

## Research Article

## Management of aquatic alligator weed (*Alternanthera philoxeroides*) in an early stage of invasion

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### Abstract

Alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is an amphibious plant that aggressively invades aquatic and terrestrial environments. It has invaded at least 14 countries and is difficult to control. The present study investigates the effectiveness of herbicides and physical removal in eliminating patches of aquatic alligator weed in an early stage of invasion. This paper firstly describes a screening trial to determine the relative efficacy of single application of three herbicides used in Australia (glyphosate, metsulfuron-methyl ± surfactant, and dichlobenil), each applied at three rates to containers of alligator weed. Control was greatest for all herbicides at rates higher than the manufacturer's recommendation (label rate). Glyphosate at 3 × label rate (3.6 kg a.i. ha<sup>-1</sup>; 10.8 g a.i. L<sup>-1</sup>) and dichlobenil at 2 × label rate (31 kg a.i. ha<sup>-1</sup>) provided the greatest level of control at 48 and 91 weeks after treatment. The presence of surfactant did not improve metsulfuron-methyl efficacy. Field studies were then carried out to evaluate the effectiveness of repeated physical removal and repeated applications of chosen herbicides to eliminate patches of aquatic alligator weed in an early stage of invasion of two urban streams in Melbourne, Australia. Glyphosate and metsulfuron-methyl (without a surfactant) were applied to patches of aquatic alligator weed in a best practice regime, consisting of up to three applications per year for up to five consecutive years. Glyphosate was applied at 3 × label rate, as well as at label rate. No alligator weed remained after two years of the herbicide application regime for patches treated with metsulfuron-methyl, while for glyphosate alligator weed remained in only one of 18 patches after three years. Physical removal eliminated 75% of patches after initial treatment and minimal follow up treatments were required where regrowth occurred. This study demonstrates that the management methods utilised are capable of eliminating patches of aquatic alligator weed in an early stage of invasion in two to three years.

**Key words:** *Alternanthera philoxeroides*, alligator weed, aquatic weed management, eradication, herbicide, physical removal

### Introduction

Alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is a perennial stoloniferous herb in the Amaranthaceae family, originating from the Parana River area of South America (Julien et al. 1995). It has subsequently spread to and increased its range within many countries including; southern USA (first detected in 1897), New Zealand (1906), China (1930s), Australia (1946), India, Burma and Indonesia (by the 1960s). More recently it has been detected in Puerto Rico, Singapore, Vietnam, Thailand, Sri Lanka, Italy and France (Dugdale and Champion 2012).

Alligator weed is an aggressive invader of both aquatic and terrestrial environments (Sainty et al. 1998). It is particularly successful in aquatic and semi-aquatic environments where it is capable of extremely rapid growth (Clements et al. 2011). In aquatic environments alligator weed roots into the soil near the water's edge or in the substrate beneath shallow water and produces mats of entangled stems that float and extend over the water surface. Floating mats of alligator weed can choke waterbodies, restricting human use, excluding desirable plant species, interfering with aquatic ecology and restricting water flow (Julien et al. 1992). Alligator weed poses a significant threat

to waterways, wetlands, floodplains and irrigation systems (van Oosterhout 2007). It can also invade terrestrial situations, such as pasture (Julien and Broadbent 1980), arable crops (Shen et al. 2005) and urban areas (Gunasekera and Bonilla 2001).

In its introduced range, alligator weed reproduces solely by clonal growth, as viable seeds are not produced. It efficiently disperses via stem fragmentation, where stem fragments or floating mats break off and disperse to surrounding areas, creating new infestations (Dugdale et al. 2010 and Julien et al. 1992). Although it prefers a warm growing season, it can tolerate winter frosts (Julien et al. 1995).

A biosecurity approach is commonly undertaken to manage invasive species, particularly weeds. One aspect of this approach for weed management is a goal to eradicate a species from an area in which it has become naturalised, provided it meets certain criteria: 1) it is deemed a species capable of invasion (i.e. it spreads into areas considerable distances away from parent plants (Richardson et al. 2000)); 2) it is in an early stage of invasion and occupies only a very small part of its potential range; and 3) it poses a significant threat to social, economic or environmental values.

Early invasion of alligator weed occurs in and around the metropolitan area of Melbourne, the capital city of Victoria, Australia, where it was first detected in 1996 (Gunasekera and Bonilla 2001). If left unchecked, it is anticipated that these infestations will act as a source population for dispersal to other areas of Victoria, where it will significantly compromise agricultural productivity, block irrigation and drainage infrastructure and reduce biodiversity and social amenity of aquatic environments. Thus, in addition to being a weed of national significance in Australia (Australian Government 2012), alligator weed has been declared a state prohibited species and targeted for eradication in Victoria (Victorian Government 2014). This situation is similar to New Zealand, where it is designated as an unwanted organism (New Zealand Government 2010). In the USA, China and the Australian state of New South Wales (where it has been a problem weed for more than 65 years, first detected in 1946), it is in a later stage of invasion, where it is much more widespread and abundant, so eradication is not feasible. In these locations suppression programs exist based on herbicide (Dugdale and Champion 2012) and biological control (Sainty et al. 1998), which aim to contain infestations and reduce the spread and impact of the weed. In China US\$72 million is

spent each year to manage alligator weed (Liu and Diamond 2005).

In Victoria, the herbicides glyphosate or metsulfuron-methyl have been the preferred methods of alligator weed management in early invasion aquatic situations. Physical removal has also been employed depending on site characteristics, environmental sensitivity and resources available. Glyphosate is labelled for use in aquatic areas but is considered to be less effective against alligator weed than metsulfuron-methyl (Dugdale and Champion 2012). The herbicide metsulfuron-methyl is commonly used in terrestrial situations, however because of its apparent success in controlling alligator weed, a permit was obtained to use it in some aquatic situations in Victoria. There are however questions of its use in aquatic environments because of toxicity, and the propensity for alligator weed to fragment and disperse after herbicide application (Clements et al. 2012; Dugdale et al. 2010). Dichlobenil is also registered for use on alligator weed in static water aquatic systems that are not used for irrigation purposes (van Oosterhout 2007).

