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Examining Clonal Propagation of the Aquatic Weed *Sagittaria platyphylla*

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Examining Clonal Propagation of the Aquatic Weed *Sagittaria platyphylla*

by L Broadhurst and C Chong

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Foreword

Aquatic weeds are a serious threat to Australian ecosystems and irrigated agriculture. They threaten many Australian river systems; adversely affecting native biodiversity, hydrological flows, and water quality and availability. Many species have been used in the aquarium trade, which has led to their introduction into many new environments.

The genus *Sagittaria*, or arrowhead, has become increasingly invasive in Australia in the past two to three decades, and two species—*S. platyphylla* and *S. montevidensis*—are now recognised as aquatic weeds with infestations in most parts of Australia. It is especially prevalent in the irrigation areas and natural river systems of the central Murray Darling Basin.

This report quantifies clonal propagation in *Sagittaria platyphylla* by determining its corm production and regenerative capacity under controlled herbicide treatment and growth environments. The research found that the production of viable vegetative propagules (corms) is prolific under conditions conducive to growth. However, glyphosate applied at field rates translocates to corms and substantially reduces regeneration potential. Desiccation over seven to 30 days reduces, but does not void, corm viability. The researchers conclude that combining herbicide application and desiccation might be a useful control for established infestations.

This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

Phase 2 of the Program, which is funded to 30 June 2012 by the Australian Government, is being managed by RIRDC. Further research will be undertaken on the genetic, reproductive and demographic facilitation of *Sagittaria* invasion.

In addition to the *Sagittaria* project, RIRDC is commissioning some 50 projects that both extends on the research undertaken in Phase 1 and moves into new areas. These reports will be published in the second half of 2012.

This report is an addition to RIRDC's diverse range of over 2000 research publications which can be viewed and freely downloaded from our website www.rirdc.gov.au. Information on the Weeds Program is available online at www.rirdc.gov.au/weeds

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

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- Ross Gledhill, of Aquatic Plant Services, Goulburn–Murray Water, Victoria
- Richard Maxwell, of the Future Farming Systems Division, Department of Primary Industries, Victoria
- Dr Ellery Mayence, from CSIRO Plant Industry, Canberra
- members of the Sagittaria Tri-State Taskforce.

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What the report is about

Aquatic weeds threaten many Australian riverine systems, adversely affecting native biodiversity, hydrological flows, water quality and availability.

The changes aquatic weeds can cause include restricting water flow and increasing sedimentation, displacing native plant species and limiting opportunities for recruitment, reducing habitat for aquatic species such as fish and tortoises, and limiting recreational activity.

Despite these threats, the demography and dispersal dynamics of many aquatic weeds are not well understood in Australian riverine systems.

This report quantifies clonal propagation in the invasive aquatic weed *Sagittaria platyphylla* by determining its corm production and regenerative capacity under controlled herbicide treatment and growth environments.

Background

Some 20 species of *Sagittaria*, or arrowhead, exist worldwide, most of them occurring naturally in North and South America. Several species are edible; others are used for the aquarium trade, which has led to their introduction into many new environments. The genus has become increasingly invasive in Australia in the past two to three decades, and two species—*S. platyphylla* and *S. montevidensis*—are now recognised as aquatic weeds, there being infestations in most parts of Australia.

The genus is especially prevalent in irrigation areas and natural river systems of the central Murray–Darling district. In Victoria *S. platyphylla* is invasive throughout natural waterways and the Goulburn–Murray Water irrigation district, infesting wetlands and streams that are tributaries of the River Murray—among them the lower Ovens River and the Goulburn River and throughout the Ramsar-listed Barmah National Park wetlands.

Aims/objectives

The aim of the research was to investigate the relative contribution of seed and vegetative propagules to the invasion front in order to help refine management strategies by doing the following:

- identifying which propagules are most likely to disperse and successfully establish in new environments
- identifying high-risk source populations requiring more strategic management
- evaluating the efficacy of current strategies for controlling *Sagittaria*.

This information will be used to improve the means and the cost-effectiveness of control, particularly for interested parties with large and ongoing investments in *Sagittaria* control.

The relative contribution of clonal reproduction to dispersal and invasive spread was assessed by evaluating the spatial genetic structure of *S. platyphylla* in the core region of its current distribution in Victorian and NSW waterways.

Methods used

This project used genetic and demographic approaches in order to understand *Sagittaria platyphylla* dispersal in the Barmah National Park wetlands in northern Victoria and adjoining Millewa Forest in

New South Wales. In this ecosystem, *S. platyphylla* appears to be at a dynamic invasion stage, with many small patches ranging from clustered to highly dispersed and relatively fewer large, continuous stands (50 to 1000 square metres), this offers the opportunity to gain an understanding of dispersal and recruitment dynamics at relatively fine scales.

Six wetlands on tributaries of the River Murray in Victoria and New South Wales were chosen as study sites. The sites were representative of the core geographic range of *S. platyphylla* infestations in natural waterways during the 2008–09 growing season. During March 2009 leaf material was randomly sampled for genetic analysis from 20 to 60 plants at each site, depending on the size of the infestation. Five replicate soil cores (25 square centimetres and 10 centimetres deep) were also randomly collected at sites 1 to 5 for germination experiments. Whole plants for growth trials (30 per site) were collected at least 10 metres apart so as to minimise the chance of sampling replicate genotypes from each site.

The leaves, soil and plants collected were used to generate information about the following:

- the genetic composition and relatedness of plants within and among the sampled sites
- the persistence of the soil seed bank
- the capacity of vegetative fragments to establish and thrive, including following herbicide application.

