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Herbicide efficacy for aquatic *Alternanthera philoxeroides* management in an early stage of invasion: integrating above-ground biomass, below-ground biomass and viable stem fragmentation

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Summary

Alternanthera philoxeroides is a problematic invasive plant in many regions of the world that is difficult to control once naturalised. It poses a threat to agricultural productivity, biodiversity and social amenity values of aquatic environments. Significant research has been conducted internationally, regarding the efficacy of different herbicides for control of A.

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philoxeroides. However, no studies have looked at key aspects of control for effective management in an early stage of invasion of aquatic environments, hindering eradication and control programmes. This study evaluates the efficacy of herbicides and surfactants on key *A. philoxeroides* response metrics, including control of above-ground biomass, below-ground biomass and production of viable stem fragments. This study concluded that glyphosate (isopropylamine salt) minimises viable stem fragment production post-herbicide application, compared with imazapyr and metsulfuron, thus reducing the potential for dispersal throughout catchments and waterways. In contrast, imazapyr and metsulfuron provided more effective control than glyphosate for *A. philoxeroides* growing on exposed embankments. We propose that an effective management strategy for early invasion of aquatic *A. philoxeroides*, using herbicides, would be to conduct initial applications of glyphosate to control overwater biomass and limit dispersal of viable stem fragments. Once infestations have been forced back to the embankment, imazapyr or metsulfuron treatments will provide longer term control.

Keywords: Alligatorweed control, dispersal, eradication, fragmentation, glyphosate, imazapyr, metsulfuron

Introduction

Alternanthera philoxeroides (Mart.) Griseb. (alligator weed) is a problematic weed species in many regions of the world that is difficult to control once naturalised. Originating from South America it has subsequently spread to and invaded aquatic and terrestrial environments in over 30 countries across Asia, Europe, North America, South America and Oceania. It is a widespread and problematic weed in regions of North America and Asia. In Oceania and Europe, infestations are in an earlier stage of invasion and where further spread is considered likely (EPPO, 2016). It is also considered to have a high invasive potential in Africa (Julien *et al.*, 1995). *Alternanthera philoxeroides* is a perennial stoloniferous herbaceous plant that is capable of inhabiting both aquatic (e.g. waterways, wetlands, floodplains, irrigation systems) and terrestrial environments (e.g. pasture, arable crops and urban areas) (Dugdale & Champion, 2012). In aquatic environments, it is capable of extremely rapid growth, where floating mats of vegetation cover waterbodies causing associated social, economic and environmental problems (Clements *et al.*, 2011; Hussner *et al.*, 2017). Once established in

catchments and waterways the plant disperses effectively through fragmentation, where stem fragments or floating mats of entangled stem material dislodge from the parent plant and drift with water currents to new locations forming new infestations (Dugdale *et al.*, 2010).

Alternanthera philoxeroides has proven difficult to control with mechanical, biological and chemical control methods. Herbicide control options have been intensively studied, with glyphosate, metsulfuron and imazapyr providing the most effective control in aquatic environments (Dugdale & Champion, 2012). The application of herbicide results in the production of stem fragments, which can be viable, and surviving root material is left from which the plant can regenerate (Dugdale *et al.*, 2010). Effective management using herbicides requires (1) preventing reproduction of above-ground and below-ground biomass at the site of infestation and (2) limiting water-borne dispersal of viable stem fragments to surrounding areas. Limiting dispersal of viable plant propagules is an important consideration of any management programme, particularly where early stage invasions are targeted for eradication (Panetta & Lawes, 2005). A significant amount of research has been conducted regarding the efficacy of different herbicides to control above-ground *A. philoxeroides* at a site or individual patch scale. However, less research has reported herbicide efficacy on below-ground *A. philoxeroides* (Dugdale & Champion, 2012). No study has simultaneously looked at key *A. philoxeroides* response metrics that must be evaluated when determining the efficacy of a particular control regime for effective *A. philoxeroides* management at the catchment scale, namely above-ground biomass, below-ground biomass and production of viable stem fragments.

Poor herbicide coverage and translocation once taken up by the plant are recognised as leading contributing factors to suboptimal levels of *A. philoxeroides* control following herbicide application (Funderburk & Lawrence, 1963; Bowmer *et al.*, 1993). Previous research has shown that metsulfuron and glyphosate applications result in the production of numerous *A. philoxeroides* stem fragments, of which up to 41 and 2%, respectively, are viable and capable of regeneration (Dugdale *et al.*, 2010; Clements *et al.*, 2012). The herbicide imazapyr has been shown to be more effective at controlling above- and below-ground *A. philoxeroides* than glyphosate, providing longer term control (Tucker *et al.*, 1994; Hofstra & Champion, 2010). However, it is unknown what effect imazapyr has on fragmentation and subsequent viability of stem fragments in aquatic environments.

