

## Impacts of a pre-dispersal seed predator on achene production in the aquatic macrophyte, *Sagittaria platyphylla*

Raelene M. Kwong<sup>1,2</sup>, Jean Louis Sagliocco<sup>1</sup>, Nathan E. Harms<sup>3</sup> and Julie G. Nachtrieb<sup>4</sup>

<sup>1</sup>Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

<sup>2</sup>Department of Ecology, Environment and Evolution, La Trobe University, Bundoora, Victoria 3086, Australia

<sup>3</sup>US Army Engineer Research and Development Center, Vicksburg, Mississippi 39180, USA

<sup>4</sup>US Army Engineer-Lewisville, Aquatic Ecosystem Research Facility, Lewisville, Texas 75057, USA (rae.kwong@ecodev.vic.gov.au)

**Summary** To aid prioritisation of potential biological control agents of *Sagittaria platyphylla* (Engelm.) J.G.Sm. for use in Australia and South Africa, we studied the effects of a pre-dispersal seed weevil, *Listronotus appendiculatus* (Boheman) (Coleoptera: Curculionidae) on achene production and plant performance.

Levels of achene production and insect damage were compared between insecticide-treated and control plants over one flowering season in an experimental study pond. Based on its potential to significantly reduce achene production under field conditions in the native range, *L. appendiculatus* may make a suitable candidate for the biological control of *S. platyphylla* by assisting in slowing the rate of spread or reinvasion back into herbicide-treated areas. However, it is unlikely to provide population-level impacts on well-established infestations.

**Keywords** *Sagittaria platyphylla*, delta arrowhead, weed biological control, *Listronotus appendiculatus*, pre-dispersal seed predation.

### INTRODUCTION

*Sagittaria platyphylla* (Engelm.) J.G.Sm. (Alismataceae) is an aquatic emergent herb indigenous to North and Central America that has become invasive in Australia and South Africa (Adair *et al.* 2012). Initially valued as an aquatic ornamental for use in ponds and aquaria, *S. platyphylla* has become a serious aquatic weed, particularly in shallow water bodies such as irrigation channels, drains, creeks and wetlands where it can rapidly form dense and extensive infestations that impede water flow and displace native species. Classical biological control is considered a desirable option for the management of *S. platyphylla* due to the difficulty and expense of controlling the weed in irrigation systems as well as the need for alternative approaches for use in sensitive aquatic habitats.

During surveys for natural enemies in North America (Kwong *et al.* 2014), the weevil, *Listronotus appendiculatus* (Boheman) was identified as a

promising candidate for the biological control of *S. platyphylla* for three reasons. Firstly, it was found across most of the plant's native distribution from temperate to subtropical climates and in open and shaded habitats. Secondly, it could reach very high densities causing high levels of damage to reproductive structures (Kwong *et al.* 2017). Thirdly, it appears to have a limited host range and is not known to attack other species outside *Sagittaria* (Muenchow and Delesalle 1992, Harms and Grodowitz 2009).

Towards refining our understanding of the potential of *L. appendiculatus* as a candidate biological control agent for *S. platyphylla*, we conducted an herbivore-exclusion experiment in the USA to: (1) measure the seasonal impact of adult and larval feeding on plant reproductive performance; and (2) to determine if plants compensate for fruit herbivory through increasing sexual or asexual reproduction.

### MATERIALS AND METHODS

The herbivore-exclusion study was conducted in an earthen, clay-lined pond (28 m × 75 m), located at the U.S. Army Engineer Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas, USA (33.067016°, -96.954724°).