The effectiveness of herbicides for management of alligator weed has been reviewed by Dugdale and Champion (2012). It is recognised that multiple herbicide applications over multiple years are required to kill any emergent alligator weed and deplete underground root storages to eventually exhaust the plant (Bowmer et al. 1991; van Oosterhout 2007). However, there is limited field information on the long term (greater than one year) effectiveness of any of these herbicides in eliminating alligator weed in an early stage of invasion.

Alligator weed is also managed by either manual or mechanical removal methods. These physical approaches require the complete excavation of all above and below ground alligator weed to prevent regrowth (Sainty et al. 1998). In aquatic situations alligator weed generally lacks a deep penetrating root system compared to the terrestrial form, most probably due to the roots obtaining the required water and nutrients directly from the water column and sediment (Geng et al. 2007; Julien et al. 1992). This growth habit therefore lends itself to physical removal. Physical removal is initially much more labour intensive than herbicide application, however due to the difficulty of controlling alligator weed with herbicide (multiple applications, over multiple years), it provides a method that can remove most, if not all alligator weed and eliminate regrowth in one instance.

**Table 1.** Herbicides and rates applied to containers of alligator weed. Five replicates per treatment.

Treatment	Product	Rate	Tank rate (herbicide a.i. / L)	a.i. (g / ha)
Metsulfuron, 0.5 × label	Esteem® 600 g / kg	5 g / 100 L	0.03	10
Metsulfuron, 1 × label	Esteem® 600 g / kg	10 g / 100 L	0.06	20
Metsulfuron, 2 × label	Esteem® 600 g / kg	20 g / 100 L	0.12	40
Metsulfuron, 0.5 × label + surfactant	Esteem® 600 g / kg + Pulse®	5 g / 100 L + 200 mL / 100 L	0.03	10
Metsulfuron, 1 × label + surfactant	Esteem® 600 g / kg + Pulse®	10 g / 100 L + 200 mL / 100 L	0.06	20
Metsulfuron, 2 × label + surfactant	Esteem® 600 g / kg + Pulse®	20 g / 100 L + 200 mL / 100 L	0.12	40
Glyphosate <sup>a</sup> , 1 × label	Roundup Biactive® 360 g / L	10 mL / L	3.6	1,206
Glyphosate <sup>a</sup> , 3 × label	Roundup Biactive® 360 g / L	30 mL / L	10.8	3,618
Glyphosate <sup>a</sup> , 6 × label	Roundup Biactive® 360 g / L	60 mL / L	21.6	7,236
Dichlobenil, 0.5 × label	Sierraron® G, 67.5 g / kg	1,150 g / 100 m <sup>2</sup>	N/A	7,763
Dichlobenil, 1 × label	Sierraron® G, 67.5 g / kg	2,300 g / 100 m <sup>2</sup>	N/A	15,525
Dichlobenil, 2 × label	Sierraron® G, 67.5 g / kg	4,600 g / 100 m <sup>2</sup>	N/A	31,050
No Herbicide	-	-	-	-

Abbreviations: a.i. = active ingredient, metsulfuron = metsulfuron-methyl

<sup>a</sup> All glyphosate present as the isopropylamine salt formulation

Further, physical removal provides an alternative to herbicide use in areas where herbicide may be deemed inappropriate. Physical removal is recommended for small infestations; particularly the initial invasion of a catchment or after a long period of chemical control has suppressed formerly high levels of biomass to low levels (van Oosterhout 2007).

The present study investigates the effectiveness of herbicides and physical removal in eliminating patches of aquatic alligator weed in an early stage of invasion. It includes a screening trial, in containers, to study the relative effectiveness of single applications of herbicides (herbicide type, herbicide rate and presence of surfactant). Field studies were then carried out to evaluate the effectiveness of repeated physical removal and chosen herbicide strategies, over multiple years, to eliminate alligator weed in an early stage of invasion of two urban streams in Melbourne, Australia.

## Materials and methods

### Screening trial

Alligator weed stem cuttings consisting of four nodes with apical shoot tips, without roots, were collected from a single patch at Patterson River (38°2'45.98"S; 145°10'11.78"E) in November 2007. Sixty-five containers (0.58 m diameter by 0.45 m tall) were half filled with topsoil that was augmented with 4 kg m<sup>3</sup> Osmocote® general purpose fertiliser (9 month slow release). A layer of washed sand was then added, before being

filled with municipal water (10 to 15 cm above soil height). Five alligator weed stems were planted into each container and left to establish for 15 weeks in a shade house. Water levels were maintained during the study period with fresh water extracted from a pond. The herbicides metsulfuron-methyl (2-(4-methoxy-6methyl-1,3,5-triazin-2-ylcarbamoyl sulfamoyl) benzoic acid), glyphosate (N-(phosphonomethyl) glycine, present as the isopropylamine salt) and dichlobenil (2, 6-dichlorobenzo nitrile) were applied to each of the alligator weed containers in March 2008 (see Table 1 for herbicides, rates of application and surfactants used). Treatments were assigned in a random manner to containers so that the trial has a completely randomised design (Cochran and Cox 1957). Although wind speed was low during treatment, a temporary barrier (tent) was erected over each container to prevent herbicide drift contaminating adjacent containers. Liquid herbicide was applied from above with a pneumatic sprayer fitted with a calibrated Even Flat Spray Tip (TP8002E). The sprayer was operated in the range of 2.8 to 3.0 bar and each treatment was sprayed for 10 seconds, when runoff was observed on aerial foliage, delivering a spray volume of 335 L ha<sup>-1</sup> of spray solution. A control treatment did not include any herbicide application.