Improved herbicide application techniques are required to enhance control and minimise production of viable stem fragments for management of *A. philoxeroides* in an early stage of invasion. The addition of surfactants to herbicide may enhance control efficacy by

improving herbicide uptake and translocation. However, no studies have reported on the effect of surfactants on herbicide efficacy for *A. philoxeroides*. Given many surfactants are toxic to aquatic biota (Siemering *et al.*, 2008), their impact needs to be evaluated for use in herbicide control programmes to reduce the risk of off-target impacts without compromising control efficacy.

This study examines the efficacy of three key herbicides and surfactants to control aquatic *A. philoxeroides* in an early stage of invasion. We evaluate the impact of the herbicides glyphosate, metsulfuron and imazapyr (and associated surfactants) on three key *A. philoxeroides* response metrics (above-ground biomass, below-ground biomass and production of viable stem fragments) in two contained experiments, of different infestation sizes, simulating *A. philoxeroides* in an early stage of invasion. Contained experiments allow all stem fragments produced following herbicide application to be accounted for, which is difficult to achieve in field experiments (Dugdale *et al.*, 2010).

Materials and Methods

Two spatially replicated experiments were established under controlled conditions in a climate controlled polytunnel in Victoria, Australia, with simulated infestations of two different size classes. In experiment one, small infestations ($< 0.1 \text{ m}^2$) were established in 15 L aquaria. In experiment two, larger infestations ($< 2 \text{ m}^2$) were established in 1200 L troughs. Plant propagation material was collected for each experiment from Dandenong Creek Wetlands (38°0'20.89"S, 145°11'28.51"E) that consisted of *A. philoxeroides* stem cuttings, 7 to 10 cm in length and 3 to 5 mm in diameter with three nodes present on each, without apical and lateral shoot tips. Both experiments were conducted and treated in the same year, as young plants, to mimic early invasion of *A. philoxeroides* growing in aquatic environments. For each experiment, plants were grown for three to four months during austral summer (38°S), where they were exposed to natural photoperiods (~14 h) with a mean ambient temperature of 20°C (SD=3).

Herbicide application occurred once in early March 2014. A selection of the treatments used in the 'small infestation' experiment was used in the 'large infestation' experiment (Table 1). All herbicides were applied from above with pneumatic sprayers (Solo – Handheld Sprayer, Part No: 418) delivering a spray volume of 600 L ha⁻¹ of spray solution. All plants were monitored for ~220 DAT and all stem fragments produced were collected and

counted. A stem fragment was defined as any piece of stem that had detached from the plant, excluding flower stalks. To test stem fragment viability, retained stem fragments were placed into nylon mesh bags (5 mm mesh). Up to 10 fragments were placed into each bag and transferred to a 1200 L aerated trough filled with municipal water to ~25 cm depth, in the same polytunnel. To test that the fragment culture facilities were suitable for subsequent fragment growth, three stem cuttings (10 to 15 cm long, 3 nodes each without apical or lateral shoots or roots) were taken from each aquarium and each trough at 0 DAT, of each treatment that did not include herbicide and placed into culture as per collected stem fragments. All stem fragments and cuttings were left in culture conditions and assessed at intervals after collection (174, 237, 258, 300, 334 and 376 DAT). If a fragment had new root > 15 mm in length (determined by colour, root tip presence and lack of root hairs) or sprouted a shoot > 15 mm in length, it was deemed viable and discarded. If there was no new growth, stem fragments and cuttings were placed back into culture conditions and left to grow until they could be deemed viable or not until 376 DAT. Few stem fragments and cuttings collected overall remained turgid and healthy without regenerating, and those that had not regenerated were excluded from analysis.

At ~220 DAT, for each experiment, a destructive harvest assessed the effect of herbicide on above-ground and below-ground *A. philoxeroides*. All apical shoot tips > 15 mm in length were counted, to give an indication of reproductive potential and then above-ground biomass was removed from each aquarium and trough and dried at 80°C until constant weight. Plant material below-ground was removed, washed and adventitious roots separated from tap roots. Tap roots were counted and their associated lengths and diameters measured to determine volume of turgid tap roots per pot. All root material was spun in a commercial salad spinner until no water droplets were produced and wet weights obtained. Specific methodology for conducting each experiment is described below.

Table 1 near here

Small infestation experiment

The experiment consisted of six randomised blocks with nine treatments in each block (See Table 1 for herbicides, rates of application and surfactants used). Blocks consisted of nine aquaria, each containing a similar amount of *A. philoxeroides*.

In November 2013, 54 plastic pots (140 mm diam/1.3 L) were filled with topsoil, a slow-release fertiliser tablet (N–P–K; 20–4.3–4.1) and topped with a 15 mm layer of sand.