Results/key findings

The main findings of the investigation are as follows:

- Seeds, rather than clonal propagules, are the main dispersal unit in *S. platyphylla*. Seed propagation is a primary leverage point in the weed's life cycle for improving effective control.
- Production of viable vegetative propagules (corms) is prolific under conditions conducive to growth. Glyphosate applied at field rates translocates to corms and substantially reduces regeneration potential. Desiccation over seven to 30 days reduces, but does not void, corm viability. Combining herbicide application and desiccation might be a useful control for established infestations.
- Genotypic diversity varied between the assessed localities. Shared genotypic groups were detected among localities, suggesting a high degree of connectivity. The genetic distribution among streams is consistent with multi-directional range expansion and long-distance seed dispersal (tens of kilometres). These results suggest that the increasing invasion of *S. platyphylla* has been facilitated by the movement of genotypes within and among streams under the prevailing habitat and flow regimes.

Implications for relevant stakeholders

Seed appears to be the main dispersal mechanism for *S. platyphylla* within and among natural streams, suggesting that seed propagation is an important leverage point for species management.

Current herbicide application rates in natural waterways have the capacity to reduce *Sagittaria* biomass and limit the recruitment ability from corms. Corms can, however, resprout vigorously when detached from the parent plant and when placed in water. Desiccation for seven to 30 days substantially reduced, but did not completely void, the corms' ability to regenerate. This suggests that herbicide and drying in combination could be used to control vegetative expansion of *Sagittaria* biomass.

The spatial genetic distribution of *S. platyphylla* reflects the movement of genotypes (genes) among streams in the river floodplain ecosystem, consistent with multi-directional range expansion. Genetic connectivity among localities is consistent with long-distance dispersal via seed (tens of kilometres).

No evidence for a persistent soil seed bank was found, suggesting that seedlings are derived predominantly from seasonally reproduced seed crops, rather than persistent seed. This is consistent with the rapid germination rates and the lack of dormancy mechanisms observed for *S. platyphylla* seed under trial conditions.

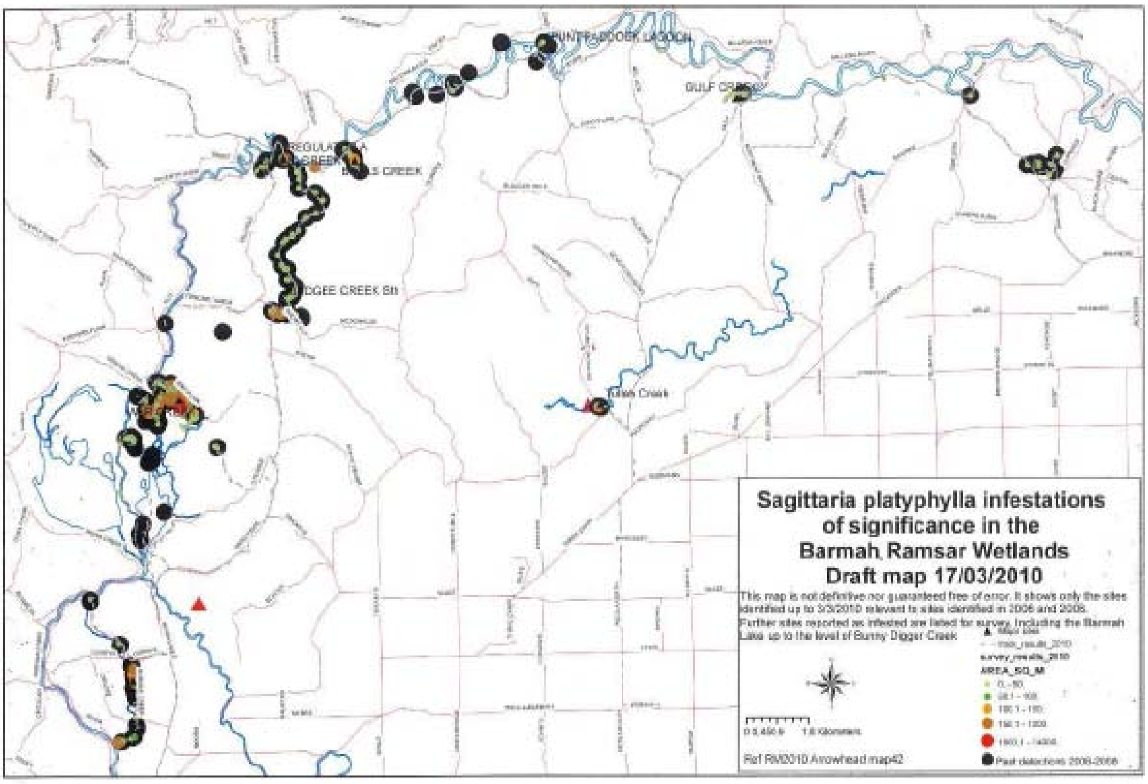
Introduction

Aquatic weeds threaten many Australian riverine systems, adversely affecting native biodiversity, hydrological flows, and water quality and availability. Among the changes aquatic weeds can cause are restricting water flow and increasing sedimentation, displacing native plant species and limiting opportunities for recruitment, reducing habitat for aquatic species such as fish and tortoises, and limiting recreational activity. Despite these threats, the demography and dispersal dynamics of many aquatic weeds are not well understood in Australian riverine systems.

Sagittaria

Some 20 species of *Sagittaria*, or arrowhead, exist worldwide, most of them occurring naturally in North and South America. Several species are edible; others are used for the aquarium trade, which has led to their introduction into many new environments. The genus has become increasingly invasive in Australia in the past two to three decades, and two species—*S. platyphylla* and *S. montevidensis*—are now recognised as aquatic weeds, there being infestations in most parts of Australia (Chong & Broadhurst 2009).