To assess herbicide efficacy the number of apical and lateral shoot tips >2 mm in length (hereafter referred to as shoot tips) were counted for each container prior to herbicide application and at 3, 5, 7, 9, 11, 48 and 91 weeks after treatment (WAT). To assess the efficacy on the parent plant only,



**Figure 1.** Alligator weed in an early stage of invasion of two urban lowland streams in Melbourne, Australia, used in the field study. Patch of alligator weed at; (A) Patterson River prior to herbicide application. (B) Merri Creek prior to physical removal.

all stem fragments produced in each container were removed at the same WAT intervals above. The viability of these alligator weed fragments was assessed and has been reported in a previous paper (Dugdale et al. 2010).

#### Screening trial statistics

After square root transformation, the number of shoot tips at each sampling was analysed using an analysis of variance appropriate for the design (Table 2). In all analyses a container was the experimental unit. The square root of the number of shoot tips just prior to herbicide application was used as a covariate for the number of shoot tips at 48 and 91 weeks after treatment. This covariate was not used at other times because it was not an effective tool for improving the precision of the results. Some reported tests are calculated using t-statistics derived from the tables of means (with standard errors) obtained from the analyses of variance outputs described in Table 2. At 91 WAT one container was removed from the analysis as an outlier. Permutation tests were also carried out for a number of shoot tips analyses, but the P values obtained were similar to those used assuming an F-distribution and hence are not reported. All these analyses were carried out using the ANOVA directive and APERMUTE procedure within GenStat 16 (Payne 2013).

#### *Field study*

Study sites were established along the margins of two urban lowland streams; Merri Creek (37°46'3.68"S 144°59'4.02"E) and Patterson River (38°3'2.16"S 145°9'45.48"E) in Melbourne, Australia, between 2008 and 2010. Each study site consisted of 4 to 18 (depending on stream and year) disjoint patches of alligator weed, within a defined stream reach (Table 3). The patches were rooted into the embankment and growing out into the water body as a floating mat, typical of aquatic alligator weed (Figure 1). Each eradication technique was applied to several entire patches of alligator weed.

In summary, thirty-three patches of alligator weed were treated with the herbicides metsulfuron-methyl applied at 1 × label rate or glyphosate applied at 1 × label rate or 3 × label rate. Twelve patches were subjected to physical removal (Table 3). In particular, a reach of the Merri Creek was selected in 2008 that contained 17 patches of alligator weed. Three patches were selected (*ad hoc*) to be treated with metsulfuron-methyl applied at 1 × label rate (0.06 g a.i. L<sup>-1</sup>) in 2008, two patches were selected to be treated with metsulfuron-methyl in 2009, 4 patches were selected for physical removal in 2008 and 8 patches were selected for physical removal in 2009 (Table 3). In 2010 a downstream reach of

**Table 2.** Analysis of variance for the container trial.

Source of variation	Degrees of freedom
Herbicide (control v dichlobenil v glyphosate v metsulfuron-methyl)	3
Herbicide rate within dichlobenil	2
Herbicide rate within glyphosate	2
Herbicide rate within metsulfuron-methyl	2
Presence of surfactant within metsulfuron-methyl	1
Interaction of herbicide rate and presence of surfactant within metsulfuron-methyl	2
Residual	52

**Table 3.** Treatment and site location details for patches of alligator weed in the field study.

Treatment	Number of patches	Site	Mean initial patch size, m <sup>2</sup> (SD)	Initial spring application	Years of application and assessment
Metsulfuron-methyl <sup>a</sup> , 1 × label rate (0.06 g a.i. L <sup>-1</sup> ) Brushoff <sup>®</sup>	3	Merri Creek	5.6 (3.9)	2008	5
	2	Merri Creek	10.1 (8.0)	2009	4
	2	Patterson River	21.6 (0.3)	2008	2
	3	Patterson River	17.0 (1.0)	2009	1
Glyphosate, 1 × label rate (3.6 g a.i. L <sup>-1</sup> present as isopropylamine salt) Roundup Biactive <sup>®</sup>	9	Merri Creek	1.2 (2.6)	2010	3
	2	Patterson River	13.9 (6.9)	2008	2
	3	Patterson River	15.3 (4.5)	2009	1
Glyphosate, 3 × label rate (10.8 g a.i. L <sup>-1</sup> present as isopropylamine salt) Roundup Biactive <sup>®</sup>	9	Merri Creek	0.8 (0.9)	2010	3
	4	Merri Creek	6.9 (4.4)	2008	5
Physical Removal	8	Merri Creek	14.5 (9.0)	2009	4

Abbreviations: SD = standard deviation

<sup>a</sup> Surfactant not used

the Merri Creek containing 18 patches of alligator weed were selected to be treated with glyphosate. Nine of these eighteen patches were selected (using random numbers) for glyphosate applied at 1 × label rate (3.6 g a.i. L<sup>-1</sup>) and the other nine patches were selected for glyphosate applied at 3 × label rate (10.8 g a.i. L<sup>-1</sup>). At Patterson River four patches were selected in 2008, two of these four patches were selected (using random numbers) for glyphosate applied at 1 × label and the other two patches were selected for metsulfuron-methyl applied at 1 × label. At Patterson River six patches, on another stretch of river, were selected in 2009. Three of these six patches were selected (using random numbers) for glyphosate applied at 1 × label and the other three patches were selected for metsulfuron-methyl applied at 1 × label (Table 3).

The application of alligator weed treatments was undertaken in a staged approach from 2008 to 2010 due to the constraints of the active alligator weed eradication program. No control patches (untreated) were used as this would have compromised the eradication program. Our methodology assumes that patches in our study area

would remain at a similar size or expand if left untreated. High flow events can dislodge aquatic plants growing along stream banks, particularly in urban stream settings. At the sites used in this study it is unlikely that elimination of alligator weed patches can be attributed to dislodgement by high flow events. Author observational data from the study sites suggest that patches of alligator weed remain without substantial size reduction after high flow events. Further, during the period of the present study, the rapid expansion of alligator weed has been demonstrated if left uncontrolled in Victoria (Clements et al. 2011).

