Control of the emerging aquatic weed Amazon frogbit with flumioxazin

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Summary Amazon frogbit is an emerging aquatic weed that causes significant impacts to freshwater systems in Australia. Currently there are limited control options available, with only one herbicide (flumioxazin) registered for its control.

Outdoor pond trials demonstrated that foliar and subsurface flumioxazin application provide excellent Amazon frogbit control (95-100% biomass reduction) at intermediate label rates. Subsurface application provided slightly better control than foliar spray. However, in deeper waterbodies foliar application will be more economical. In a field trial, a subsurface injection of flumioxazin (200 ppb) in a farm dam covered by a dense Amazon frogbit infestation provided ~99% control with a single application within three months.

Overall, our work demonstrated that flumioxazin is an excellent control tool to manage Amazon frogbit and will greatly enhance the management of this invasive aquatic weed.

Keywords floating macrophytes, *Limnobium laevigatum*, aquatic herbicide, aquatic weed management.

INTRODUCTION

Invasive aquatic weeds cause significant environmental and socio-economic impacts worldwide. Amazon frogbit *Limnobium laevigatum* (Humb. & Bonpl. ex Willd.) Heine (Hydrocharitaceae; also known as sponge plant) is a free-floating aquatic plant originating from freshwater habitats of tropical and subtropical South America, Central America, and the Caribbean (Cook and Urmi-König 1983). The plant also produces inconspicuous white flowers, forming a fleshy capsule with up to 100 seeds per capsule. A popular aquarium species, it has been introduced in multiple locations around the world, including California, Japan, southern Africa and Australia (Howard *et al.* 2016, Anderson and Akers 2011, Kadono 2004). Amazon frogbit is a fairly recent arrival in Australia, first detected in 2003, it is now present in multiple states (QLD, NSW, WA) and is rapidly expanding its range (Atlas of Living Australia 2022).

Amazon frogbit has morphologically distinct growth forms. Starting as small seedlings that resemble duckweed, the plant develops larger spongy (aerenchymatic) floating leaves that lay flat on the water surface. Once the water surface is covered, it extends its leaves vertically and can become up to 50 cm tall, resembling water hyacinth (Cook and Urmi-König 1983). Amazon frogbit readily reproduces asexually which allows it to rapidly overgrow entire water bodies. But the plant also produces inconspicuous white flowers, forming seed pots that can contain 20-30 seeds. Seeds can germinate immediately or persist in the environment for at least three years (Weerasinghe 2020).

The impact of Amazon frogbit species is not fully documented. However, frogbit can form dense mats with up to 2000-2500 plants per square meter (Weerasinghe 2020) and the similarity of its growth habit to water hyacinth indicate that it can cause significant environmental and socio-economic impacts. Amazon frogbit readily outcompetes other aquatic plants (Perryman 2013) and the rapid growth results in a thick cover of the water surface, affecting water quality and interfering with recreational and commercial use of freshwater systems. Like other free floating aquatic weeds, the thick floating mats prevent gas exchange and light to penetrate the underlaying water column, thereby modifying aquatic habitats and making them unsuitable for native flora and fauna (Perna and Burrows 2005).

Currently there are limited options available to manage this highly invasive aquatic weed (Anderson and Akers 2011). The herbicides imazamox and penoxulam were found to be effective in controlling Amazon frogbit in the USA (Willis *et al.* 2018), but these herbicides are currently not registered for aquatic use in Australia. The congeneric *Limnobium spongia* (Bosc) Steudel native to the USA can be controlled with diquat, triclopyr and 2,4-D; glyphosate was not effective for control (Madsen *et al.* 1998). Flumioxazin is a new herbicide registered in Australia for control of aquatic weeds (Clipper, Sumitomo Inc.). Preliminary field trials showed that flumioxazin effectively controls Amazon frogbit (authors' observations), but there is no published data on the effect of application techniques (foliar vs. subsurface) and application rate on control efficacy. To address this knowledge gap, we conducted a mesocosm (pond) trial to analyse the efficacy of flumioxazin in controlling Amazon frogbit and carried out a small-scale field trial to measure control efficacy in a real-world scenario.

MATERIALS AND METHODS

Mesocosm trial The experiment was conducted in an outdoor area at the Ecosciences Precinct, Dutton Park, Queensland, Australia, in February- April 2017 (late summer to autumn). Frogbit was cultured in 35 plastic crates (60 x 35 x 37 cm; ~70 L) filled with de-chlorinated tap water, aerated to prevent stratification. Mesocosms were fertilized monthly with 5 g of soluble fertiliser (Thrive, Yates, Australia) containing macro nutrients (NPK 25:5:8.8) and trace elements to support healthy plant growth. Once Amazon frogbit plants covered the entire water surface, crates were randomly assigned to five treatments (seven replicates each): control (no herbicide), low subsurface, high subsurface, low foliar, and high foliar (see Table 1 for rates). The dosages represent low and medium application rates listed on the of the flumioxazin Clipper (Sumitomo) label.