The genus is especially prevalent in irrigation areas and natural river systems of the central Murray–Darling district. In Victoria *S. platyphylla* is invasive throughout natural waterways and the Goulburn–Murray Water irrigation district, infesting wetlands and streams that are tributaries of the River Murray—among them the lower Ovens River and the Goulburn River and throughout the Ramsar-listed Barmah National Park wetlands (see Figures 1 and 2) (Goulburn–Murray Water 2010).



Source: R Maxwell, Department of Primary Industries, Victoria.

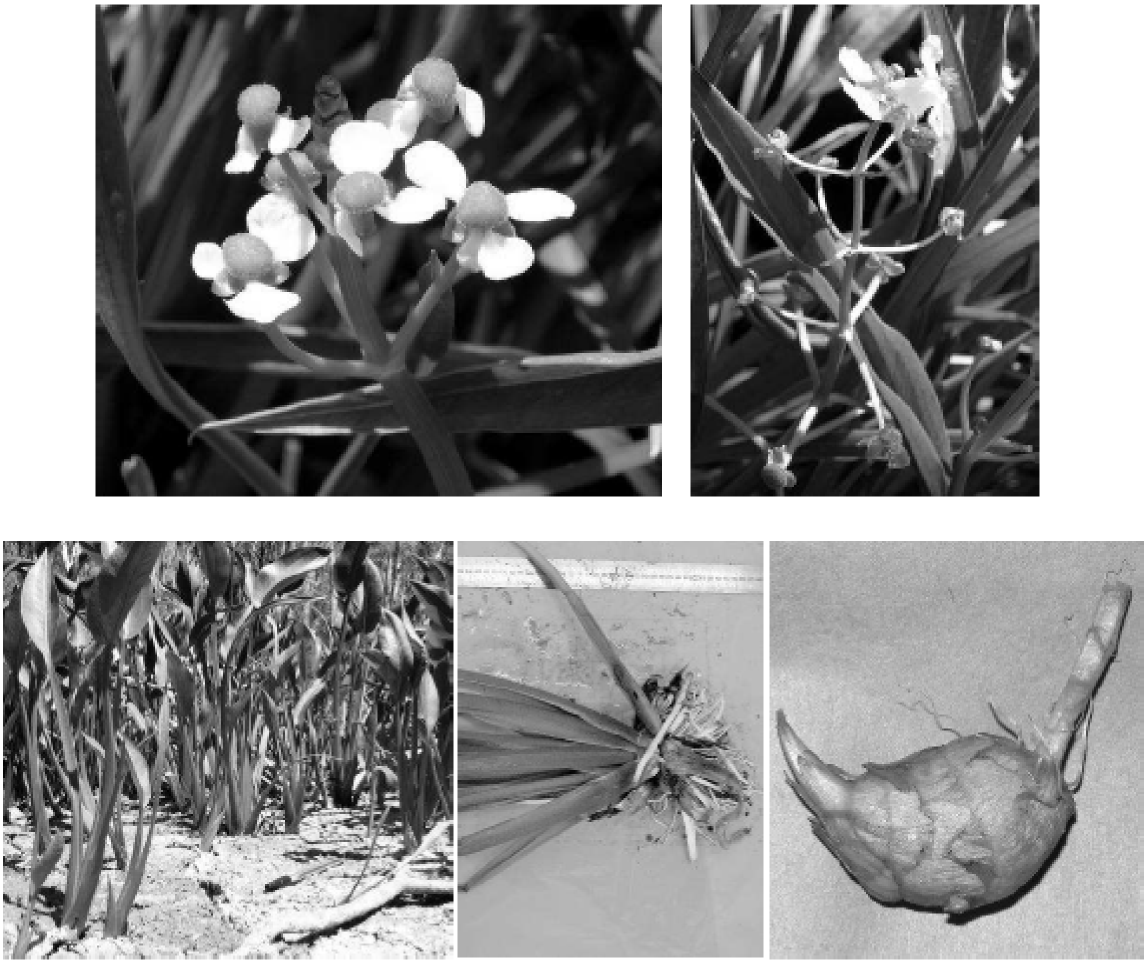
Figure 1 Distribution of *S.* platyphylla infestations in the Barmah National Park wetlands, northern Victoria, 2010




Images courtesy of C Chong, CSIRO Plant Industry, Canberra.

Figure 2 Uninvaded (left) and invaded waterways, in northern Victoria

Like many aquatic weeds, *S. platyphylla* can reproduce by sexual (seed) as well as clonal (ramets and corms) means (see Figure 3), providing two distinct mechanisms for dispersal. Seed are produced after flowering, which occurs predominantly in summer. Being small and buoyant, the seeds are capable of ‘rafting’ to suitable sites on relatively light currents, collecting in still pools and river bends. This mechanism can bring about the redistribution of genes along and among waterways. Clonal growth occurs from spring to autumn via ramet (shoot) production from rhizomes and from corms at the ends of auxiliary stolons. Ramets increase standing biomass, propagating genotypes already at a site; corms also perpetuate standing genotypes but are capable of moving following dispersing if detached from the mother plant during adequate stream flow. Field observations of seasonal emergent regrowth suggest that multiple sources—seeds, dispersed vegetative ramets and existing overwintered ramets and corms—can contribute to many cohorts.



Note: The upper panel shows female (left) and male (right) flowers on bisexual inflorescences; the lower panel shows emergent, rhizome and corm development.
Images courtesy C Chong, CSIRO Plant Industry, Canberra.

Figure 3 Reproductive structures in *S. platyphylla*

Project aims

The aim of the research was to investigate the relative contribution of seed and vegetative propagules to the invasion front in order to help refine management strategies by doing the following:

- identifying which propagules are most likely to disperse and successfully establish in new environments
- identifying high-risk source populations requiring more strategic management
- evaluating the efficacy of current strategies for controlling *Sagittaria*.