#### Herbicide application

Herbicide was applied to patches of alligator weed based on the annual treatment program described by van Oosterhout (2007). Specifically, herbicide was applied whenever there was any foliar alligator weed present in spring (November), summer (January) and autumn (March) for up to five consecutive years (Table 3). All herbicide was applied with a pneumatic single nozzle hand wand applicator to aerial foliage, until runoff

occurred. Prior to herbicide application a netting barrier consisting of polyethylene netting (15 mm diamond mesh) attached to steel stakes was constructed around each patch of alligator weed to prevent any alligator weed stem fragments from entering or exiting the treatment areas.

### Physical removal

Physical removal was conducted by experienced contractors (Thiess Services Pty Ltd). All above ground alligator weed was removed, followed by stems and roots that were traced back into the substrate and removed by hand or with mattocks. A floating boom with a netting skirt hanging from it was positioned to encircle each patch against the bank to catch any alligator weed fragments produced during excavation. Once removed, all alligator weed and associated soil was placed into bags and transported to a deep burial site for safe disposal.

### Efficacy of herbicide and physical removal treatments

To assess the efficacy of all herbicide and physical removal treatments, the area occupied by each alligator weed patch was measured at three month intervals (November, January and March) each year, for up to five years post initial treatment (Table 3). For the assessments, the presence or absence of alligator weed was recorded and, when present, the area occupied was determined by measuring the maximum length and width of the patch (including all stem material visible both above the water surface and above the sediment for the portions that were growing on the embankments) and approximating it to the shape of an ellipse, from which an area was calculated. A visual estimate of alligator weed percent coverage, defined as the vertical projection of all plant material on the ground surface, within the ellipse was made. The area and cover values were then multiplied to give an area metric calculation, termed 'area occupied' by alligator weed. The effectiveness of physical removal and herbicide treatments were examined for up to five years (Table 3).

### Field study statistics

At Merri Creek in 2010, glyphosate rate treatments were applied randomly to the 18 available patches. Thus a cause and effect hypothesis test can be constructed to examine the effect of glyphosate

rate on the time until alligator weed was absent (first recording occasion after the final time alligator weed was observed). However, hypothesis tests based on the normal distribution are not appropriate and standard non-parametric tests are ineffective due to the ordinal form of the data. In this case, a standard approach is to use proportional odds models (McCullagh and Nelder 1989), which are commonly referred to as ordinal logistic regression models. More specifically, the effect of glyphosate rate on the efficacy of control at Merri Creek was tested by fitting an ordinal logistic model, with an estimated over-dispersion parameter, for the number of days until alligator weed was absent from each patch to the logarithm of the initial area of infestation and the rate of glyphosate application, and then using an analysis of deviance F test for testing the glyphosate effect adjusted for the logarithm of the initial area of infestation. The initial area of infestation is included as a covariate to improve the power of the hypothesis test. Prior to fitting the ordinal logistic model, the number of days until alligator weed was absent from each patch is pooled into 4 groups, namely (i) week 10, (ii) week 39, (iii) week 50, 59 or 93 and (iv) week 103. This allows several observations in each group, so that the model numerically converges and so that the F approximation is more reasonable.

To determine any relationship between patch size and the efficacy of physical removal at Merri Creek, the effect of initial patch size and year of removal was determined by fitting a generalised linear model with Poisson errors, logarithmic link and over-dispersion parameter that includes effects for the logarithm of the initial area of infestation and the number of regrowth occasions. Permutation tests are calculated using analysis of deviance F statistics. All modelling and testing was carried out using the generalised linear model facilities in GenStat 16 (Payne 2013).

## **Results**

### *Screening trial*

Prior to any herbicide application, alligator weed plants growing in the containers had a moderately dense growth habit (62 of the 65 containers had >75% cover). The stem material extended over the water surface and as a tangled mat beneath it, typical of the aquatic form of alligator weed. The plants were prostrate (<0.1 m), and had an average of 65 (SD 15) shoot tips. Over the duration of the experiment, treatments without herbicide showed

**Table 4.** Effect of herbicide rate on the number of shoot tips in the container trial for (A) Glyphosate, (B) Metsulfuron-methyl and (C) Dichlobenil. P values are bolded when  $P < 0.05$ ; values are square-root transformed, except back transformed means in parentheses. WAT = Weeks after treatment. SED = standard error of difference between square-root transformed means. Values for control are the same in parts A, B, and C, of table.

A. Glyphosate

WAT	Residual degrees of freedom	Control (n=5)	1 × label (n=5)	3 × label (n=5)	6 × label (n=5)	SED	P value	
							Control vs 1 × label	Rate 1 vs 3 vs 6 × label
3	52	9.24 (85)	0.8 (1)	0.0 (0)	0.4 (0)	0.63	<b><math>1.1 \times 10^{-18}</math></b>	0.50
5	52	7.9 (63)	0.3 (0)	0.0 (0)	0.0 (0)	0.36	<b><math>3.5 \times 10^{-27}</math></b>	0.67
7	52	7.7 (59)	0.5 (0)	0.0 (0)	0.0 (0)	0.40	<b><math>6.2 \times 10^{-24}</math></b>	0.32
9	52	7.0 (49)	0.2 (0)	0.0 (0)	0.0 (0)	0.23	<b><math>4.4 \times 10^{-34}</math></b>	0.61
11	52	7.5 (56)	2.5 (6)	0.2 (0)	0.2 (0)	0.47	<b><math>1.7 \times 10^{-14}</math></b>	<b><math>2.6 \times 10^{-6}</math></b>
48	51	12.3 (152)	6.8 (47)	0.3 (0)	2.3 (5)	1.53	<b>0.00067</b>	<b>0.00025</b>
91	50	14.22 (202)	3.1 (10) <sup>#</sup>	-0.6 (0)	1.7 (3)	1.50	<b><math>1.3 \times 10^{-9}</math></b>	0.052

<sup>#</sup> This value becomes 5.1 (26) if outlier was not removed from the analysis.

