X$)	$\hat{\mu}_Y$	se($\hat{\mu}_Y$)	$\hat{\mu}_Z$	se($\hat{\mu}_Z$)	$\hat{\mu}_{Nf}$	
Generation	First generation		First generation		Second generation		First	Second
Species								
S. platyphylla								
$\hat{\mu}_X$ from pot trial	76.3[a]	7.17					74	3888
$\hat{\mu}_X$ from container trial	90.2[b]	14.68	0.43	0.046	442	80.8	87	4594
S. calycina	232.0[b]	33.29	0.45	0.061	1060	125.2	235	31108
S. latifolia	22.0[b]	7.94	0.02	0.018	na	na	1.10	2.13[c]
S. subulata	2.0[b]	2.30	na	na	na	na	-	-
D. minus	3.4[a]	2.62	0.18	0.044	19	16.9	1.35	1.32
A. plantago-aquatica	0.7[a]	1.09	0	-	na	na	0	0
E. cordifolius	na	na	0	-	na	na	0	0
Rice	0[a]	-	na	na	na	na	0	0
C. acanthocarpa	0[a]	-	0	-	na	na	0	0

[a] Calculated using pot trial

[b] Calculated using container trial

[c] Calculated assuming $\hat{\mu}_Z = (33/4) * \hat{\mu}_X$

Notes:

A. plantago-aquatica: No adults produced from 180 eggs

E. cordifolius: No adults produced from 120 eggs.

Rice: No eggs produced from 50 adults (25 females) in first generation pot oviposition trial

C. acanthocarpa: No eggs produced from 60 adults (30 females) in first generation pot oviposition trial and no adults produced from 120 eggs.

Appendix 1. Reproductive cycle assessment

Table 4. Relative reproductive risks scores on the reproductive performance of *Listronotus appendiculatus* on non-target plant species relative to that on *Sagittaria platyphylla* and *S. calycina*

Standard plant	cf *S. platyphylla* ($\widehat{\mu_x}$ from pot trial)		cf *S. platyphylla* ($\widehat{\mu_x}$ from container trial)		Cf. *S. calycina* ($\widehat{\mu_x}$ from container trial)	
Generations used	First generation	Two generations combined	First generation	Two generations combined	First generation	Two generations combined
S. platyphylla $\widehat{\mu_x}$ from pot trial	1	1	0.85	0.92	0.31	0.35
$\widehat{\mu_x}$ from container trial	1.18	1.09	1	1	0.37	0.38
S. calycina	3.20	2.83	2.71	2.60	1	1
S. latifolia	0.015	0.023	0.013	0.023	0.005	0.008
D. minus	0.018	0.018	0.016	0.018	0.006	0.007
A. plantago-aquatica	0	0	0	0	0	0
E. cordifolius	0	0	0	0	0	0
Rice	0	0	0	0	0	0

Appendix 1. Reproductive cycle assessment