On 6 June 2012, 24 plots (0.5 × 0.5 m) were randomly placed approximately 3–5 m apart along the margins of the study pond within *S. platyphylla* stands so that each half of the pond contained 12 plots. Plots were randomly assigned a treatment of either non-herbivore (treated) or herbivore (control). Herbivores were eliminated from non-herbivore plots by foliar application of a mixture of Karate® insecticide (Syngenta Crop Protection, Greensboro, NC) at the rate of 0.1 mL L<sup>-1</sup> in a water solution (0.030 mL insecticide applied per plot) and Thoroughbred® non-ionic-organosilicone surfactant (Estes Incorporated, Wichita Falls, TX) at the rate of 1 mL L<sup>-1</sup>. The insecticide application was extended 0.5 m beyond the plot boundaries to ensure full coverage and applied when there was no breeze to ensure insecticide did not drift

onto control plots. Plots were treated with insecticide every two weeks beginning 8 June and ending 13 September 2012. Herbivore plots were sprayed with water in the same manner and frequency as non-herbivore plots. No differences in initial plant height or water depth were detected among treatments (ANOVA,  $F_{1,21} = 3.28$ ,  $P = 0.08$  for plant height;  $F_{1,21} = 0.46$ ,  $P = 0.5$  for water depth). Initial plant density in herbivore plots was marginally greater than in non-herbivore plots (Table 2). A small-scale greenhouse trial on potted *S. platyphylla* determined that the insecticide/surfactant mixture elicited no phyto-toxic or phyto-stimulation responses (R. Kwong unpubl. data).

**Fruit herbivory** Study plots were examined approximately every four weeks for fruit production and herbivore damage. The total number of undamaged and damaged fruit (surface or internal damage) per inflorescence was recorded. An additional assessment of internal damage was measured as the proportion of fruit per plot with damaged receptacles, which was assessed following the removal of achenes from each fruit (see *Achene herbivory* section below). The number of immature fruit per plot that had been completely destroyed by feeding activity was also recorded.

**Achene herbivory** Following fruit herbivory assessments, fruit from each plot were pooled and air dried. Achenes from mature fruit were dislodged from their receptacles and all extraneous plant material and insect larvae removed. To estimate mean achene weight per fruit, the pooled achenes per plot were weighed and averaged across the number of mature fruit per plot. Individual achene weight was determined by counting the number of achenes (intact + eaten + unfertilised) present in a 0.025 g subsample of the pooled achenes for each plot. The number of achenes per fruit was then calculated as the ratio of the achene weight per fruit and the individual achene weight. Weevil larvae targeted the achene embryos and did not feed upon unfertilised achenes. Therefore, when estimating the proportion of damaged achenes per fruit, unfertilised achenes were subtracted from the total number of achenes.

**Plant response** To evaluate end of growing season differences in plant density and biomass due to herbivore-plant interactions, all 24 plots were harvested at 3.5 months after commencement of the trial (22 September 2012). The total number of *S. platyphylla* plants and tubers per plot were counted. Foliage and tuber material were dried separately to constant weights in an oven at 55°C for a minimum of 48 h.

**Post-trial herbivory** To assess the continued activity of weevils on fruit damage and achene production, *S. platyphylla* was sampled on two occasions (22 October and 21 November 2012) in an adjacent stand occurring within the same pond. This stand had not previously been sprayed with insecticide or water. On each sampling occasion, 12 quadrats were haphazardly placed within the stand and were assessed for the following measures: number of fruit per plot, proportion of fruit per plot with internal damage, number of achenes per fruit and proportion of damaged achenes per fruit. Estimates for number of achenes and percentage of damaged achenes per fruit followed the same procedures outlined in *Achene herbivory* above.

**Statistical analysis** All statistical analyses were conducted using GenStat 16th Edition (VSN International Ltd., www.vsnl.co.uk). The exclusion experiment was a randomised block design with blocking based on two halves of the pond. Estimated means for non-herbivore (insecticide) and herbivore (control) treatments and for the different sampling dates were compared using analysis of variance (ANOVA) or linear mixed models. The choice of statistical technique depended on the complexity of the structure of the data and the degree of balance. For some response variables, missing values such as the absence of fruit on a particular sampling date, created an unbalanced design (Quinn and Keough 2002). Proportion data were analysed using generalised linear mixed models (GLMMs), with the link function  $\text{logit}(p) = \log(p/1-p)$  applied to the expected proportions, thus necessitating the results being presented as back-transformed means on the original scale. The analysis of plant density at the end of the trial used pre-trial plant density as a covariate.