B. Metsulfuron-methyl

WAT	Residual degrees of freedom	Control (n=5)	0.5 × label (n=10)	1 × label (n=10)	2 × label (n=10)	SED	P value	
							Control vs 0.5 × label	Rate 0.5 vs 1 vs 2 × label
3	52	9.24 (85)	2.7 (7)	2.8 (8)	2.5 (6)	0.44	<b><math>9.2 \times 10^{-17}</math></b>	0.87
5	52	7.9 (63)	1.4 (2)	1.4 (2)	1.1 (1)	0.26	<b><math>8.4 \times 10^{-27}</math></b>	0.40
7	52	7.7 (59)	1.0 (1)	0.5 (0)	0.1 (0)	0.28	<b><math>1.8 \times 10^{-25}</math></b>	<b>0.010</b>
9	52	7.0 (49)	0.4 (0)	0.2 (0)	0.0 (0)	0.16	<b><math>1.4 \times 10^{-36}</math></b>	0.072
11	52	7.5 (56)	1.8 (3)	2.4 (6)	1.5 (2)	0.33	<b><math>4.1 \times 10^{-19}</math></b>	<b>0.028</b>
48	51	12.3 (152)	10.6 (112)	9.3 (86)	3.1 (10)	1.08	0.19	<b><math>7.2 \times 10^{-9}</math></b>
91	51	14.22 (202)	8.9 (80)	10.0 (99)	7.9 (62)	1.06	<b>0.00014</b>	0.16

C. Dichlobenil

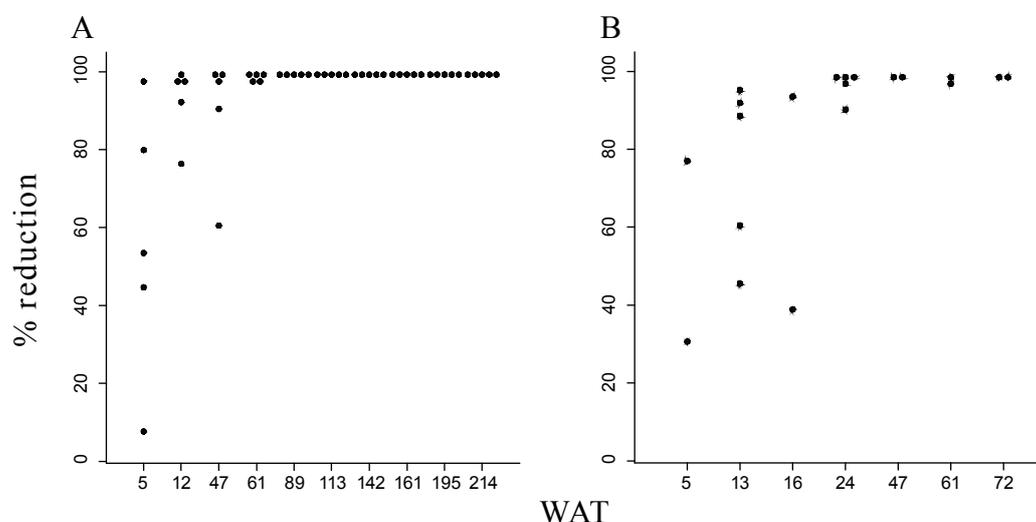
WAT	Residual degrees of freedom	Control (n=5)	0.5 × label (n=10)	1 × label (n=10)	2 × label (n=10)	SED	P value	
							Control vs 0.5 × label	Rate 0.5 vs 1 vs 2 × label
3	52	9.24 (85)	0.0 (0)	0.0 (0)	0.0 (0)	0.63	<b><math>2.3 \times 10^{-25}</math></b>	1.00
5	52	7.9 (63)	0.0 (0)	0.0 (0)	0.0 (0)	0.36	<b><math>6.4 \times 10^{-28}</math></b>	1.00
7	52	7.7 (59)	0.0 (0)	0.2 (0)	0.0 (0)	0.40	<b><math>2.5 \times 10^{-25}</math></b>	0.85
9	52	7.0 (49)	0.0 (0)	0.0 (0)	0.0 (0)	0.23	<b><math>1.1 \times 10^{-34}</math></b>	1.00
11	52	7.5 (56)	0.0 (0)	0.0 (0)	0.0 (0)	0.47	<b><math>1.1 \times 10^{-21}</math></b>	1.00
48	51	12.3 (152)	6.0 (36)	2.4 (6)	0.3 (0)	1.53	<b>0.00013</b>	<b>0.0016</b>
91	50	14.22 (202)	5.6 (31)	4.0 (16)	0.1 (0)	1.50	<b><math>5.1 \times 10^{-7}</math></b>	<b>0.0018</b>

an increase in the production of shoot tips (Table 4). At the conclusion of the trial, observations of the root mass of the plants showed that stems had produced multiple adventitious roots that extended into the top ~ 10 cm of the substrate, but true tap roots were rare (probably because of the anoxic nature of the sediment below this depth and roots obtaining the required water and nutrients directly from the water column and sediment).

All of the herbicide treatments considerably reduced alligator weed abundance relative to controls, which was still notable 91 WAT (Table 4). For each herbicide there was an effect of

herbicide rate, with higher rates resulting in fewer shoot tips, although this was not apparent until 48 WAT for dichlobenil (Table 4).

Within glyphosate treatments, the 3 × label rate treatment provided the greatest level of control and was considerably more effective than 1 × label rate at 11 and 48 WAT ( $P < 0.01$ ; Table 4). By 91 WAT no difference in herbicide rate was observed between glyphosate treatments ( $P = 0.052$ ), and abundance remained considerably less than controls (Table 4A). The 6 × label rate treatment did not provide any further control than the 3 × label rate treatment and was less effective at 48 and 91 WAT (Table 4A).