Two *A. philoxeroides* stem cuttings were inserted into each pot. Individual pots were placed into separate 15 L plastic aquaria (300 × 250 × 200 mm) and filled with municipal water to 1 cm above the top of the pots. Aquaria were placed on a bench in a polytunnel and left to grow for 16 weeks, during this time water was aerated continuously and water levels maintained.

For herbicide application, aquaria were assembled by treatment (six replicate aquaria) and positioned inside individual spray tents in an adjacent polytunnel, to prevent herbicide drift. Water levels in aquaria remained at 1 cm above the top of the pots during treatment. Following herbicide treatment aquaria were left for 24 h (to prevent herbicide washing off treated plants), after which time they were emptied and refilled with municipal water to remove any chemical residue in the water. Aquaria were then placed back into the polytunnel and repositioned within blocks.

To assess the efficacy of herbicide on the potted plant, prior to treatment and on five occasions post-herbicide treatment (0, 29, 55, 82, 187 and 215 DAT), plant cover was assessed visually by estimating the area of each aquarium occupied using the following cover scale: 0 = no cover, 1 = ≤ 5%, 2 = 6 to 24%, 3 = 25 to 49%, 4 = 50 to 74%, 5 = 75 to 94%, 6 = ≥ 95%. At 215 DAT, all above- and below-ground biomass from each aquarium was harvested, washed and dry weights obtained.

To determine stem fragmentation rate, prior to treatment and on three occasions post-herbicide treatment (0, 29, 55 and 82 DAT) all fragments in the aquaria were collected and counted. To collect stem fragments, the potted plant was removed and the contents of each aquarium poured through a 2 mm sieve. The potted plant was then repositioned in the aquaria and refilled with water.

Large infestation experiment

The experiment consisted of 3 replications of 4 treatments (Table 1). Each trough was an experimental unit. Treatments were assigned to troughs in a completely random manner.

Twelve 1200 L round troughs (1.8 m diameter × 0.5 m depth) were positioned in the same polytunnel facility adjacent to the small infestation experiment. An embankment was constructed within each trough to model the gradient from riparian to aquatic habitat found along the margin of waterbodies. The embankment area consisted of a semicircle of 0.8 m² (sectioned off with 15 cm plastic edging), where ~0.2 m³ of topsoil was placed sloping from the top of the plastic edging (15 cm) up the trough wall (30 cm in height). For each trough five slow-release fertiliser tablets were inserted 10 cm below the sediment surface and topped

with a layer of sand. In early December 2014, 30 *A. philoxeroides* stem cuttings were inserted into each establishment area. Troughs were filled with municipal water to 20 cm depth and left for 12 wk. Water levels were maintained and each trough aerated continuously.

For herbicide application, temporary barriers (tents) were erected over each trough to prevent herbicide drift between troughs. The water level in each trough was 20 cm deep during application. Troughs were left for 24 h following herbicide application, after which time troughs were emptied and refilled with municipal water.

A single stem fragment collection occurred at 188 DAT. To simulate mechanical disturbance that occurs in the field, prior to fragment collection each trough was sprayed with a jet nozzle (Gardena® – Art. 8121, hard jet spray pattern set to maximum flow) for 20 s at 11 L min⁻¹ (Gardena® – Art. 8188-20, flow meter) to induce disturbance. All stem fragments produced were counted and a subsample of 50 randomly selected stem fragments collected from each trough was placed into culture, to determine viability. The number of viable stem fragments for each trough was estimated by multiplying the proportion of viable stem fragments from the subsample by the total number of fragments produced.

To assess the efficacy of herbicide on the parent plant in each trough, plant area was assessed at intervals post-herbicide treatment (0, 34, 57, 84, 188 and 222 DAT). Plant area was determined for two zones in each trough on each date; the rooted area on the embankment (referred to as the ‘embankment area’ hereafter) and the area of *A. philoxeroides* floating over the water surface (referred to as the ‘overwater area’ hereafter). The potential total area occupied was estimated by measuring the maximum length and width of the *A. philoxeroides* present in each trough (including all stem material visible both above the water surface and above the sediment) and calculating the area of an ellipse with those dimensions. A visual estimate of *A. philoxeroides* percent coverage within the potential total area, defined as the vertical projection of all plant material on the ground (or water’s surface), within the ellipse was then made. Finally, potential total area and cover percentage were multiplied, and divided by 100, to give an estimate of the ‘area occupied’ by *A. philoxeroides* in each trough (Clements *et al.*, 2014). This process was then repeated for the ‘embankment area’ alone. Subtracting the ‘embankment area’ from the ‘total area occupied’ determined the ‘overwater area’.

At 222 DAT prior to destructive harvest, the number of apical shoot tips that were present in the ‘embankment area’ and ‘overwater area’ were counted in each trough. All above-ground biomass present in the ‘embankment area’ and ‘overwater area’ was harvested

and dry weights obtained. Below-ground biomass was measured by subsampling the 'embankment area' of each trough. Six soil core samples (round tube, 16.5 cm diameter and 15.7 cm deep) were taken evenly across the 'embankment area' (~16% of the 'embankment area' of each trough was subsampled). Each soil core was washed through a 2 mm sieve and adventitious roots and tap roots obtained.