## RESULTS

The removal of herbivores from plots using an insecticide spray treatment had no discernible influence on the number of fruit produced by plants at the individual or plot level throughout the duration of the trial (Table 1, Figure 1a,b). Nor did the application of insecticide appear to deter insect pollinators from visiting flowers as the percentage of unfertilised achenes between herbivore and non-herbivore plots were comparable (Table 1.).

**Fruit herbivory** The insecticide treatment reduced fruit herbivory on *S. platyphylla*, measured as piercing or chewing surface damage and internal weevil larva feeding damage. Temporal variation in herbivory occurred and treatment differences appeared rapidly (13 June 2012) for both surface and internal damage (Table 1, Figure 1c,d). Surface damage was highest at the start

of the trial irrespective of treatment and was likely due to superficial feeding by adult *L. appendiculatus* and the pentatomid bug *Euschistus ictericus* (Linnaeus), which was also observed on the fruits of *S. platyphylla* at the site. Across the duration of the trial, on average 30 and 34% of fruit in herbivore plots showed signs of surface and internal damage respectively and insecticide treatment reduced these levels of damage by 39% for surface damage and 83% for internal fruit damage (Table 1). Weevil larvae tunneled into and consumed tissue within the receptacle. Overall, receptacle damage in herbivore plots occurred in almost half of the fruit, but ranged from 46.7% (95% CI = 31.2, 62.8) at the start of the trial and increased to 65.9% (95% CI = 52.6, 77.1) by the completion. In contrast, no receptacle damage was recorded in the insecticide plots (Table 1). When immature fruit were heavily attacked, they

were completely destroyed, while no immature fruit were destroyed in the non-herbivore plots (Table 1).

**Achene herbivory** Inflorescence-feeding insects decreased the total weight of achenes per fruit across the duration of the trial (Table 1). This was due to an overall 22% reduction in the mean total number of achenes (fertilised and unfertilised) per fruit from 518 in non-herbivore plots to 403 in herbivore plots. Of total achenes, the number that remained intact and viable (i.e. not damaged by larvae) was 38% lower in herbivore plots compared to non-herbivore (i.e. 291 compared to 467) (Table 1, Figure 1e,f). In addition to feeding on receptacle tissue, larvae also consumed the embryos of fertilised achenes. Overall, larvae destroyed an average of 14.3% of achenes in herbivore plots compared to only 0.06% of damage in

**Table 1.** Comparisons of fruit and achene production and levels of herbivory by the pre-dispersal seed predator, *Listronotus appendiculatus* between herbivore (control) and non-herbivore (insecticide-treated) plots of *Sagittaria platyphylla*. Values are means with 95% confidence intervals [minimum, maximum] shown in parentheses.

Outcome measures Trial with repeated measures [T1-T5]	Comparisons between treatment groups		
	Herbivore (control)	Non-herbivore (insecticide-treated)	p-value
<b>Fruit herbivory measures</b>			
Number of fruit per inflorescence [means]	5.84 [5.42, 6.25]	5.37 [4.97, 5.76]	0.168
Number of fruit per plot [back-transformed means]	23.76 [17.50, 32.26]	26.50 [19.64, 35.75]	0.689
% Fruit per inflorescence with surface damage [back-transformed mean]	29.92 [23.19, 37.65]	18.12 [13.68, 23.61]	0.012
% Fruit per inflorescence with internal damage [back-transformed mean]	34.00 [27.93, 41.14]	5.80 [4.02, 8.32]	<0.01
% Fruit per plot destroyed at immature stage [back-transformed means]	9.28 [5.27, 13.30]	0.00	<0.01
% Damaged receptacles per plot [back-transformed means]	49.32 [38.36, 60.36]	0.00	<0.001
<b>Achene production measures per plot on mature fruit</b>			
Achene weight per fruit (mg) [means]	0.0578 [0.0492, 0.0664]	0.0747 [0.0663, 0.0830]	0.004
Total number of achenes (fertilised and unfertilised) per fruit [means]	403 [356, 449]	518 [472, 563]	0.001
Number of intact (undamaged) achenes per fruit [means]	291 [240, 343]	467 [417, 517]	<0.001
% of damaged achenes per fruit [back-transformed means]	14.18 [9.63, 20.41]	0.062 [0.030, 0.130]	<0.001
% of unfertilised achenes per fruit [back-transformed means]	9.94 [7.30, 13.39]	7.22 [5.29, 9.78]	0.086