This information will be used to improve the means and the cost-effectiveness of control, particularly for interested parties with large and ongoing investments in *Sagittaria* control.

Materials and methods

This project used genetic and demographic approaches in order to understand *Sagittaria platyphylla* dispersal in the Barmah National Park wetlands in northern Victoria and adjoining Millewa Forest in New South Wales. In this ecosystem, *S. platyphylla* appears to be at a dynamic invasion stage—with many small patches ranging from clustered to highly dispersed and relatively fewer large, continuous stands (50 to 1000 square metres)—offering the opportunity to gain an understanding of dispersal and recruitment dynamics at relatively fine scales.

Study sites and sampling strategies

Six wetlands on tributaries of the River Murray in Victoria and New South Wales—at 35.9–36.0°S and 144.9–146.2°E (see Table 1) were chosen as study sites. The sites were representative of the core geographic range of *S. platyphylla* infestations in natural waterways during the 2008–09 growing season. Creek infestations were assessed by surveying the entire length of the creek, whereas river infestations were assessed by surveying the length from the River Murray to the upstream point beyond which no new infestations were detected for 20 kilometres. During March 2009 leaf material was randomly sampled for genetic analysis from 20 to 60 plants at each site, depending on the size of the infestation. Five replicate soil cores (25 square centimetres and 10 centimetres deep) were also randomly collected at sites 1 to 5 for germination experiments. Whole plants for growth trials (30 per site) were collected at least 10 metres apart so as to minimise the chance of sampling replicate genotypes from each site.

Table 1 Sampling sites and areal extent of infestations of *S. platyphylla*: Barmah National Park and Millewa Forest

Sampling site	Geographic location (degrees)		Infested area (m^2)[a]
	Southing	Easting	
1. Ovens River	36.049	146.189	>1000
2. Tongalong Creek	35.850	145.259	19–59
3. South Budgee Creek	35.857	145.007	662–1000
4. Moira Lake (NSW)	35.955	144.951	<100
5. Barmah Creek	36.005	144.947	1137–1781
6. Goulburn River	36.132	145.002	<100

a. Range reflects fluctuations in infestation size recorded between 2008 and 2010 ground surveys.
Source: C Chong, CSIRO Plant Industry, Canberra, and R Maxwell, Department of Primary Industries, Victoria.

The leaves, soil and plants collected were used to generate information about the following:

- the genetic composition and relatedness of plants within and among the sampled sites
- the persistence of the soil seed bank
- the capacity of vegetative fragments to establish and thrive, including following herbicide application.

Genetic composition and relatedness

DNA extracted from 158 representative samples from the six wetlands was genotyped using AFLP (amplified fragment length polymorphisms) markers, and the resultant data were used to generate the number of genotypes within and among tributaries, the levels of relatedness among genotypes, and spatial genetic structure maps.

Soil seed bank

Soil samples were germinated under glasshouse conditions and monitored for eight months for germination and growth of *S. platyphylla*. No *S. platyphylla* seedlings were detected during this period.

Capacity of vegetative fragments to establish and thrive

Plants without corms were collected from each site and planted in 14 cylindrical polycarbonate vessels (five plants per vessel) filled with about 1.1 cubic metres of equal parts of compost–earth–sand mix and watered to soil saturation with water levels maintained at 10-centimetre stem base submergence (see Figure 4). The plants were used for a sequence of experiments.

Figure 4 Glasshouse establishment of *S. platyphylla* collected from five River Murray tributaries across the weed's core invasive range

Growth rates and corm and ramet production

After 16 weeks five ramets from each vessel were unearthed and rinsed. The mass, number of leaves, and number and weight of corms were recorded for each plant. Recovered corms were then stored in the dark at 4°C for 30 days before a corm viability assessment. Half of the vessels were later treated with glyphosate, the remaining untreated plants being used to determine the total ramet production per soil volume and unit area and total corm production per number of ramets and soil volume.

Glyphosate resilience

After 16 weeks' growth half the poly vessels were randomly selected for glyphosate treatment; the remainder were kept as controls. The treated plants received a single foliar application of Weedmaster Duo© (Nufarm, 360 gL_{-1} glyphosate $NaOOCCH_2$-NH-14C-H_2-PO_3-H_2) delivered at a rate equivalent to current field rates of application in waterways in Barmah National Park (R Gledhill, Goulburn–Murray Water, pers. comm., June 2009). All sprayed and unsprayed vessels were monitored for regrowth for four weeks, which was considered sufficient time for herbicide translocation to below-ground structures (Tanphiphat & Appleby 1990; Shaner 2009). Data collected included the extent of biomass dieback (foliar or to stem base) and the emergence of new ramets.

Effect of glyphosate on corm viability and recruitment

Twenty corms harvested from each vessel were randomly selected, individually weighed and assigned to one of two treatment blocks for planting—control (unsprayed) in soil and sprayed in soil. Two corms per vessel were planted at one of five time intervals (zero, seven, 15, 30 and 60 days), the corms being stored individually in the dark at room temperature until planted. Twenty-eight corms per time interval were planted. Another 28 corms previously subjected to 30 days' cold after-drying (as just described) were planted at the initial time period. Corms were individually planted about 2 centimetres deep in 1200–cubic centimetre pots filled with equal parts of compost–earth–sand mix and watered to capacity. To determine propagules' capacity to sprout without soil, additional corms—one from each growth vessel—were placed in individual clear plastic vessels containing about 1200 centimetres of tap water. (The water-only treatment is considered a pilot investigation only.) All corm weights were recorded at harvest and at planting so as to monitor the rate of biomass loss. Shoot emergence from the corms was checked daily and recorded. Data were analysed in order to quantify the differences in corm mass, survival and recruitment under the glyphosate and control treatments.

