Herbicide application creates stem fragments capable of dispersal and regeneration in aquatic alligator weed

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Summary Alligator weed (*Alternanthera philoxeroides*) is subject to an eradication program in Victoria. In aquatic situations the herbicides glyphosate or metsulfuron-methyl are used. Anecdotal observations suggest that herbicide application results in the production of many alligator weed stem fragments and that some of these are viable and capable of colonisation. We applied herbicide to alligator weed growing in flooded containers and collected the resulting stem fragments. Herbicide treatment resulted in many more stem fragments than no herbicide treatment, e.g. 382 stem fragments m^{-2} for glyphosate treated plants versus 7 m^{-2} for untreated plants. Some stem fragments collected were viable resulting in viable stem fragment production of 66 viable stem fragments m^{-2} for metsulfuron compared to 9 m^{-2} for glyphosate and 3 m^{-2} for dichlobenil and untreated plants. In the field, a high proportion of stem fragments were viable (60–80%) for both glyphosate and metsulfuron-methyl for patches >5 m^2. For patches <5 m^2, viability was low (5%). If we are to eradicate aquatic alligator weed utilising herbicides, strategies to minimise production of viable stem fragments need to be considered.

Keywords *Alternanthera philoxeroides*, metsulfuron-methyl, glyphosate, eradication, allofragments, autofragments.

INTRODUCTION

Alligator weed is established in aquatic habitats around the metropolitan area of Melbourne and in two rural locations in Victoria. Alligator weed is classified as a State Prohibited Weed in Victoria, and is therefore subject to an eradication program implemented by the Victorian state government. The eradication program is based on the herbicides glyphosate or metsulfuron-methyl, although dichlobenil has been used in the past. A substantial decrease in biomass is observed at each site after herbicide application, but regrowth is usually observed, either at the same location or at other nearby locations. Given that alligator weed only reproduces by asexual means outside of its native range, this represents vegetative regrowth rather than germination from seed. Anecdotal observations by us and Prichard (2002) suggest that herbicide application results in the production of many alligator weed stem fragments and that a proportion of these are viable and capable of colonisation, thus contributing to the spread of alligator weed.

Alligator weed exhibits phenotypic plasticity across the transition from dry to flooded environments. The aquatic ecotype forms a mat of entangled stems and has adventitious roots that may be rooted into the substrate or the bank or floating free in deeper water. This growth form aids the dispersal of alligator weed by the production of stem fragments that drop into the water and move with water currents to new locations (Julien *et al.* 1992).

This paper reports on a container trial and a field trial to determine the effect of herbicide treatments on the production of stem fragments in aquatic alligator weed, and their subsequent viability (ability to regenerate). Additional information and different analyses of the results from this research have been published elsewhere (Dugdale *et al.* 2010).

MATERIALS AND METHODS

Container trial Sixty-five containers (0.58 m diameter by 0.45 m tall) were half filled with topsoil, augmented with 4 kg m^{-3} slow release fertiliser. A layer of clean sand was added to bring the containers to three-quarters full, before being filled with water (10 to 15 cm above soil height). Alligator weed stem cuttings were collected and five were planted into each container. Water levels were maintained with water from a dam and the alligator weed was left to establish for 15 weeks. Herbicide was applied to each of the alligator weed containers in March 2008, with each of the following treatments replicated five times: metsulfuron-methyl, 0.03, 0.06 and 0.12 g a.i. L^{-1} with and without Pulse® surfactant; glyphosate, 3.6, 10.8 and 21.6 g a.i. L^{-1}; dichlobenil, 78, 155 and 311 g a.i. 100 m^{-2}). Units differ because dichlobenil is applied in granular form while the other herbicides tested are applied in liquid form. Although wind speed was low during treatment, a temporary barrier (tent) was erected over each container to prevent herbicide drift. Liquid herbicide was applied until runoff was observed.

To quantify stem fragment production and determine stem viability all fragments in each container were collected and counted 3 weeks after treatment (WAT) then at fortnightly intervals until 11 WAT. To simulate mechanical disturbance that occurs in the field, prior to each collection the containers were sprayed with a jet nozzle of a garden hose at mains pressure for 5 to 10 seconds. At each collection a subsample of five stem fragments (representing a range of sizes) from each container was placed into culture to determine viability (occasionally less than five stem fragments were present), along with five stem cuttings taken from each of the control containers at 3 WAT. Culture consisted of transferring the stems to 750 mL glass jars filled with municipal water and stored in a glass house. Water level in the jars was maintained and the fragments were left at least 195 days. At the end of the storage period the viability of each fragment was determined. If it had new root growth ≥5 mm long or new shoot growth it was deemed viable.