non-herbivore plots (Table 1;  $F = 166.15$ ;  $df = 34.6$ ;  $P < 0.001$ , time  $F = 4.15$ ;  $df = 59.8$ ;  $P = 0.005$ ; interaction  $F = 6.48$ ;  $df = 136.4$ ;  $P < 0.001$ ), although two periods of greater damaged were observed in herbivore plots on the 11 July (22%) and the 21 September (39%).

**Plant response** Over the duration of the trial, there was around a three-fold increase in plant density in non-herbivore plots and a four-fold increase in herbivore plots, however no treatment effect was evident in the number of plants per plot at the end of the trial

when corrected for initial plant density (Table 2). Tuber abundance and total dry weight of tubers per plot did not differ between herbivore and non-herbivore plots, however above ground plant biomass was 35% less in non-herbivore than in herbivore plots, although the effect was marginally significant (Table 2).

**Post-trial herbivory** Fruit production continued at the site for the two consecutive post-trial assessment dates, although the percentage of fruit attacked by weevil larvae reduced by 74% (Table 3) and very little feeding damage was observed to the achenes.

**Table 2.** Comparison of plant density and above and below-ground plant biomass between insecticide-treated (non-herbivore) and control (herbivore) plots of *Sagittaria platyphylla*. Values are means with 95% confidence intervals [minimum, maximum] shown in parentheses.

Outcome measures	Comparisons between treatment groups		
	Herbivore (control)	Non-herbivore (insecticide-treated)	p-value
<b>Plant density per plot pre and post</b>			
Pre-trial number of plants [means]	15.2 [11.4, 19.0]	20.7 [16.9, 24.5]	0.05
End of trial number of plants [means]	61.9 [52.3, 71.5]	59.3 [49.7, 68.9]	0.69
<b>End of trial plant response per plot</b>			
Number of tubers [means]	50.1 [12.1, 88.1]	113.1 [22.5, 203.7]	0.12
Dry tuber biomass (g) [back-transformed means]	8.16 [3.87, 17.23]	21.3 [10.1, 45.0]	0.07
Above ground biomass (g) [means]	67.7 [52.0, 83.4]	44.1 [28.4, 59.8]	0.04

**Table 3.** Post-trial herbivory assessments on an untreated (non-insecticide) stand of *Sagittaria platyphylla*. Comparisons of fruit and achene production and levels of herbivory by the pre-dispersal seed predator, *Listronotus appendiculatus* between two dates. Values are means with 95% confidence intervals [minimum, maximum] shown in parentheses.