Statistical analyses

In the small infestation experiment, measurements were analysed as a 6 replicate, 9 treatment randomised block analysis of variance, with an individual aquarium being the experimental unit. Preliminary analyses indicated that there was little effect of different surfactants for the same herbicide, and thus treatment effects were divided into (1) the effect of herbicide type (none, glyphosate as isopropylamine salt, glyphosate as isopropylamine + mono-ammonium salt, metsulfuron or imazapyr) and (2) the effect of different surfactants for the same herbicides (i.e. a combined examination of (i) no surfactant, Bonus® or BS1000® for metsulfuron and (ii) no surfactant, Bonus® or Hasten® for imazapyr). For all measurements except cover scores at different times, prior to analysis of variance, a data transformation was chosen, using graphs of fitted values versus residuals, so that the residual variation did not change as the mean increased. Cover scores at 0 DAT (prior to the application of herbicide), was used as a covariate whenever its use appreciably improved the precision of the comparisons between herbicides and surfactants.

In the large infestation experiment, measurements were analysed as a 3 replicate, 4 treatment completely randomised analysis of variance, with an individual trough being the experimental unit. For many measurements, prior to analysis of variance, a data transformation was chosen, using graphs of fitted values versus residuals, so that the residual variation did not change as the mean increased. Area occupied in the 'embankment area' or 'overwater area' at 0 DAT, was used as a covariate whenever its use appreciably improved precision of results.

In both experiments, for some measurements there were only a few discrete values, or there were many zero values, and thus *P* values were calculated non-parametrically. In these cases, while the *F* statistic was calculated in the usual way, *P* values were calculated using a permutation test with 9999 permutations. The analyses of variance were implemented using the ANOVA directive and the permutation tests were implemented using the APERMTEST procedure, both in GenStat 16 statistical package (Payne, 2013).

Results

Small infestation experiment

Prior to chemical application, *A. philoxeroides* growing in aquaria had cover scores between 3 (25 to 49%) and 4 (50 to 75%). The plants were rooted into the pot and had plant material extending over the water surface as floating prostrate growth, typical of the aquatic form of *A. philoxeroides* in an early stage of invasion. Over the duration of the experiment, treatments without herbicide increased in plant cover, averaging cover scores of 3.3 at 0 DAT and 4.5 at 215 DAT (Table 2).

All herbicide treatments substantially reduced above-ground plant cover until 82 DAT. After this time, plant cover increased in all herbicide treatments except imazapyr. Imazapyr reached a minimum plant cover at 187 DAT. By 215 DAT, imazapyr treated plants had progressed from witches broom presentation (clusters of small apical shoot tips) to healthy shoots (cover score = 2.0), but this regrowth was much less than glyphosate and metsulfuron treated plants (cover score = 2.6 to 3.0). Generally, at 215 DAT, above-ground biomass in all herbicide treatments was about 75% less than the no herbicide treatment (Table 2).

The volume of turgid taproots was reduced by at least 85%, compared with the no herbicide treatment, in the glyphosate (isopropylamine salt; referred to as 'IS' hereafter), metsulfuron and imazapyr herbicide treatments at 215 DAT. Glyphosate as the isopropylamine salt + mono-ammonium salt (referred to as 'ISMS' hereafter) produced little reduction in the volume of turgid taproots, as compared with the no herbicide treatment. Below-ground wet weight biomass was, at best, only moderately reduced by any herbicide treatment (Table 2).

The herbicides metsulfuron and imazapyr increased the number of viable stem fragments by about 15 fold compared with the no herbicide treatment, which had 1.5 stem fragments per aquarium. Metsulfuron and imazapyr produced similar numbers of viable stem fragments, at similar times. However, the viable stem fragments in the imazapyr treatment took about 30% more time to sprout new roots or shoots than the viable stem fragments in the metsulfuron treatment (Table 2). Glyphosate (IS) produced similar numbers of viable stem fragments to the no herbicide treatment and about 90% less viable stem fragments compared with metsulfuron and imazapyr ($P < 0.001$). Glyphosate (ISMS) produced about three times the number of viable stem fragments than the no herbicide treatment (Table 2). Plants treated with glyphosate (IS) produced stem fragments (which were subsequently viable) earlier than

imazapyr and metsulfuron, on average taking 29 DAT, while imazapyr and metsulfuron took 44 and 50 DAT, respectively. Different formulations of glyphosate resulted in stem fragmentation at different times, with fragmentation from glyphosate (IS) occurring twice as fast as glyphosate (ISMS) (Table 2).