**Figure 2.** Temporal reduction in alligator weed (% area occupied), compared to area occupied at time of initial herbicide application, for (A) Metsulfuron-methyl (0.06 g a.i. L<sup>-1</sup>) at Merri Creek, n=5; and (B) Metsulfuron-methyl (0.06 g a.i. L<sup>-1</sup>) at Patterson River, n=5. Each point in the figure represents the result from a single patch at a sampling occasion. Values between 98-100% are expressed as 98% for clarity. X axis represents intervals of herbicide application and measurement at each site. Note different scales on X axis. WAT = weeks after treatment.

**Table 5.** Effect of surfactant (Pulse®) in metsulfuron-methyl treatments on the number of shoot tips in the container trial. P values are bolded when  $P < 0.05$ ; values are square-root transformed, except back transformed means in parentheses. WAT = Weeks after treatment. SED = standard error of difference between square-root transformed means.

WAT	No surfactant	Surfactant	SED	P value
3	2.7 (7)	2.6 (7)	0.36	0.89
5	1.4 (2)	1.3 (2)	0.21	0.65
7	0.6 (0)	0.4 (0)	0.23	0.37
9	0.4 (0)	0.0 (0)	0.13	<b>0.0074</b>
11	2.1 (4)	1.8 (3)	0.27	0.27
48	7.8 (61)	7.5 (56)	0.88	0.25
91	9.9 (97)	8.0 (64)	0.87	0.11

All metsulfuron-methyl treatments reduced alligator weed abundance to near zero by 7 to 9 WAT, however, by 48 WAT regrowth had occurred. By 91 WAT no difference in herbicide rate was observed between metsulfuron-methyl treatments ( $P = 0.16$ ), and abundance remained considerably less than controls (Table 4B). The addition of a surfactant to metsulfuron-methyl treatments had no effect on control efficacy at all intervals ( $P > 0.1$ ), except at 9 WAT ( $P = 0.0074$ ; Table 5).

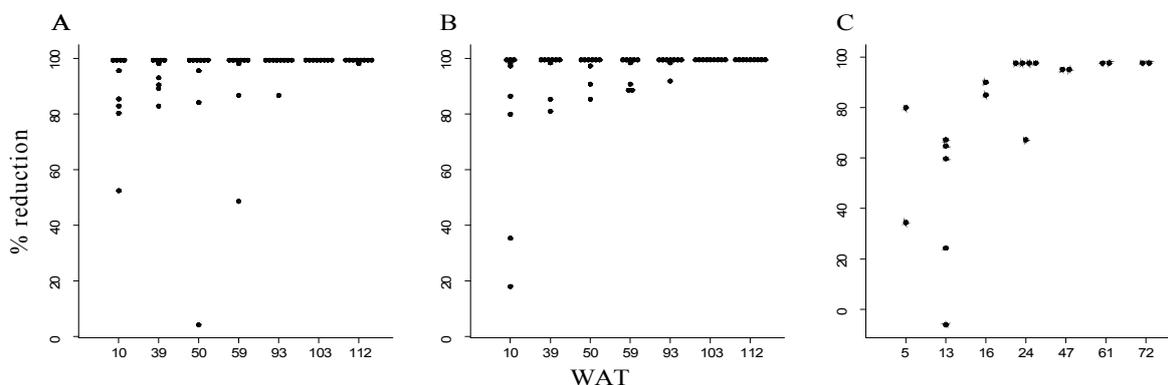
Dichlobenil provided excellent control, reducing alligator weed abundance by 100% for all rates up to 11 WAT, which was maintained at 48 and 91 WAT for 2 × label rate (Table 4C). The dichlobenil treatment at 2 × label rate was more effective than the 1 × and 0.5 × label rate treatments at these times ( $P = < 0.01$ ; Table 4C).

No viable plant material was present at 48 and 91 WAT for glyphosate at 3 × label rate and dichlobenil at 2 × label rate. Metsulfuron-methyl provided less control than glyphosate and dichlobenil irrespective of herbicide rate. The rate of decline of shoot tips was slower for metsulfuron-methyl treatments compared to glyphosate and dichlobenil. The presence of a surfactant did not improve metsulfuron-methyl efficacy. To reduce abundance of shoot tips to near zero, metsulfuron-methyl treatments took between 7 and 9 WAT, glyphosate and dichlobenil treatments responded much earlier, within 3 and 5 WAT (Table 4).

#### Field study

##### Metsulfuron-methyl

At the time of initial herbicide application the five patches of alligator weed subjected to metsulfuron-methyl at Merri Creek ranged in size from 1.4 to 15.7 m<sup>2</sup>. All patches recorded regrowth, which



**Figure 3.** Temporal reduction in alligator weed (% area occupied), compared to area occupied at time of initial herbicide application, for (A) Glyphosate 1  $\times$  label rate (3.6 g a.i. L<sup>-1</sup>) at Merri Creek, n=9; (B) Glyphosate 3  $\times$  label rate (10.8 g a.i. L<sup>-1</sup>) at Merri Creek, n=9; and (C) Glyphosate 1  $\times$  label rate (3.6 g a.i. L<sup>-1</sup>) at Patterson River, n=5. Each point in the figure represents the result from a single patch at a sampling occasion. Values between 98-100% are expressed as 98% for clarity. X axis represents intervals of herbicide application and measurement at each site. Note different scales on X axis. WAT = weeks after treatment.

occurred following one to four herbicide applications. After two years of three applications per year, no regrowth was recorded out to four or five years of monitoring (Figure 2A). This shows that applications of metsulfuron-methyl three times per year for two years can reduce the area occupied by alligator weed to near zero (99.7% reduction, SD 0.05) by the end of the second year of application and that no regrowth occurred in subsequent years. This is supported by data obtained at Patterson River where metsulfuron-methyl achieved a 99.9% reduction (n=2) over two seasons of treatment, and 97.1% reduction in one year of treatment, (n=5) (Figure 2B). Further monitoring and treatment at Patterson River was abandoned, as stem fragments from nearby patches of alligator weed (outside of the trial patches) overtopped the mesh barriers during a flood making it impossible to determine if regrowth was derived from within the trial patches or reinvasion from fragments entering into the trial patches.