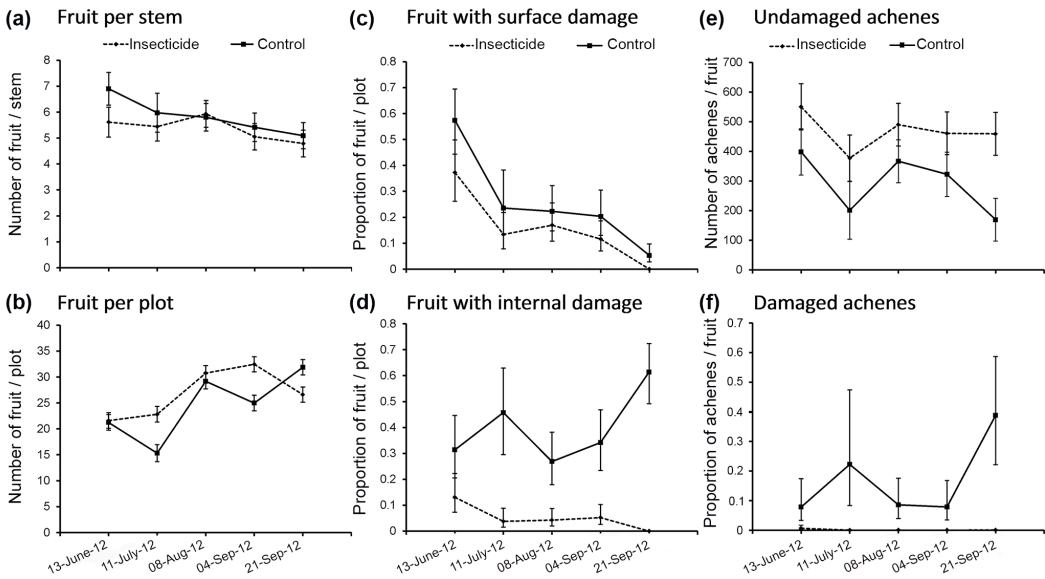
Outcome measures	Comparisons between treatment groups		
	22 October 2012	21 November 2012	p-value
<b>Trial with repeated measures [T1–T5]</b>			
<b>Fruit herbivory measures</b>			
Number of fruit per plot [back-transformed means]	23.00 [15.9, 30.1]	17.00 [8.73, 25.3]	0.24
% Fruit per inflorescence with internal damage [back-transformed mean]	20.63 [14.00, 29.40]	5.39 [2.05, 13.42]	0.01
<b>Achene production measures per plot on mature fruit</b>			
Number of intact (undamaged) achenes per fruit [means]	351 [263, 440]	435 [331, 539]	0.21
% of damaged achenes per fruit [back-transformed means]	3.8 [1.8, 11.0]	0.05 [0.04, 0.23]	<0.001

DISCUSSION

**Impact on reproductive performance** Our investigations demonstrated that the pre-dispersal seed predator *L. appendiculatus* negatively affected the reproductive performance of *S. platyphylla* in three ways. Firstly, when larvae attacked immature fruit they often completely destroyed the fruit leaving behind a mass of frass and the remnants of achenes. Hence, an average of 10% of all fruit produced per plant was destroyed prior to achene maturation. Secondly, feeding damage of receptacle tissue disrupted the development of achenes resulting in an overall 22% reduction in the number of achenes produced per fruit. In herbivore plots, an average of 50% of fruit across the 3-month trial sustained insect damage to the receptacle. Finally, direct consumption of the fertilised achene embryos resulted in a further 14% destruction of achenes per fruit. Therefore, the remaining number of achenes produced per fruit was reduced from 467 in non-herbivore plots to 291 in herbivore plots, which represents an average overall reduction of 38%. During our field surveys conducted at 38 sites from 2010 to

2012 (Kwong *et al.* 2017), achene destruction varied markedly from site to site which may be associated with a variety of factors including: (a) habitat or climate factors operating at a local or regional scale; (b) episodic rather than chronic impacts; and/or (c) levels of parasitism or predation of the pre-dispersal seed predators or of the plant’s pollinators. While parasitism levels were not quantified during our insect-exclusion experiment, we have documented a number of parasitic wasp species from *L. appendiculatus* larvae (N. Harms unpubl. data). In addition, the predatory reduviid bugs (Heteroptera: Reduviidae) were observed at some sites, piercing fruit and feeding on *L. appendiculatus* larvae. In contrast, weevil populations were in such high abundance at other sites, such as Yazoo Wildlife Management Reserve in Mississippi, that few of the sampled fruit produced achenes.