There was no evidence ($P > 0.05$; data not shown) of any effect of different surfactants, for the same herbicide with any measurement (above-ground biomass, below-ground biomass and production of viable stem fragments) tested in the small infestation experiment.

Table 2 near here

Large infestation experiment

Prior to chemical application, *A. philoxeroides* growing in troughs had rooted into the embankment and had formed a mat of floating horizontal stems, which supported vertical stems over most of the area within each trough, though a closed canopy had not formed. The average area occupied in each trough prior to application was 1.69 m² (SD=0.21). Treatments without herbicide increased in area due to expansion of *A. philoxeroides* growing overwater at 34 DAT; further expansion over the period of the experiment was minimal as *A. philoxeroides* occupied most of the area within each trough at this time.

All herbicide treatments steadily reduced above-ground plant cover in the ‘embankment area’ until 188 DAT, with no detectable difference between the three herbicides. At 188 DAT, plant cover in the ‘embankment area’ had been reduced by about 75% by each of the herbicide treatments (Table 3). At 222 DAT, there was recovery in plant cover in the glyphosate treatment, with the plant cover doubling to that occurring at 188 DAT (Table 3). This regrowth was reflected in apical shoot tips and biomass at 222 DAT being about 60% of those in the no herbicide treatment. Visual observation indicated that much of this new regrowth was at the water’s edge. For metsulfuron and imazapyr treatments, plant cover in the ‘embankment area’ was steady from 188 DAT to 222 DAT (Table 3). Nevertheless, both metsulfuron and imazapyr treated *A. philoxeroides* were showing some signs of regrowth with few small apical shoot tips (> 15 mm) present.

At 222 DAT, there was evidence that both imazapyr and metsulfuron reduced the number and volume of turgid taproots and the total root biomass in the ‘embankment area’, but there was little difference between these measurements for these two herbicide treatments

(Table 3). Visual observation indicated that for metsulfuron treatments the herbicide had destroyed tap roots, where all that remained was blackened, rotting roots or the remaining outer sheaths of rotted tap roots. There was also evidence that imazapyr destroyed tap roots. However, turgid healthy looking tap roots remained. There was no detectable difference in root measurements between the glyphosate treatment and the no herbicide treatment (Table 3). Visual observation in the glyphosate treatment indicated that there were many small tap roots, particularly from soil cores located close to the water's edge. However, there was some evidence that herbicide had destroyed tap roots, where all that remained was the outer sheaths of rotted tap roots.

'Overwater area' occupied by *A. philoxeroides* differed significantly between herbicides at 84 DAT ($P = 0.004$) in the large infestation experiment. Glyphosate reduced overwater *A. philoxeroides* to minimal levels by 84 DAT, but metsulfuron and imazapyr treatments remained at similar levels to controls at this time (Table 3). After 188 DAT, following the simulated disturbance event, nearly all the floating stem material in the metsulfuron and imazapyr treatments was converted to stem fragments, a large number of which were subsequently determined as viable (Table 3).

Glyphosate (IS) produced 72% fewer viable stem fragments than metsulfuron and imazapyr. However, glyphosate still produced ~60 viable stem fragments per m² (~101 per trough). The average time to sprouting of viable stem fragments was about 20% greater after imazapyr treatment than any other treatment (Table 3). By 222 DAT, the glyphosate treatment had more overwater regrowth with more stems and apical shoot tips than metsulfuron and imazapyr, which were similar. Imazapyr and metsulfuron had minimal overwater biomass at 222 DAT (Table 3).

Table 3 near here

Discussion

In both the small and large infestation experiments, glyphosate, metsulfuron and imazapyr all reduced above-ground plant cover by a maximum of 75% compared with no herbicide. This is comparable to the level of control that is expected from a single herbicide application for rooted *A. philoxeroides* (Dugdale & Champion, 2012). Consistent with previous findings, the above-ground results for metsulfuron were mostly similar to imazapyr. As expected,

metsulfuron and imazapyr provided longer duration of control than glyphosate, before regrowth was observed (Hofstra & Champion, 2010).