### Glyphosate

The 18 patches subjected to glyphosate application along Merri Creek ranged in initial patch size from 0.02 to 7.9 m<sup>2</sup> (89% of patches were <2.5 m<sup>2</sup>), prior to treatment. No differences ( $P = 0.60$ ) in efficacy were detected between glyphosate at 1  $\times$  label rate (3.6 g a.i. L<sup>-1</sup>) and 3  $\times$  label rate (10.8 g a.i. L<sup>-1</sup>), based on the number of days until alligator weed was absent from each patch. The rate at which alligator weed declined is shown in Figures 3(A) and 3(B). All patches recorded regrowth, which occurred following one to seven

applications. By the end of the third year of treatment and monitoring (112 weeks), alligator weed was still present in only one patch (glyphosate at 1  $\times$  label rate); this was the largest patch at the start of the trial (7.9 m<sup>2</sup>) and was reduced by 99% (0.08 m<sup>2</sup>). No other patches remained active irrespective of herbicide rate. At Patterson River a similar result was achieved where glyphosate at 1  $\times$  label rate achieved an average of 99.95% reduction (n=2) after two seasons of treatment, and an average of 92.9% reduction after one year of treatment (n=5) (Figure 3C). These patches at Patterson River were abandoned after two years of monitoring as described above.

### Physical removal

The alligator weed patches subjected to physical removal along Merri Creek varied in size, ranging from 3.5 to 30.5 m<sup>2</sup> prior to treatment in 2008 and 2009. Following physical removal, regrowth was recorded from three out of the 12 patches (25%), out to four-five years of monitoring. The patches that recorded regrowth ranged in initial patch size from 11.2 to 23.6 m<sup>2</sup>. Two patches needed one instance of follow up removal in the first year after initial treatment, with no subsequent regrowth. One patch needed follow up removal three times over two consecutive years. There was no effect of year ( $P = 0.77$ ) or initial patch area ( $P = 0.19$ ) in determining whether or not regrowth occurred. However, the power of this test was lacking because only three out of 12 patches had any regrowth. It is reasonable to assume that larger patches of alligator weed are

more likely to produce regrowth following physical removal as the abundance of viable propagules and root material increases with patch size.

## Discussion

### *Screening trial*

Improved control was achieved for all herbicides at rates greater than the manufactures recommended rate (label rate) 48 WAT (Table 4). This suggests that there is scope to revise herbicide labels or for users to apply to the statutory authority for minor use permits to allow for improved management of alligator weed. However, the use of herbicides in natural environments must consider more than just the sensitivity of the target weed to the active ingredient and additives in the chemical product.

Only two previous studies have reported excellent (90–100% reduction in abundance) long-term (~ 52 weeks) control of alligator weed after a single herbicide application. These studies used dichlobenil (Blackburn and Durden 1974) and metsulfuron-methyl (Hofstra and Champion 2010). Excellent long-term control (under the above definition) was achieved with single applications of dichlobenil (rates above 15.5 kg a.i. ha<sup>-1</sup>) and metsulfuron-methyl (40 g a.i. ha<sup>-1</sup>) in the current trial, 48 WAT (however regrowth had occurred by 91 WAT for metsulfuron-methyl treatments). Our results validate those of Hofstra and Champion (2010), who used 36 g a.i. ha<sup>-1</sup> of metsulfuron-methyl on plants of similar age to those used in our study. However, they also report control was much less effective for plants that had been cultured for multiple growing seasons prior to metsulfuron-methyl application (the plants in the current study were cultured for 15 weeks).

Alligator weed abundance was reduced by >90% in the current trial with a single application of glyphosate at 10.8 g a.i. L<sup>-1</sup> (3 × label; 3.6 kg a.i. ha<sup>-1</sup>). Dugdale and Champion (2012) report that in four separate studies using a single application of glyphosate, less than 60% control was achieved after ~ 52 weeks using rates up to 7.2 g a.i. L<sup>-1</sup> (6.4 kg a.i. ha<sup>-1</sup>). It is unlikely that we achieved greater control than the other studies simply because of the high rate we used; Hofstra and Champion (2010) used glyphosate at 6.4 kg a.i. ha<sup>-1</sup> and achieved <60% control in outdoor containers very similar to ours. It is possible that our excellent control with a single application of glyphosate was achieved because we removed all of the stem fragments that were generated from the herbicide application. However, this is unlikely given we

showed that only ~ 2% of these were viable for glyphosate (Dugdale et al. 2010). It is more likely that excellent control was achieved because the alligator weed very rarely formed tap roots in our containers; instead it produced many adventitious roots. Given a key mode of regeneration after herbicide application is from roots, this is a likely source of difference. Observations from past field management programs support this, as extensive areas of floating aquatic alligator weed were effectively controlled with a single glyphosate application (Sainty et al. 1998). This suggests that the results presented in the screening trial may only be representative of newly colonising plants, in an early invasion stage, established from floating asexual fragments.

The excellent control achieved with 3 × glyphosate and 2 × dichlobenil in the container trial prompted us to test the former in the field study. Although dichlobenil was not tested in the field trial (because its use is limited to standing water situations, of which there are currently too few sites containing alligator weed in Victoria to use as experimental sites), these results suggest dichlobenil is likely to present a viable option for alligator weed management.

Metsulfuron-methyl performance against alligator weed was not reduced when used without a surfactant in the container trial, further, patches were eliminated when metsulfuron-methyl was used in the field without a surfactant. The Australian product label instructs that a surfactant be used, so this result was unexpected. Although we have not found any publications that report on the effect of surfactants on metsulfuron-methyl efficacy against alligator weed, control of the woody weed *Diodia ocimifolia* and weeds of wheat (*Aegilops cylindrica*, *Bromus secalinus* L. and *Bromus tectorum* L.) was not improved by addition of non-ionic surfactants (Olson et al. 2000, Ooi 1999). Given many surfactants are toxic to aquatic biota (Brausch et al. 2007; Siemering et al. 2008), using metsulfuron-methyl without a surfactant may reduce the risk of off-target impacts without compromising control efficacy.