**Plant response to herbivory** Herbivores and pathogens affect plants in different ways and as such, plants exhibit a diversity of responses that can vary from susceptibility (the inability of the plant to resist



**Figure 1 (a-f).** Effects of insecticide treatment to exclude the curculionid pre-dispersal seed predator, *Lis-tronotus appendiculatus* on *Sagittaria platyphylla* over a 14-week period. Fruiting head (fruit) production: (a) the number of fruit per stem; and (b) the total number of fruit per plot fruit. Fruit damage: proportion of fruit per plot with (c) surface and (d) internal damage. Achenes: (e) the number of undamaged achenes per fruit, and (f) the proportion of achenes damaged per fruit. Insecticide treatment commenced on 8 June 2012. Dashed line represents non-herbivore plots (insecticide-treated), solid line represents herbivore plots (control, treated with water). Means (a) and back-transformed means (b) to (f) are plotted with 95% CI.

herbivore damage) to resistance (plant traits or life-history mechanisms that enable plants to avoid, defend or tolerate herbivore damage), or overcompensation (plants that have higher fitness when they are damaged compared with related plants that are undamaged (Agrawal 2000)). In terms of seed predation, plants may avoid or minimise losses of seed to predators in at least two ways (Atlan *et al.* 2010): phenological escape and predator satiation. Phenological escape results from asynchrony between predator and prey populations. In this case, fruit production occurs at a time when herbivore populations are minimal, either before or after peak seed predation. Predator satiation occurs where plants produce an overabundance of fruit thereby reducing the probability of an individual fruit being attacked (Janzen 1971). We found evidence for both of these strategies. *Sagittaria platyphylla* used a phenological avoidance strategy by continuing fruit production for up to two months following inactivity by *L. appendiculatus*. Secondly, *S. platyphylla* produced an abundant supply of fruit across the season with the proportion of fruit attacked never reaching more than 60%. However, rates of fruit predation were found to be highly variable in natural populations, ranging from no attack to almost 100% of fruit attacked (Kwong 2016), suggesting that predator satiation may not occur at all sites or at all times.

In addition to avoidance and satiation strategies, another response to herbivory may be through compensation, by replacing tissue lost to herbivory or reallocating resources to other plant parts. In our study, we expected to find a compensation for fruit herbivory through the production of more fruit, yet we found no evidence for an increase in fruit production at either the plant or plot level. As with other studies where seeds from non-attacked fruit were heavier than from insect-attacked fruit (English-Loeb and Karban 1992), we expected to find an increase in weight of intact achenes, yet we found no evidence for this.

The reallocation of resources to vegetative biomass is seldom reported in the literature as a response to herbivory of reproductive structures, therefore we expected to find no effect of this in our study. Yet in our study, aboveground plant biomass was 1.5 times greater in herbivore compared to non-herbivore plots. A likely explanation for the possible reallocation of resources in our study might be associated with insect herbivore damage to leaves and petioles, which would have been reduced following insecticide application. At the study site, leaf damage in the form of small shot holes was caused by adults of the weevil, *Listronotus lutulentus* (Boheman), while leaf petioles were damaged by larvae of both *L. lutulentus* and *L. appendiculatus* boring into and pupating at the base of

petioles. The increase in aboveground plant biomass in herbivore plots was not associated with plant density as plant density levels were similar between herbivore and non-herbivore plots. We therefore hypothesise that leaf and petiole herbivory may have induced a plant growth compensatory response.