Results

Growth rates and corm and ramet production

Sagittaria platyphylla exhibits rapid growth: stem density increased from the initial five ramets per vessel to 167 stems per vessel during the 16-week growth trial. This equates to about 10 stems a week. In the same period the number of corms increased from zero per vessel to 478 per vessel, equating to about 30 a week.

Glyphosate resilience

Herbicide treatment resulted in complete dieback of all above-ground biomass within seven days of application (see Figure 5, at 5b and 5c). At harvest, viability and recruitment potential were indeterminable from visual inspection of biomass alone, while the corms of treated plants showed slight to moderate signs of necrosis but were otherwise turgid and intact (see Figure 5e). Of the corms replanted immediately after harvest, 85 per cent of non–herbicide treated corms (24 corms) were viable and established as healthy seedlings. Days to emergence ranged from five to 36. In contrast, only 10 per cent of the herbicide-treated corms (three corms) showed signs of resprouting, and all three died before reaching seedling establishment at four weeks. These data strongly suggest that glyphosate does translocate to corms.

Effect of glyphosate on corm viability and recruitment

Effect of desiccation on corm mass

Non–herbicide treated corms subjected to delayed replanting (post-harvest desiccation) lost 55.8 per cent of their mass after seven days (compared with 71 per cent for herbicide-treated corms), 57.4 per cent after 15 days (78.6 per cent for herbicide-treated) and 61.7 per cent after 30 days (79.4 per cent for herbicide-treated). No further mass loss was observed for the 60-day treatment for either the non–herbicide treated corms or the herbicide-treated ones. The proportion of corm biomass lost over the 60-day period differed between the herbicide and non-herbicide treatments (one-way ANOVA, $F_{13, 266}$ = 1.62, P <0.01).

Table 2 Percentage of *S. platyphylla* corm biomass lost following desiccation

Treatment	Days				
	0	**7**	**15**	**30**	**60**
Non-herbicide	..	55.8 (0.31)	57.4 (0.33)	61.7 (0.33)	No change
Herbicide	..	71.0 (0.31)	78.6 (0.35)	79.4 (0.29)	No change

.. Not applicable.
Note: Standard errors are in parentheses.

Effect of desiccation on corm viability

For corms subjected to post-harvest desiccation at room temperature, no corm—regardless of herbicide treatment—exhibited viability for the seven-, 15-, 30- or 60-day periods. Only non–herbicide treated corms replanted immediately after harvest exhibited viability.

Recruitment after 30 days' cold, dry storage

After dry storage at 4°C and darkness for 30 days, 39.3 per cent of corms (11 out of 28) showed successful recruitment and seedling establishment. Further assessment of the temporal longevity of corms could be valuably done by collection and abscission from shoots, subsequent burial and exposure to field soil temperature conditions, followed by seasonal germination tests.

Recruitment from water-borne corms

Eleven of the 14 corms placed in small beakers filled with water initiated new shoots. Shoot and root development was vigorous and remained viable for the four weeks of the study period. Corms placed in vessels containing water initially sink, then float to the surface when shoots develop, and then sink again as roots develop. To characterise the spatial dispersal distribution of vegetative propagules, the pattern of movement of viable corms vertically as well as laterally in the water body could be further assessed.