### *Herbicide field study*

All herbicides (glyphosate at 3.6 and 10.8 g a.i. L<sup>-1</sup> and metsulfuron-methyl at 0.06 g a.i. L<sup>-1</sup>) applied up to three times per year were very effective in reducing the amount of alligator weed present in the field. The area occupied by alligator weed was reduced by ≥99% (e.g. <0.35 m<sup>2</sup> patch size) using either herbicide within two years.

Following this period of treatment a reduced number of applications were required as the area occupied by alligator weed was at very low levels or absent. Regular monitoring during this period (three to five years following initial treatment) is crucial, even when alligator weed is absent, to enable early detection and treatment of any regrowth before it can regenerate below ground reserves or stems capable of dispersal. The improved control with glyphosate at 10.8 g a.i. L<sup>-1</sup> compared to 3.6 g a.i. L<sup>-1</sup> that we recorded in the container trial was not apparent in the field. There are at least two possible explanations for this: Firstly, the alligator weed in the field is likely to have had a more developed root system at the time of initial treatment, which would have provided a source of regeneration after each herbicide application; secondly, the alligator weed patches were monitored and resprayed at three-month intervals between spring and autumn, so any regrowth was destroyed before it could grow enough for differences in patch size between rates to become apparent. Therefore, we do not recommend using elevated rates of glyphosate on alligator weed when applied three times per year. However, this result suggests a more efficient management program can be developed by using higher rates of herbicide. For example, it may be possible to achieve equivalent levels of control of alligator weed with elevated rates of either glyphosate or metsulfuron-methyl with one or two applications per year, compared to applying these herbicides three times per year at label rate. Further research is required to test this approach and determine if improved herbicide regimes can be established for alligator weed management.

Results from our field trial support previous findings where multiple applications per season of glyphosate (Schooler et al. 2008) or metsulfuron-methyl (Hofstra and Champion 2010, Schooler et al. 2008, Schooler et al. 2010) provide good to excellent (80–100%) long-term control (~ 52 WAT) of above or belowground alligator weed (Dugdale and Champion 2012). The literature suggests that multiple annual applications of metsulfuron-methyl is the preferred herbicide treatment (Hofstra and Champion 2010; Schooler et al. 2008), and is usually preferred over glyphosate for alligator weed management programs (Bowmer et al. 1991; Champion 2008; Dugdale and Champion 2012; Sainty et al. 1998; van Oosterhout 2007). However, recent container studies have shown that glyphosate application results in fewer viable stem fragments than metsulfuron-methyl post herbicide application, indicating a reduced risk

of dispersal and likelihood of new infestations establishing in aquatic situations (Clements et al. 2012; Dugdale et al. 2010).

#### *Physical removal field study*

Physical removal provides a method to control alligator weed that should not result in dispersal of fragments, or rely on multiple applications over multiple years. We have demonstrated that manual removal is effective at eliminating individual patches of aquatic alligator weed, although regular follow-up assessments are crucial so that repeat control can occur before the plant can replenish its underground reserves. Manual excavation has been shown to be effective for eradication of small patches of terrestrial alligator weed (Sainty et al. 1998), but as far as we are aware this is the first study to report on the effectiveness of manual removal of aquatic alligator weed. One key driver of successful physical removal of alligator weed is the proficiency of the personnel conducting the management works. Although most of the biomass of aquatic alligator weed is in the mats that float over the water, most of the effort in manual removal is required on below ground parts of the plant (roots, particularly tap roots and stem material) in the sediment near the water's edge. If all of the below ground plant material is not removed, rapid regrowth will occur. Further, Wilson et al. (2007) has shown that alligator weed develops a different morphology after physical removal, where plants that were subjected to shoot removal just above the soil level (to mimic mowing) had a higher below ground root biomass, a higher ratio of root to stem biomass and positioned its leaves closer to the ground, consequently making subsequent management efforts more difficult in aquatic and riparian environments. A disadvantage of manual removal is that it is very labour intensive. To remove each patch of alligator weed in this study took between 4.5 to 10.5 person hours per square metre. This means manual removal is costly in the initial year of treatment but if conducted properly, few resources are required in subsequent years.

#### *Management implications*

The results of the herbicide field studies demonstrate that we can eliminate patches of alligator weed with three applications per year of glyphosate or metsulfuron-methyl (at label rate) in Victoria, and thus validates the best practice guidelines of van Oosterhout (2007). A notable departure of the control method we used compared

to that recommended, is that we sprayed patches with herbicide whenever there were any alligator weed shoots present. van Oosterhout (2007) recommends skipping herbicide application when stems are <10 cm long and have <5–6 sets of leaves, or patches are <30 cm diameter (in the case of prostrate regrowth). Our data shows that very effective suppression can be achieved when alligator weed is treated without regard to ensuring that it is of a minimum size. Further, we do not recommend skipping applications when the stems are <10 cm long as alligator weed regrowth can be very rapid in aquatic environments creating a large number of stems for potential dispersal, and allowing below ground reserves to be replenished, both of which will impair an eradication program. The container trial suggests it may be possible to develop a more efficient herbicide control program using elevated rates of either glyphosate or metsulfuron-methyl to reduce the number of applications required each year, but we did not verify this in the field. Further research is required to evaluate this approach.

The results also demonstrate that physical removal is effective at eliminating patches of alligator weed. Because alligator weed has been known to regrow for up to 10 years after last being recorded (van Oosterhout 2007), we have not declared that any of the patches in this study have been eradicated. Despite this, the results can be used to guide ongoing suppression of alligator weed leading to eradication.

This study demonstrates that the methods used in Victoria's alligator weed eradication program are capable of eliminating patches of alligator weed in two to three years and indicates the eradication program has the tools required to succeed.

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## Management of aquatic alligator weed

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