In the 'embankment area' of the large infestation experiment, there appeared to be a corresponding reduction in below-ground plant material for metsulfuron and imazapyr, but the proportional decline was much smaller than above-ground plant material. With glyphosate there was no evidence of a reduction in below-ground material at 222 DAT. In contrast, in the small infestation experiment, there was about an 85% reduction in volume of turgid taproots, for glyphosate (IS), metsulfuron and imazapyr, although the biomass reduction was much less. These data indicate that effective control of below-ground tap roots can be achieved utilising glyphosate when treating small infestations in an early stage of invasion (Clements *et al.*, 2014). Reduction in below-ground biomass after herbicide application has been demonstrated in a limited number of mesocosm studies (Schooler *et al.*, 2007; Hofstra & Champion, 2010) and field studies (Bowmer *et al.*, 1991; Schooler *et al.*, 2008). In terrestrial field situations, glyphosate (IS) and imazapyr reduced *A. philoxeroides* below-ground biomass by 80% and 16% respectively, two months after treatment (Bowmer *et al.*, 1991). Schooler *et al.* (2008) showed long term (15 months after herbicide application) effects where glyphosate (IS) and metsulfuron reduced below-ground biomass by 81% and 96% respectively. Metsulfuron and imazapyr have been shown to provide effective control of below-ground biomass in mesocosm studies (flooded tanks) and were both more effective than glyphosate on mature plants (Hofstra & Champion, 2010). Schooler *et al.* (2007) found root biomass was reduced by only ~25%, 5 weeks after glyphosate treatment. It is not well understood how the effect of herbicides differ for aquatic versus terrestrial *A. philoxeroides* (Dugdale & Champion, 2012). The degree with which a herbicide damages below-ground plant material will have a large effect on longer term control. Clearly there are large differences between studies, including our own, in the effectiveness of these herbicides in reducing below-ground plant material.

Glyphosate (IS) provided good control of attached overwater plant material in both experiments. Glyphosate completely destroyed overwater stem material prior to the induced disturbance event in the large infestation experiment, with most stems becoming flaccid and translucent and sinking to the bottom of the troughs. In contrast, overwater floating *A. philoxeroides* remained at high levels for metsulfuron and imazapyr treatments up until 188 DAT (prior to the simulated disturbance event). Both herbicides showed similar response to treatment where almost complete defoliation of leaf material occurred, however, stem material remained turgid and floating on the water surface. Following the induced

disturbance event, most of the overwater biomass that remained in the imazapyr and metsulfuron troughs fragmented and consequently separated the floating weed mats ('overwater area') from the 'embankment area'. As a consequence, nearly all the floating stem material in the metsulfuron and imazapyr treatments was converted to stem fragments, for which a large number were subsequently viable (Table 3). After induced disturbance, minimal attached overwater biomass remained in the metsulfuron and imazapyr treatments. This process effectively simulates what occurs in a natural system when a period of flow dislodges *A. philoxeroides* and disperses viable stem fragments downstream.

In the small infestation experiment, simulated disturbance occurred continually from soon after herbicide application. In this experiment, large numbers of viable stem fragments were produced by metsulfuron and imazapyr treatments. The common theme of the two experiments, simulating different size infestations, is that applications of metsulfuron and imazapyr induce large amounts of viable stem fragments at the time of disturbance.

Glyphosate produced viable stem fragments after induced disturbance but at a much lower rate than with metsulfuron and imazapyr. In the small infestation experiment, the number of viable stem fragments produced was only about 10% of that produced with metsulfuron and imazapyr, whilst in the large infestation experiment the production was about 25%. In fact, in the small infestation experiment, the number of viable stem fragments produced by glyphosate (IS) over the 215 day study period was not much more than that produced naturally by the no herbicide treatment. In contrast, in the large infestation experiment, the number of viable stem fragments in the glyphosate treatment was about 25 times the number in the no herbicide treatment. This is likely to be associated with differences in the methodology of the two studies. Two relevant differences are the size of infestation being simulated, and the induced disturbance regimes. Larger *A. philoxeroides* stem fragments have been shown to have greater potential for regeneration (Dong *et al.*, 2012). These results suggest that as patches get larger and denser, more viable stem fragments will be produced from glyphosate treatments. Therefore, the results suggest that applying glyphosate to small infestations in an early stage of invasion will limit production of viable stem fragments and the potential for dispersal throughout catchments.

Imazapyr treated stem fragments take longer to sprout new roots or shoots (20 to 30% longer; ~50 days longer) compared with metsulfuron and glyphosate. The extra time until sprouting may provide a false sense of effective *A. philoxeroides* control in the short term, by enabling stem fragments to disperse throughout catchments before lodging and rooting to create new infestations. However, these stem fragments may also be subjected to increased

environmental stressors during this time (e.g. overwintering, transport to less suitable habitats), reducing their fitness and rendering them less viable.

In the small infestation experiment, glyphosate as isopropylamine salt formulation provided much more effective control of aquatic *A. philoxeroides* (production of viable stem fragments and below-ground tap roots) than glyphosate as isopropylamine + mono-ammonium salt formulation. Further investigation is required to understand this result.

No benefit for control of aquatic *A. philoxeroides*, in an early stage of invasion, could be detected by adding surfactants to any herbicide treatment. These results were similar to previous findings where metsulfuron combined with the surfactant Pulse® had no additional benefit (compared with metsulfuron alone) in controlling above-ground aquatic *A. philoxeroides* (Clements *et al.*, 2014) or reducing viable stem fragment production (Dugdale *et al.*, 2010).