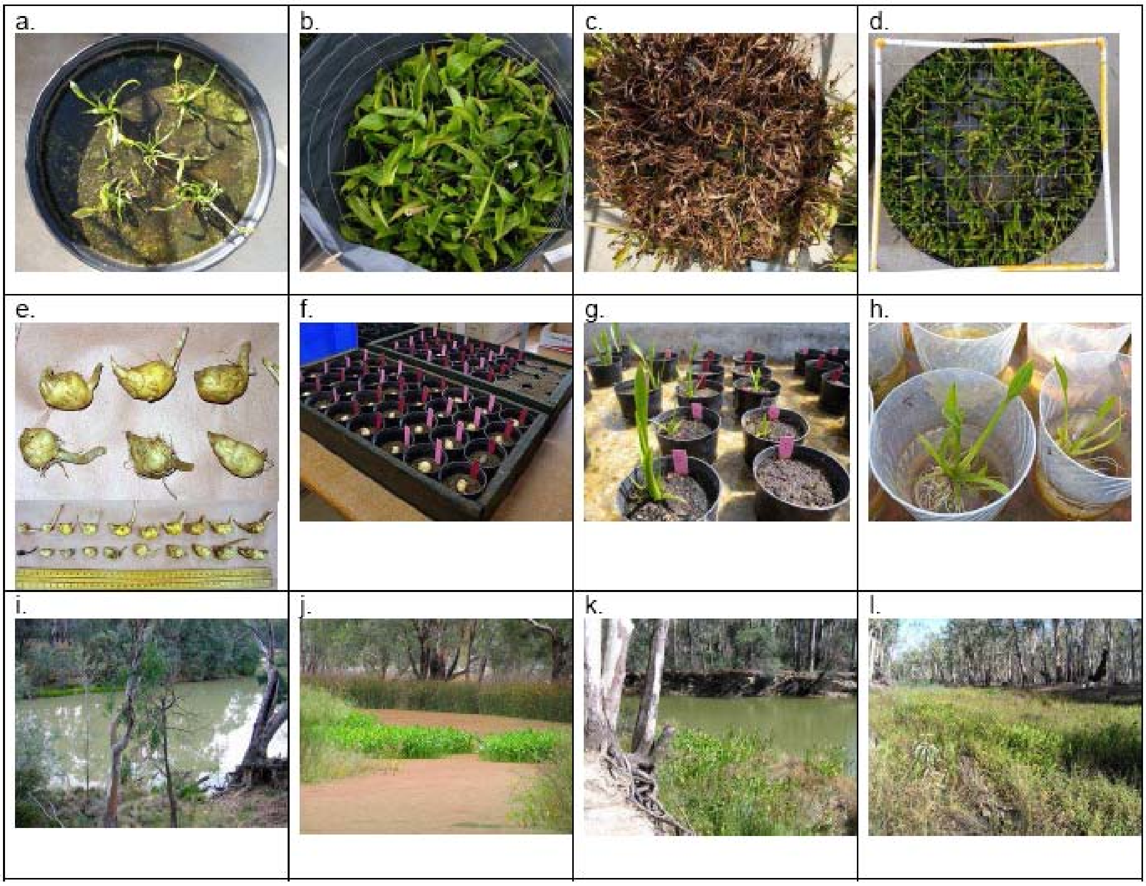
Genetic composition and relatedness

AFLP markers determined that all analysed plants, regardless of stream location, were genotypically unique, indicating that seeds are the major dispersal unit for *S. platyphylla*. Genetic diversity differed among the streams sampled (the proportion of polymorphic loci 0.045 to 0.81—see Table 3), Moira Lake and the Goulburn River having lower diversity.

Table 3 Genetic diversity in *S. platyphylla* sites with standardised sample sizes

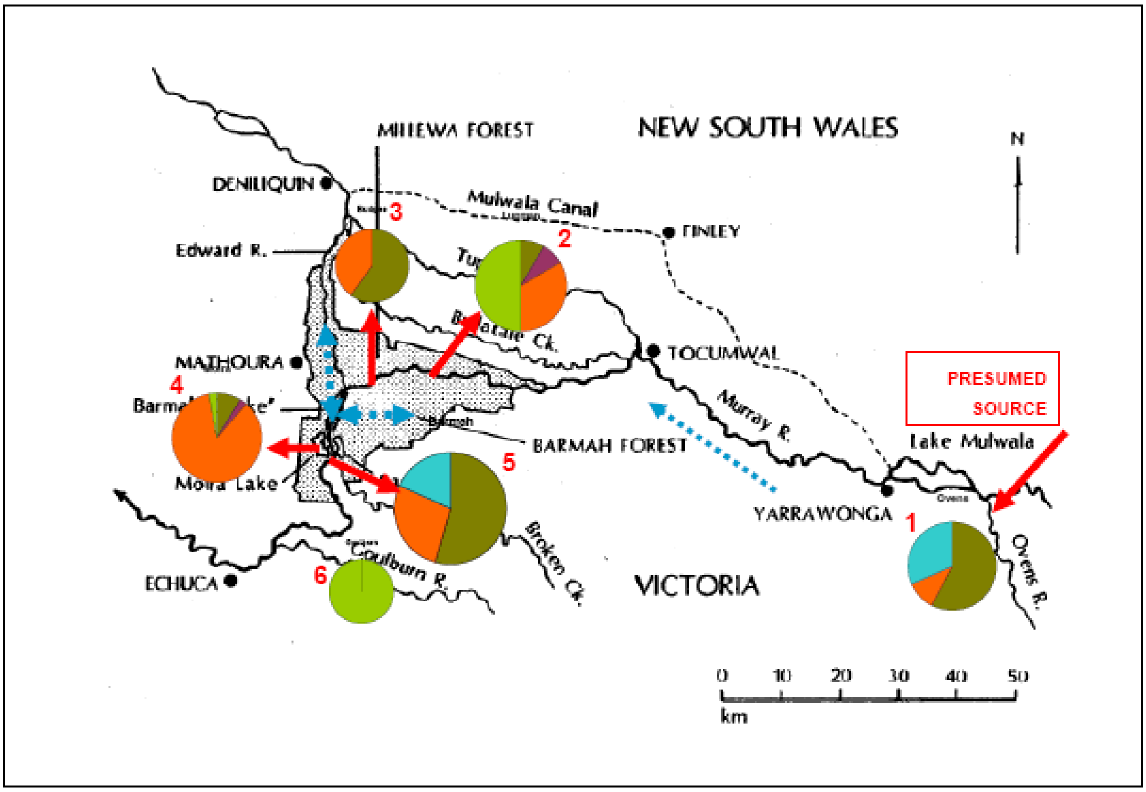
Locality	Band richness	Proportion polymorphic loci
1. Ovens River	1.58	0.774
2. Tongalong Creek	1.61	0.760
3. South Budgee Creek	1.63	0.690
4. Moira Lake	1.52	0.617
5. Barmah Creek	1.59	0.812
6. Goulburn River	1.40	0.446

Note: Band richness, *Br*, is analogous to allelic richness. Loci are polymorphic at $\alpha = 0.01$ for $1.01 < Br < 1.99$.



Notes: a) ramet production at three weeks; b) ramet production at 16 weeks; c) shoot dieback after glyphosate application; d) quantifying stem density on harvest at 16 weeks; e) harvested corms unsprayed (upper) and glyphosate-treated (lower); f) weighed corms prepared for replanting; g) new shoot growth from replanted corms; h) shoot and root development in water. *S. platyphylla* in March 2009 at i) Goulburn River, (j) Moira Lake, k) Tongalong Creek, and l) Barmah Creek.

Figure 5 Aspects of the research



Note: Map shows spatial distribution of genotypic clusters (colours) inferred from Bayesian genotypic assignment of AFLPs. Circle radius is proportional to sample size. Dashed blue arrows represent directionality of natural stream flow.

Figure 6 Genetic clustering of *S. platyphylla* in River Murray waterways

An analysis of molecular variance showed significant genetic structuring among localities, independent of geographic position (P<0.001). Bayesian probabilistic assignment of individuals to genotypic groups based on allelic frequencies, rather than spatial location, showed that similar genetic groups occur in multiple locations, suggesting connectivity among streams (see Figure 6). The data suggest that range expansion of *S. platyphylla* has been promoted by genetic admixture and dispersal over relatively large distances (tens of kilometres).