To assess the potential of *L. appendiculatus* for the biological control of *S. platyphylla* in introduced countries we need to determine what influence seed herbivory has on the population dynamics of the weed. *Sagittaria platyphylla* is a perennial, long-lived species due to its prolific clonal growth via stolons and tubers. While we do not yet understand the importance of seed production on population growth rate, we suspect that seedling recruitment is unlikely to play an important role in the population dynamics of established populations. *Sagittaria platyphylla* has proven difficult to control with conventional methods due to submersed rosettes and subterranean tubers that are protected from contact herbicide application. However, populations can rapidly recolonise from the existing seed bank when conditions become favourable, such as during draw-down events in warmer months (Flower 2004). Hence, the reduction in seed banks caused by *L. appendiculatus* may assist in reducing the amount of seed available for re-colonisation following herbicide application.

From a biocontrol perspective, we predict that *L. appendiculatus* on its own is unlikely to reduce existing stands of *S. platyphylla* in the long term, but rather should be seen as one of a complementary suite of control agents that attack different life history stages of the weed.

#### ACKNOWLEDGMENTS

We are grateful to the Rural Industries Research and Development Corporation (Project No. PJR-007053), Murrumbidgee Irrigation Ltd and the Victorian Department of Economic Development, Jobs, Transport and Resources (formerly Department of Environment and Primary Industries) (Australia) for project funding.

#### REFERENCES

- Adair, R., Keener B., Kwong, R., Sagliocco, J-L. and Flower, G. (2012). The biology of Australian weeds 60. *Sagittaria platyphylla* (Engelmann) J.G. Smith and *Sagittaria calycina* Engelmann. *Plant Protection Quarterly* 27, 47-58.
- Agrawal, A.A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science* 5, 309-13.
- Atlan, A., Barat, M., Legionnet, A.S., Parize, L. and Tarayre, M. (2010). Genetic variation in flowering phenology and avoidance of seed predation in

- native populations of *Ulex europaeus*. *Journal of Evolutionary Biology* 23, 362-71.
- English-Loeb, G.M. and Karban, R. (1992). Consequences of variation in flowering phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). *Oecologia* 89, 588-95.
- Flower, G.E. (2004). The biology and control of arrowhead. Report to Goulburn-Murray Water, Tatura, Victoria. [http://www.g-mwater.com.au/downloads/arrowhead/giles\\_flower\\_arrowhead\\_report.pdf](http://www.g-mwater.com.au/downloads/arrowhead/giles_flower_arrowhead_report.pdf) (accessed 18 April 2016).
- Harms, N.E. and Grodowitz, M. (2009). Insect herbivores of aquatic and wetland plants in the United States: a checklist from literature. *Journal of Aquatic Plant Management* 47, 73-96.
- Janzen, D.H. (1971). Seed predation by animals. *Annual Review of Ecology, Evolution and Systematics* 2, 465-92.
- Kwong, R., Sagliocco, J-L., Harms, N., Shearer, J.F., Keener, B. and Green, P. (2014). Prospects for the biological control of delta arrowhead (*Sagittaria platyphylla*), an invasive aquatic species in Australia. Proceedings of the XIV International Symposium On Biological Control of Weeds, eds F.A.C. Impson, C.A. Kleinjan and J.H. Hoffmann, pp. 53-67. (University of Cape Town, South Africa).
- Kwong, R.M. (2016). The invasive aquatic macrophyte *Sagittaria platyphylla* (Alismataceae): Is it a suitable target for classical biological control? PhD dissertation, La Trobe University.
- Kwong, R.M., Sagliocco, J-L., Harms, N.E., Butler, K.L., Green, P.T. and Martin, G.D. (2017). Biogeographical comparison of the emergent macrophyte, *Sagittaria platyphylla* in its native and introduced ranges. *Aquatic Botany* 141, 1-7.
- Muenchow, G. and Delesalle, V. (1992). Patterns of weevil herbivory on male, monoecious and female inflorescences of *Sagittaria latifolia*. *American Midland Naturalist* 127, 355-67.
- Quinn, G.P. and Keough, M.J. (2002). 'Experimental design and data analysis for biologists'. Cambridge University Press.