The viability of stem fragments produced following herbicide application is an important factor in determining the effectiveness of an aquatic *A. philoxeroides* management programme at the catchment scale, particularly when *A. philoxeroides* is in an early stage of invasion. The production of viable stem fragments has the potential to increase the distribution of the weed throughout catchments where control has been enacted. We suggest that, where there is a possibility of dispersal and colonisation of stem fragments, metsulfuron and imazapyr should be avoided for the control of *A. philoxeroides* overwater. Even with glyphosate, barriers to minimise stem fragment dispersal should be utilised (Hussner *et al.*, 2017).

Our results lead us to propose that an effective management strategy for aquatic *A. philoxeroides* would be to conduct initial applications of glyphosate to control overwater biomass and limit dispersal of viable stem fragments. Once infestations have been forced back to the embankment utilising glyphosate and there is negligible overwater *A. philoxeroides* present, imazapyr or metsulfuron treatments will provide more effective and longer term control than glyphosate, reducing the number of applications required each year to control aquatic *A. philoxeroides*. We suggest that this approach should be tested in the field.

Conclusions

Glyphosate (isopropylamine salt) minimises production of viable stem fragments post-herbicide application, compared with imazapyr and metsulfuron, for control of *A. philoxeroides* growing overwater. Therefore, the potential for dispersal throughout

catchment and waterways is reduced. However, regrowth is rapid from plant material rooted in the embankment following glyphosate treatments, particularly after initial treatment. In contrast, imazapyr and metsulfuron both provide more effective control than glyphosate for *A. philoxeroides* rooted on embankments. Further experiments to develop and determine effective control measures for *A. philoxeroides* in an early stage of invasion of aquatic environments need to integrate control of above-ground biomass, below-ground biomass and production of viable stem fragments.

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Table 1 Experimental treatments used in the small infestation experiment and large infestation experiment. Experimental treatments used in the large infestation experiment are in bold and are a selection of those used in the small infestation experiment

Treatment ^a	Product name®	Formulation ^a	Product concentration	Herbicide rate ^b kg a.i. ha ⁻¹
None	-	-	-	-
Glyphosate	Roundup Biactive	360 g L⁻¹ (IS)	1 L 100 L⁻¹	2.17
Glyphosate + Surfactant ¹	Credit + Bonus	540 g L ⁻¹ (ISMS) + Surfactant ¹	670 mL 100 L ⁻¹ + 670 mL 100 L ⁻¹	2.30
Imazapyr ¹	UniMaz	250 g L ⁻¹ (IS)	500 mL 100 L ⁻¹	0.75
Imazapyr ¹ + Surfactant ¹	UniMaz + Bonus	250 g L ⁻¹ (IS) + Surfactant ¹	500 mL 100 L ⁻¹ + 500 mL 100 L ⁻¹	0.75
Imazapyr¹ + Surfactant²	UniMaz + Hasten	250 g L⁻¹ (IS) + Surfactant²	500 mL 100 L⁻¹ + 500 mL 100 L⁻¹	0.75
Metsulfuron	Brushoff	600 g kg⁻¹	10 g 100 L⁻¹	0.036
Metsulfuron + Surfactant ¹	Brushoff + Bonus	600 g kg ⁻¹ + Surfactant ¹	10 g 100 L ⁻¹ + 100 mL 100 L ⁻¹	0.036
Metsulfuron + Surfactant ³	Brushoff + BS1000	600 g kg ⁻¹ + Surfactant ³	10 g 100 L ⁻¹ + 100 mL 100 L ⁻¹	0.036

^a Abbreviations: IS = Glyphosate present as isopropylamine salt; ISMS = Glyphosate present as isopropylamine salt and mono-ammonium salt.

Surfactant¹ = Bonus® 114 g L⁻¹ alkylethoxyphosphate, trolamine salt, Nufarm Australia Ltd., Laverton North, VIC.

Surfactant² = Hasten® 704 g L⁻¹ ethyl and methyl esters of vegetable oil with 196 g L⁻¹ non-ionic surfactant, Victorian Chemical Company Pty. Ltd., Coolaroo, VIC.

Surfactant³ = BS1000® 1000 g L⁻¹ alcohol alkoxylate, Crop Care Australasia Pty. Ltd., Murarrie, QLD.

^b All herbicide applied at 600 L ha⁻¹. **Table 2** Effect of herbicides on *A. philoxeroides* abundance measures in the small infestation experiment; values in parentheses are back-transformed means. The residual degrees of freedom for each measurement = 37 to 39 (depending on the use of covariates and exclusion of aquaria from the analysis)