Discussion

Seasonal control of *Sagittaria platyphylla* using glyphosate typically kills above-ground shoots, but achieving plant death requires the delivery of a lethal herbicide dose to the perenniating organs. In *Sagittaria* this constitutes the underground corms, from which new shoots and rhizomes can initiate and potentially disperse as next-generation vegetative propagules. The efficacy of herbicide relative to minimising recruitment through vegetative propagules is, however, not previously documented. Importantly, the genetic structure of the plants and the relative contribution of vegetative and seed recruitment to dispersal and the expanding range of *S. platyphylla* infestations are unknown. These knowledge gaps make it difficult to develop effective management strategies at a population-based scale or to predict changes in population structure in response to a variety of growth and disturbance scenarios.

This investigation quantified clonal propagation in *S. platyphylla* by determining corm production and regenerative capacity under controlled herbicide treatment and growth environments. As a complementary analysis we assessed the evidence for population structure and characterised genetic diversity in streams representing the core region of the weed's distribution in Victorian waterways.

Synthesis of research findings

Spatial genetic structure

AFLP markers determined that all sampled individuals were unique genotypes. This suggests that seeds, rather than clonally generated propagules, are the primary dispersal unit for *S. platyphylla*.

Infestation patterns

New infestations can contain a high degree of genetic diversity and are probably seed derived. Multiple similar genetic groups were detected across the different stream localities sampled, suggesting that multi-directional range expansion of *S. platyphylla* has occurred in the river floodplain. This is consistent with natural hydro-geomorphic flood patterns in Barmah National Park.

Growth and dispersal ability

S. platyphylla demonstrates strong reproductive and dispersal capacity for both seed and vegetative structures. Seed and vegetative reproductive modes appear, however, to contribute to invasion dynamics in spatially discrete ways. Vegetative reproduction in established infestations promotes individual longevity, rapid biomass increase and persistence, whereas regeneration via seed contributes to maintained genetic diversity and dispersal between streams.

Resilience following herbicide treatment

Glyphosate treatment does translocate to corms but is not entirely successful at killing underground structures: about 10 per cent are potentially capable of resprouting following herbicide application at field rates. Given the ability for rapid growth found in this study, this 10 per cent represents a significant impediment to *Sagittaria* control. Interactions between chemical type, application rate and micro-habitat conditions such as water availability should also be considered when evaluating the herbicide's efficacy in stopping corm regeneration (that is, successful establishment as new shoots) under field conditions.

Management implications

Seed appears to be the main dispersal mechanism for *S. platyphylla* within and among natural streams, suggesting that seed propagation is an important leverage point for species management.

Current herbicide application rates in natural waterways have the capacity to reduce *Sagittaria* biomass and limit the recruitment ability from corms. Corms can, however, resprout vigorously when detached from the parent plant and when placed in water. Desiccation for seven to 30 days substantially reduced, but did not completely void, the corms' ability to regenerate. This suggests that herbicide and drying in combination could be used to control vegetative expansion of *Sagittaria* biomass.

The spatial genetic distribution of *S. platyphylla* reflects the movement of genotypes (genes) among streams in the river floodplain ecosystem, consistent with multi-directional range expansion. Genetic connectivity among localities is consistent with long-distance dispersal via seed (tens of kilometres).

No evidence for a persistent soil seed bank was found, suggesting that seedlings are derived predominantly from seasonally reproduced seed crops, rather than persistent seed. This is consistent with the rapid germination rates and the lack of dormancy mechanisms observed for *S. platyphylla* seed under trial conditions.

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Appendix A Workshop findings

The *Sagittaria* Research Workshop was held on 5 May 2010 at CSIRO's Black Mountain Laboratories in Canberra. The following organisations were represented:

- CSIRO—Linda Broadhurst, Caroline Chong and Ellery Mayence
- Department of Primary Industries, Victoria—Daniel Clements, Jean Louis Sagliocco, Nigel Ainsworth and Raelene Kwong
- Industry & Investment NSW—Stephen Johnson
- Goulburn–Murray Water—Ross Gledhill and Tim Nitschke.

The workshop provided an opportunity for discussing several themes:

- the current state of biological knowledge in relation to *Sagittaria*
- knowledge gaps—identification and development of priorities
- initiation of a collaborative report or paper to consolidate these data if appropriate
- actions for broad-scale management of *Sagittaria*
- raising the profile of *Sagittaria* as a weed of national significance.

Biological knowledge

Discussion centred around the life cycle of *Sagittaria* in order to help identify critical leverage points for management (see Figure A.1). Information on matters such as flowering (timing, length of season, environmental cues, and so on), seed production (timing, volumes, and so on), vegetative growth, establishment (rates, locations, and so on), genetic differentiation and diversity was sought from participants, including expert opinion where hard data are not available.