The plants were treated with flumioxazin (Valor, Sumitomo Inc) in late summer, 22 February. For subsurface application, aliquots (10 - 20 mL) of a flumioxazin stock solution were injected into the water column. For foliar treatments, plants were sprayed with a paint gun (8 - 16 mL stock solution) with a plastic shroud in place to prevent spray drift; water was added to the low foliar treatment dose (8 mL) to keep spray volume consistent (equivalent to 750L ha^{-1}). The paint gun produced small droplets that achieved even coverage with the applied volume. On the treatment day, the water temperature was 27.01 °C and the water was acidic (pH 5.1) and had a specific conductance of 152 $\mu S\ cm^{-1}$.

Plant health was visually assessed twice weekly for the remainder of the experiment. At the end of the experiment (20 April; eight weeks after treatment), all remaining frogbit was harvested from each crate to measure wet mass (WM). We tested for differences between treatments with ANOVA and Tukey's HSD after transforming data (LN) to meet the requirements of parametric tests. Statistical analysis was carried out in R (v4.0.5, The R Foundation).

Field trial A field experiment was performed in a small dam (27°48'27.3"S ,153°01'47.4"E; 495 m^2 surface area, 520 m^3 Volume) on a private property in Jimboomba, QLD. The dam was partially shaded by tall eucalypt trees and the entire dam surface area was covered with a thick mat of Amazon frogbit. To determine pre-treatment biomass, four samples of Amazon frogbit (0.25 m^2) were collected from random locations in the dam. Plant samples were dried for 72 hours at 50 (°C) and weighed to determine dry mass (DM). On 11 September 2019 (early spring), 103 g flumioxazin a.i. (Valor, Sumitomo Inc) was mixed in 200 L of water in a truck-mounted quick spray unit. The herbicide solution was injected 50 cm below the water surface in 15 evenly spaced treatment spots (~ three meters distance between injection spots) to achieve a target concentration of 200 ppb flumioxazin in the water column. Plant health and water chemistry parameters were monitored regularly for the next three months and at the end of the trial the entire remaining frogbit biomass was harvested to determine dry mass.

RESULTS

Mesocosm trial On the day of treatment, frogbit covered the entire water surface of the experimental crates forming a tall canopy (mean plant height 15.1 cm ± 3.3 SD); the average wet mass was 15.4 kg m^{-2} (± 2.5 SD). Frogbit plants exhibited herbicide damage (blackened leaf veins) in all treatments (except control) within 24 hours. Plant health deteriorated rapidly over the next 7 days. Visual damage was most severe in the high subsurface application and frogbit was severely compromised, disintegrated and began to sink within 2 weeks. From DAT40, most plants in the high subsurface were dead and there was no further change in conditions until week 8 (harvest). The decline in health over time was similar in all herbicide treatments. Plant damage was lower in low subsurface and high foliar treatments and the low foliar application caused the least amount of visual damage.

At the end of the experiment, there was a significant difference in canopy height (ANOVA: df=4, F = 39.0, $p<0.0001$) and biomass (ANOVA: df=4, F = 27.97, $p<0.0001$) between treatments (Fig. 1, Table 1). Final canopy height in the control ponds was similar to starting conditions and the biomass had increased by 22% (Fig. 1, Table 1). Herbicide application significantly reduced plant height and biomass in all treatments compared to the control (Fig. 1, Table 1). High subsurface application was the most efficient treatment and achieved complete control in all but one crate. Control efficacy in terms of biomass and plant height reduction declined in order from high subsurface to high foliar, low subsurface with least control achieved with the low foliar treatment (Fig. 1, Table 1). However, the differences in final biomass between herbicide treatments were not statistically significant except for the low foliar treatment (Fig. 1).

Field trial Before treatment, Amazon frogbit covered the entire water surface of the dam with an average dry mass of 611 g m^{-2} ± 72 SD, providing an estimate of around 5 t of plant wet mass for the entire dam. After subsurface flumioxazin application, Amazon frogbit leaves started darkening within 48 hours of exposure. However, the thick plant mat took considerable time to break down. Two months after treatment, some of the plants had decayed and sunk and with about half of the dam area had having an open water surface. After three months, only a small amount of decaying and fragmented frogbit remained in the dam and was harvested; the total biomass was 1.3 kg DM of frogbit (~21.1 kg wet mass) for the entire dam, giving a control efficacy of 99.6% with the single herbicide application.

Dam water physico-chemical variables changed over the period of the experiment from DAT0 to three months after treatment. Water temperature increased over time from 14.6 to 27.6 °C following the seasonal warming of air temperature from spring to summer. Initially, the water in the dam was acidic (pH 5.5) but the pH started to increase in the last month of the trial, coinciding with the opening of the water surface, until it was neutral (pH 7.0) at the end of the trial. Specific conductance increased steadily over time from 47 to 493 µS cm^{-1}.