Measurement ^a	Herbicide	SED	P value
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	None (y)	Glyphosate IS (y)	Glyphosate ISMS (y)	Metsulfuron (z)	Imazapyr (z)	(y) v (y)	(y) v (z)	(z) v (z)	Herbicides
<i>Aboveground plant material</i>									
Cover (0-5 score) 215 DAT ^b	4.5	2.8	3.0	2.6	2.0	0.36	0.29	0.21	< 0.001 ^c
Minimum observed cover between 29 and 215 DAT ^b	3.8	1.6	1.8	2.1	1.3	0.26	0.21	0.15	< 0.001 ^c
Log ₁₀ (Aboveground biomass dry weight (g) 215 DAT) ^b	1.33 (21)	0.54 (3)	0.77 (6)	0.69 (5)	0.57 (4)	0.155	0.127	0.090	< 0.001
<i>Belowground plant material</i>									
Log ₁₀ (Volume of turgid taproots (mm ³) 215 DAT+ 100)	2.8 (510)	2.1 (10)	2.7 (420)	2.3 (80)	2.3 (90)	0.21	0.17	0.12	0.004 ^c
Log ₁₀ (Belowground biomass wet weight (g) 215 DAT) ^b	0.91 (8)	0.72 (5)	0.77 (6)	0.68 (5)	0.68 (5)	0.102	0.84	0.059	0.060
<i>Viability of stem fragments</i>									
Log ₁₀ (Total number of viable stem fragments + 2) ^{bd}	0.54 (1.5)	0.64 (2.4)	0.83 (4.7)	1.42 (24.1)	1.36 (21.0)	0.120	0.098	0.070	< 0.001
Log ₁₀ (Average collection time of viable stem fragments in days)	1.86 (73)	1.46 (29)	1.77 (59) ^e	1.70 (50)	1.64 (44)	0.047	0.039	0.027	< 0.001
Log ₁₀ (Average time to sprouting of stem fragments (DAT))	2.30 (200)	2.42 (260) ^f	2.38 (240) ^e	2.31 (210)	2.47 (290)	0.030	0.024	0.017	< 0.001

^a Abbreviations: Glyphosate IS = glyphosate present as isopropylamine salt; Glyphosate ISMS = glyphosate present as isopropylamine salt and mono-ammonium salt; DAT = days after herbicide treatment; SED = standard error of difference between means; *P* values are in bold when *P* < 0.05.

^b Used cover score at 0 DAT as covariate.

^c Calculated using permutation test.

^d Eighty-nine percent of stem cuttings (16 of 18) collected to test stem fragment culture facilities were viable.

^e One aquarium excluded since there were no viable stem fragments from the aquarium.

^f One aquarium with an exceptionally large sprouting time was excluded as an outlier.

Table 3 Effect of herbicides on *A. philoxeroides* abundance measures in the large infestation experiment; values in parentheses are back-transformed means. The residual degrees of freedom for each measurement = 8 (except when a covariate for cover score was used, in these cases the residual degrees of freedom = 7

Measurement ^a	Herbicide				SED	<i>P</i> value
	None	Glyphosate IS	Metsulfuron	Imazapyr		

<i>Aboveground plant material</i>						
Embankment area (m ²) DAT						
84 ^b	0.65	0.33	0.29	0.41	0.067	0.004
188 ^b	0.56	0.18	0.13	0.15	0.038	<0.001
222 ^b	0.67	0.37	0.13	0.16	0.055	<0.001
Log ₁₀ (Aboveground embankment biomass (g) dry weight 222 DAT) ^c	2.43 (270)	2.25 (179)	2.03 (108)	1.85 (71)	0.093	<0.001
<i>Belowground plant material</i>						
Log ₁₀ (Volume of turgid taproots (mm ³) 222 DAT + 100) ^c	3.4 (11)	3.8 (14)	2.9 (8)	3.1 (10)	0.33	0.059

Log ₁₀ (Belowground embankment biomass wet weight (g) 222 DAT + 100)	2.27 (87)	2.20 (59)	2.17 (48)	2.17 (48)	0.029	0.024
<i>Overwater plant material</i>						
Overwater area (m ²) DAT						
84 (prior to disturbance)	1.33	0.19	1.20	0.94	0.228	0.004
188 (after disturbance)	1.15	0.08	-0.01	0.00	0.068	0.003^d
222	1.34	0.45	0.01	0.004	0.076	< 0.001^d
Log ₁₀ (Overwater biomass dry weight (g) 222 DAT) ^c	2.57 (373)	1.90 (79)	1.12 (13)	1.53 (34)	0.148	<0.001
Log ₁₀ (Total number of viable stem fragments + 2) after 188 DAT ^e	0.79 (4)	2.01 (101)	2.56 (362)	2.56 (362)	0.171	<0.001
Average time to sprouting of stem fragments (DAT)	240	269	253	315	15.5	0.006

^a Abbreviations: Glyphosate IS = glyphosate present as isopropylamine salt; DAT = days after herbicide treatment; SED = standard error of difference between means; *P* values are in bold when *P* < 0.05.

^b Used cover score at 0 DAT as covariate.

^c Used overwater area at 0 DAT as covariate.

^d Calculated using permutation test.

^e All stem cuttings (15 of 15) collected to test stem fragment culture facilities were viable.

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