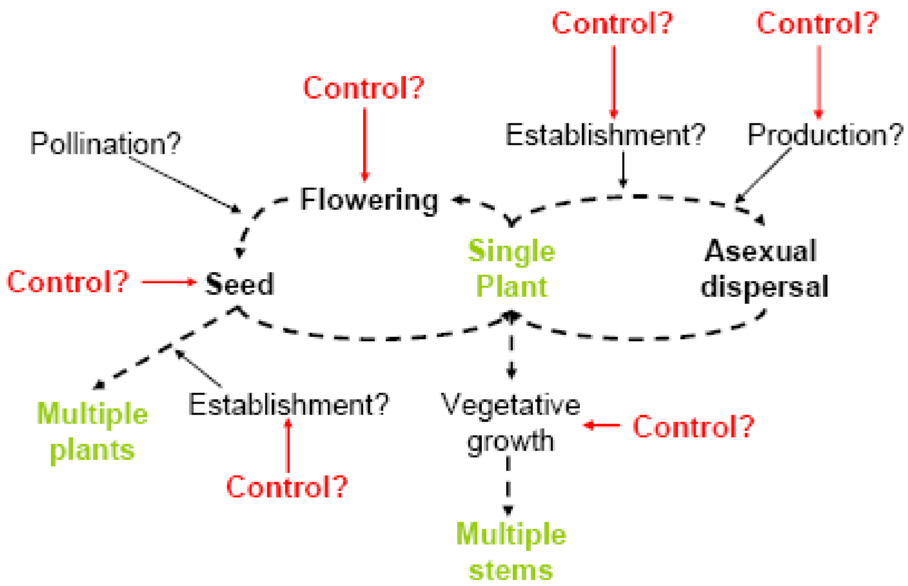

Figure A.1 The life cycle of *Sagittaria*: critical leverage points

The members of the research group identified 16 research questions that they consolidated into the following research areas (in the order of priority shown):

1. *The efficacy of herbicide for controlling and managing* Sagittaria. Herbicide application remains a major component of current *Sagittaria* management strategies. The main research areas identified were as follows:

   – The generation of glyphosate-tolerant genotypes is unlikely to be a risk for recurrent application to regenerative plants, but application to seedlings and rosettes might lead to selection for resistance, which could be passed on to subsequent generations. Screening of seedlings under various application rates would offer a relatively rapid assessment of the potential to generate glyphosate tolerance.

   – If a risk of generating glyphosate-tolerance is identified, modelling should provide an indication of how quickly the tolerance could be spread. This could be integrated with the 'Molecular control of reproduction in weeds' research, which is performing genetic simulations in order to assess the impacts of chemically induced self-incapability on wild radish (*Raphinus raphanistrum*) populations.

   – Research is also required to improve our understanding of the physiological and demographic processes following glyphosate application. This includes determining the rate and pathways of herbicide translocation throughout single and multiple plants and how the timing of applications to various life history stages influences control. Competition dynamics between seedlings and corms in the season following herbicide application are currently being investigated by Raelene Kwong of the Department of Primary Industries, Victoria.

2. Sagittaria *demography and physiology.* A better understanding of the demography of *Sagittaria* is needed in order that managers and researchers can have improved information on the invasion risks posed by various life history stages. Caroline Chong of CSIRO has identified seed as the primary dispersal risk, suggesting that further research on this life history stage is required. Raelene Kwong is investigating seed viability, including seed bank dynamics. Further research is needed in order to understand the seasonality of seed production and environmental cues that stimulate germination and establishment. Raelene Kwong is also investigating rosette production and growth. An improved understanding of physiological changes such as photosynthesis as plants emerge above the water line might also yield useful information about herbicide translocation and effectiveness.

3. *Biocontrol.* Biocontrol agents are being investigated by Jean Louis Sagliocco and Raelene Kwong, both of the Department of Primary Industries in Victoria; this will involve visiting the home range of *Sagittaria* to look for potential agents. It will also require clarification of taxonomic questions about *Sagittaria* in Australia—particularly in New South Wales—to ensure that biocontrol agents for the appropriate *Sagittaria* species are collected and deployed once they have been assessed for release. Taxonomic relationships could also help narrow the home range where suitable biocontrol agents might be found. Testing of biocontrol agents should include genotypes from a broad range of invaded environments, to ensure that the agents are effective over large spatial and environmental scales and so protect against possible selection in Australia generating new genotypes against which the agents are no longer effective.

4. *Ecological and economic impacts.* There is little information available with which to determine the ecological impacts of *Sagittaria* on other plant, vertebrate and invertebrate species in wetlands and riparian systems. There is also a need to provide information about *Sagittaria*'s economic impacts in terms of the provision of water resources to the agricultural, industrial and public sectors in invaded states and territories.

5. *Experimental management.* Several experimental management treatments were discussed, among them the following:

- Caroline Chong and Ellery Mayence, both from CSIRO, have determined that corms are extremely sensitive to desiccation, and it might be possible to dry channels and rotary hoe in order to expose corms to drying. This would necessitate the gathering of demographic information about the timing of peak corm production and the quantification of lateral growth from rosettes to test the effectiveness of this treatment.

- Trials using protocols developed for control of submerged aquatic weeds should be conducted. This includes the application of gels to hold herbicides close to submerged plant parts.

- Other chemicals should be trialled in the search for a fail-safe should glyphosate tolerance become a problem for *Sagittaria* control in the future.

Examining Clonal Propagation of the Aquatic Weed *Sagittaria platyphylla*

by G Charles, Australian Weeds Research Centre

This report quantifies clonal propagation in the invasive aquatic weed *Sagittaria platyphylla* by determining corm production and regenerative capacity under controlled herbicide treatment and growth environments. The relative contribution of clonal reproduction to dispersal and invasive spread was assessed by evaluating the spatial genetic structure of *S. platyphylla* in the core region of its current distribution in Victorian waterways.

This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

Solutions to weeds in Australia require a long-term, integrated, multi-stakeholder and multi-disciplinary approach. RIRDC is seeking project applications that involve collaboration between stakeholder groups, and where possible, including external contributions both monetary and in-kind.

This report is an addition to RIRDC's diverse range of over 2000 research publications which can be viewed and freely downloaded from our website www.rirdc.gov.au.. Information on the Weeds Program is available online at www.rirdc.gov.au/weeds. Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Cover photos: Uninvaded and invaded waterwayhs. Sourced from this report. Courtesy C. Chong, CSIRO Plant Industry, Canberra.

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