Table 1. Flumioxazin application rates, final Amazon frogbit canopy height (maximum plant height), wet biomass and % control in terms of biomass reduction compared to before treatment. Values are means ± SD. Lettering indicates a statistically significant difference at p<0.05 (Tukey's HSD).

treatment	flumioxazin a.i. rate	canopy height cm	wet mass kg m^{-2}	% control
control	0	14.3 ± 3.6a	19.7 ± 2.3 a	-26
low foliar	105 g ha^{-1}	2.4 ± 2.2b	4.1 ± 3.7	74
high foliar	210 g ha^{-1}	0.5 ± 1.1c	0.7 ± 1.3	96
low subsurface	100 ppb (µg L^{-1})	0.8 ± 1.2bc	1.7 ± 2.9	87
high subsurface	200 ppb (µg L^{-1})	0.0 ± 0.0c	0.0 ± 0.0	100

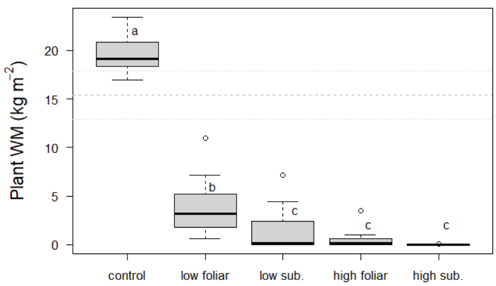

Figure 1. Final Amazon frogbit biomass eight weeks after herbicide application with different application techniques and dosage. Lettering indicates a statistically significant difference at p<0.05 (Tukey's HSD); 'low/high sub.' stands for low/high subsurface application, respectively. Horizontal lines indicate mean biomass (dashed) before treatment ± SD (dotted).

DISCUSSION

Our research demonstrated that flumioxazin is an excellent herbicide tool to manage Amazon frogbit in Australian freshwater systems at low to intermediate label rate applications. In outdoor mesocosm trials we found little difference in control efficacy (87 – 100%) between foliar and subsurface application. Only the low foliar spray provided significantly less control efficacy than the other treatments. But even at this low 105 g ha^{-1} foliar spray a 74% reduction in biomass was achieved with a single application. While foliar application was slightly less efficient than subsurface application, it will still be more economical in deeper water bodies as only the surface area is treated instead of dosing the entire water body volume. Therefore, a smaller amount of product is needed with foliar application. Despite taking care to achieve even herbicide coverage when spraying the Amazon frogbit, the slightly lower foliar efficacy could be result of uneven coverage when applying the herbicide, a common issue with foliar spraying of aquatic weeds (Willis *et al.* 2018, Mudge *et al.* 2012). Additionally, it is possible that uptake of flumioxazin by Amazon frogbit is more efficient through the root system and the leaves that are in contact with the water surface, than through the tougher ticker cuticle of emergent leaves. Lastly, the total amount of flumioxazin applied to the crates in the subsurface treatment was ~ three times higher, so plants can take up more product.

The field trial further demonstrated that flumioxazin is an efficient control tool for managing Amazon frogbit in a real-world scenario, removing more than 99% of the biomass with a single subsurface application of 200 ppb. However, it took three months to achieve control in the field site compared to a few weeks in the mesocosm experiment. We hypothesize that the shading through trees in the field site slowed down the control. Nevertheless, final control was the same as in the small-scale experiment. The removal of the dense frogbit cover on the water surface dramatically improved the water quality. Before herbicide application the water in the dam was highly acidic (low pH) and would have been unsuitable for a wide range of aquatic organisms. After the removal of frogbit the pH became neutral, greatly improving habitat quality.

Flumioxazin provides similar or better control than other aquatic herbicides reported in the literature. While imazamox provided around 90% biomass control at intermediate application rates (Willis *et al.* 2018) the application rate (280g ha^{-1}) was still more than twice that of the flumioxazin dose (105 g ha^{-1}) from the current study; imazamox is not registered for aquatic use in Australia. The congeneric *L. spongia* was controlled well with diquat in the USA (Madsen *et al.* 1998). While some diquat products are registered for aquatic use in Australia, diquat is a broad spectrum herbicide that potentially can cause considerable non-target damage to native macrophytes. From the authors' experience, flumioxazin is far more specific and carries a lower risk of damaging other aquatic plants. Flumioxazin also hydrolyses rapidly once applied to the water (Mudge *et al.* 2010, Katagi 2003), therefore, no long-term non-target damage should not be expected. Glyphosate products are registered for aquatic weed control in Australia. However, the literature suggests that it only provides poor control of the congeneric *L. spongia* (Madsen *et al.* 1998) and therefore similar poor control of Amazon frogbit is anticipated, suggesting that flumioxazin will provide far better control.

Future research should investigate dose-response relationships for foliar and subsurface flumioxazin application to control Amazon frogbit in more detail and determine minimum contact times compared to breakdown rates.

ACKNOWLEDGMENTS

Sumitomo Inc. supplied the Valor herbicide used in the trials. The Logan City Council facilitated access to the Jimboomba field site and provided operational support with manpower and spray equipment. The Queensland Government and Queensland local governments funded the research.

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