After appropriate transformation, the results of each treatment were analysed using an analysis of variance appropriate for the design.

Field trial For each of the 2007 and 2008 growing seasons, four patches of alligator weed growing along the margins of water bodies in Melbourne were selected. A barrier consisting of polyethylene netting (15 mm diamond mesh) held up by steel stakes was constructed around the water-ward side of each patch, so as to prevent movement of alligator weed stem fragments into and out of each patch.

In December 2007 and December 2008 glyphosate was applied to two patches at a tank rate of 10 mL L^{-1} (3.6 g a.i. L^{-1}) and metsulfuron-methyl, without surfactant, was applied to another two of the patches at a tank rate of 10 g 100 L^{-1} (0.06 g a.i. L^{-1}). All herbicide was applied with a pneumatic sprayer, from above the foliage, until runoff occurred. Two weeks after these applications, alligator weed stem fragments were collected from within the barriers, and then at approximately weekly intervals for 5 to 7 weeks. On each collection date 5–20 fragments, representing a range of sizes and apparent health, were collected.

Sampled stem fragments were placed in 750 mL glass jars filled with municipal water and stored in a glass house. Assessment and culture conditions were the same as for the container trial except the culture period was shorter (12 to 16 weeks).

A parsimonious general linear model was developed to relate the percent viability of fragments to herbicide type, year, site and area of patch, using F-tests.

RESULTS

Container trial Within the fragment culture facilities, new shoot and root growth was observed arising from all of the stem cuttings taken from the controls. Most stem fragments placed into culture either grew (regenerated new roots and/or shoots) or rotted to become semi-amorphous. Typically, much of the original fragment senesced and remaining live nodes produced new root and shoot growth from axillary buds. However, some of the fragments that did regenerate did so from stem fragments in an advanced state of decay where all of the inter-nodal material was flaccid, slimy and semi-amorphous. The nodal material, although looking very decayed, remained turgid and regenerated roots and shoots. Only a very small proportion of stems appeared to remain turgid and healthy without regenerating.

All three herbicides induced large amounts of fragmentation, compared to the untreated plants, with the greatest fragmentation occurring with glyphosate (Figure 1). The viability of the metsulfuron-methyl treated plants was similar to the viability of the untreated plants (41 and 23%, respectively). However, very few stem fragments were produced from untreated plants (10 from five control tubs over 11 weeks) so their subsequent viability should be used with caution. When the number of stem fragments produced was taken into account the number of viable fragments produced from the metsulfuron-methyl treated plants was about 20 times greater than in plants that were not treated with herbicide (Figure 1b). The viability of stem fragments of dichlobenil and glyphosate treated plants was, on average for each herbicide, 1 and 2%. While the difference in number of viable stem fragments from glyphosate and untreated plants did not reach traditional statistical significance levels ($P > 0.1$), it was estimated that about three times as many viable fragments occurred with glyphosate treated plants than with untreated plants and no difference in the number of viable fragments occurred between dichlobenil treated plants and controls (Figure 1b).

With the exception of dichlobenil herbicide, there was no evidence of any effect of herbicide rate or the use of surfactant on any measurement ($P > 0.05$). Therefore, except for dichlobenil, it appears that applying herbicide at different rates, within the range of different rates tested here, will not reduce the number of stem fragments produced, nor reduce their viability.

Field trial The patches of alligator weed collapsed after herbicide application and stem fragments became abundant. For any specific year and patch area there was no significant difference in viability between herbicides ($P = 0.35$). In 2007, stem fragments from sites

that were less than 5 m^2 had about 5% viability, while stem fragments from sites that were greater than 5 m^2 had about 60% viability (Figure 2). In 2008, plants from sites that were greater than 5 m^2 had about 80% viability. No sites treated in 2008 were less than 5 m^2.

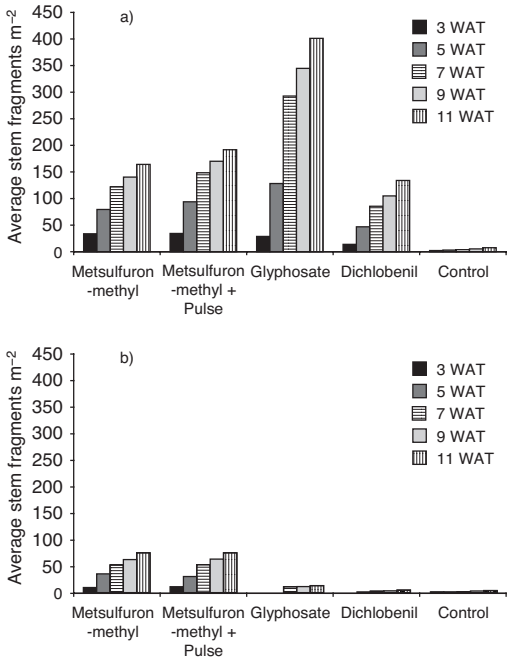

Figure 1. Cumulative number of total fragments (a) and viable fragments (b) produced over each collection date from alligator weed grown in containers and treated with herbicides. Herbicide rates are combined.

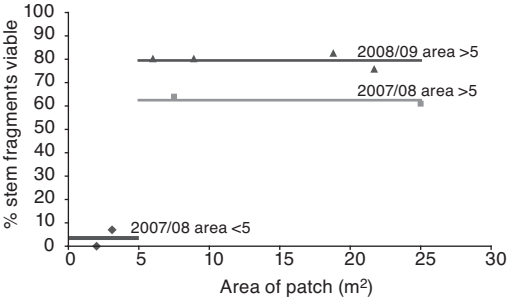

Figure 2. Relationship between viability of fragments and area of patch for patches treated in each of the years 2007 and 2008. Lines are calculated using parsimonious general linear model.

DISCUSSION

In the container trial high fragmentation occurred for all herbicides and irrespective of rate of application, confirming our anecdotal observations from the field. Both the container and field trials confirmed the viability (capable of regeneration) of a proportion of these fragments, and the fragments are probably capable of colonisation. This is likely to compromise eradication programs that use these herbicides, unless the problem of viable fragmentation is addressed.

In the container trial the actual number of fragments, viability rates of those fragments and number of viable fragments differed greatly with herbicide. In contrast, the field trial showed that many stem fragments were viable regardless of whether glyphosate or metsulfuron-methyl was used. The field trial results also show that the viability of fragments derived from small patches of aquatic alligator weed was much less than those from larger patches. A further difference between the results in the container trial and the field trial is the overall viability of stem fragments, particularly for plants treated with glyphosate. For glyphosate the overall viability of stem fragments was 60 to 80% in the field trial, but only 2% (inclusive of all treatment rates) in the container trial. It is clear that, in the field, there are other factors that have a larger impact on fragment viability than the type of herbicide used.

Surprisingly, we can rule out the absence of surfactant with metsulfuron-methyl in causing the high rate of viability in the field trials because the results of our container trial show that it has no effect on fragmentation rate or subsequent fragment viability (Figure 1).

A possible explanation for the differences between the field and container results is incomplete herbicide coverage on the plant due to differences in the density and architecture of the plants between field infestations and container trial plants. At the time of herbicide treatment the alligator weed plants growing in the containers had a dense growth habit (62 of the 65 containers had >75% cover) and were relatively prostrate (<100 mm tall). This was unlike aquatic alligator weed in the field, which was denser (seven of the eight patches had >95% cover), more upright (0.3 to 0.8 m tall) and luxuriant. Hence a more even and thorough coverage of herbicide could be achieved on the prostrate, contained plants compared with the erect field plants where the outer canopy-forming leaves and stems protected the plant material below. In these situations, results from contained trials might not be a good guide to results in the field.

Within the field patches, it is expected that the smaller sites received a more complete coverage of herbicide. We have observed that when herbicide is

applied to large, dense patches of aquatic alligator weed, very little reaches the interior of the patch. Therefore, a portion of the aerial biomass is not effectively treated, but is protected by the overlying canopy. We think that the stems from this part of the biomass contribute to the high rate of stem viability. Underwater stems are also protected from the aerially applied herbicide. Langeland (1986) also noted that a large number of axillary buds on glyphosate treated alligator weed subsequently grew without symptoms of the herbicide, and it was speculated that much of the glyphosate is either not translocated into the buds or metabolised before reaching them. This is supported by several studies that show downward translocation of a number of foliar-applied herbicides to alligator weed is poor (Earle *et al.* 1951, Funderburk and Lawrence 1963, Bowmer *et al.* 1993, Tucker *et al.* 1994). Based on this information, we suggest that the stem fragment viability reported in this study is a result of poor herbicide coverage during treatment combined with poor translocation of the herbicide once within the plant.

The shedding of plant organs takes place at predetermined abscission zones and may occur as organs senesce, or in response to environmental factors such as stress (Roberts *et al.* 2002). Given the rapid shedding of leaves and stems that occurs post-herbicide application in alligator weed, we, like Langeland (1986), postulate that rapid formation of an abscission layer at the stem nodes may provide a mechanism that prevents sufficient herbicide translocation to protected plant parts.

We recommend that the number of propagules entering the water after herbicide application of large patches needs to be restricted. Barriers to prevent fragment escape, as used by Prichard (2002) in New South Wales, should be used where there is a high risk of dispersal to downstream areas.

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