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Alligatorweed Produces Viable Stem Fragments in Response to Herbicide Treatment

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ABSTRACT

Alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) is subject to an eradication program in Victoria, Australia. In aquatic situations the herbicides glyphosate (N-(phosphonomethyl) glycine, present as the isopropylamine salt) or metsulfuron-methyl (2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) benzoic acid) are used. Anecdotal observations suggest that herbicide application results in the production of many alligatorweed stem fragments and that some of these are viable and capable of colonization. We applied herbicide to alligatorweed growing in waterlogged containers (glyphosate, mesulfuron-methyl and dichlobenil (2,6-dichlorobenzonitrile)) and collected the resulting stem fragments. Herbicide treatment resulted in many more stem fragments than no herbicide treatment, with 382 stem fragments m^{-2} for glyphosate-treated plants, 165 m^{-2} for metsulfuron-methyl, 129 m^{-2} for dichlobenil, and 7 m^{-2} for untreated plants. A proportion of stem fragments collected were viable and varied between herbicides (2, 41, 1, and 23% for glyphosate, metsulfuron-methyl, dichlobenil and untreated plants, respectively). Overall viable stem fragment production was greatest for metsulfuron-methyl-treated plants, which produced 66 m^{-2} viable stem fragments compared to 9 m^{-2} for glyphosate and 3 m^{-2} for dichlobenil and untreated plants. We also applied glyphosate or metsulfuron-methyl to patches of alligatorweed in field situations. A high proportion of stem fragments were viable (60 to 80%), regardless of herbicide used, for patches >5 m^2. For patches <5 m^2, viability was low (<5%). We postulate that viable fragments are produced by a combination of poor herbicide coverage to lower stems and poor translocation of herbicide within the plant. Successful aquatic alligatorweed eradication programs will require actions to manage viable stem fragment production.

Key words: allofragments, *Alternanthera philoxeroides*, autofragments, diclobenil, eradication, glyphosate, metsulfuron-methyl.

INTRODUCTION

In 1995 alligatorweed was discovered being propagated as a leafy vegetable by members of the Sri Lankan community in Australia in the mistaken belief that it was sessile joy weed (*Alternanthera sessilis* [L.] R. Br. ex DC.; Gunasekera and Bonila 2001). In Victoria it was first recorded in backyards in 1996 and then was found naturalized in creeks in 1997 (Gunasekera pers. comm.). It is now naturalized in many aquatic habitats around the metropolitan area of Melbourne and at two locations in rural Victoria.

Alligatorweed 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. The aquatic ecotype is characterized by having greater biomass, shoot to root ratio, stem density, stem diameter, and leaf area per stem than the terrestrial ecotype (Julien et al. 1992, Geng et al. 2007). This growth form aids the dispersal of alligatorweed by the production of stem fragments that drop into the water and move with water currents to new locations. Furthermore, during periods of flood whole mats of aquatic alligatorweed can be dislodged and deposited downstream (Julien et al. 1992), thus facilitating dispersal.

Many aquatic plants reproduce and disperse vegetatively through unspecialised fragments (Barrat-Segretain 1996). Production of stem fragments occurs through autofragmentation (self-induced abscission of shoots) and allofragmentation (breakage as a result of disturbance, such as flood flows, herbivory, weed cutting; Riis et al. 2009). The abundance of stem fragments after herbicide application may represent a further cause of allofragmentation. For stem fragments from aquatic plants to successfully disperse, they must be capable of both regeneration (the ability of the fragment to grow new roots and shoots) and colonization (the ability of the fragment to develop roots attached to the sediment; Barrat-Segretain et al. 1998).

Given its limited distribution in Victoria and its renowned weedy characteristics (Julien et al. 1992, 1995, Burgin and Norris 2008), alligatorweed 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 currently based on the herbicides glyphosate and metsulfuron-methyl, although dichlobenil has previously been used. A substantial decrease in biomass is observed at each site after herbicide application, but regrowth usually occurs either nearby or at the same location. Anecdotal observations suggest that herbicide appli-

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cation results in the production of many alligatorweed stem fragments and that a proportion of these are viable and capable of colonization, thus contributing to the spread of alligatorweed (Prichard 2002). If eradication programs based on herbicide application inadvertently increase viable stem fragments, then the eradication programs will be compromised.

We conducted a container trial to determine the effect of herbicide treatments on the production of stem fragments in aquatic alligatorweed and their subsequent viability (ability to regenerate), as well as a field trial to determine if viable alligatorweed stem fragments are produced in the field after herbicide application.

MATERIALS AND METHODS

Contained Trial

We used 65 containers (0.58 m dia * 0.45 m tall) half filled with topsoil augmented with 4 kg m^{-3} Osmocote® general purpose fertilizer (9 mo slow release). A layer of clean sand was then added to bring the containers to three-fourths full before being filled with water (10 to 15 cm above soil height). Alligatorweed stem cuttings consisting of the apical tip plus four nodes without roots were collected from Patterson River (38°2'45.98"S; 145°10'11.78"E) in November 2007; five stems were planted into each container within 3 h of collection. Water levels were maintained with water extracted from a pond, and the alligatorweed was left to establish for 15 weeks. Plant cover was assessed prior to herbicide application and at intervals after. Prior to herbicide application, two cuttings were collected at random from each of the five control containers to test viability. Herbicide was applied to each of the alligatorweed containers in March 2008 (see Table 1 for herbicides, rates of application and surfactants used); treatments were randomly assigned to containers. Although wind speed was low during treatment, a temporary barrier (tent) was erected over each container to prevent herbicide drift. 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 s, when runoff was observed, delivering a spray volume of 335 L ha^{-1} of spray solution. A control treatment did not include any herbicide application.

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 s. 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 fewer than five stem fragments were present). Culture consisted of transferring the five stems into one 750 mL glass jar filled with municipal water and stored in a glass house. The number of nodes and apices and length of the longest root present on each fragment was recorded. Water level in the jars was maintained, and the fragments were left at least 195 d (from the autumn collection until spring when assessments were carried out). No nutrients were added to the jars and algae growth was sparse. At the end of the storage period the number of turgid nodes and apices and length of longest root present on each fragment was again recorded. In addition, the viability of each fragment was determined. If it had new root growth ≥5 mm long or new shoot growth it was deemed viable. At the end of the culture period a random subsample of 26 stems deemed to be viable were potted in soil and grown to confirm that they were capable of growing into new plants.

Contained Trial Statistics

After appropriate transformation of each measurement, so that the residual variation did not change as the mean changed, the results of each measurement were analyzed using an analysis of variance appropriate for the design (Table 2). In the analysis of variance the effect of herbicide rate was separately evaluated for each of the three types of herbicide. Because of the different concentrations and modes of action

Table 1. Herbicides and rates applied to containers of alligatorweed. There were five replicates per treatment.

Treatment[1]	Product	Rate (product)	Active ingredient (g/ha)
Metsulfuron-methyl at half label rate	Esteem® 600 g / kg	5 g/100 L	10
Metsulfuron-methyl at label rate	Esteem® 600 g/kg	10 g/100 L	20
Metsulfuron-methyl at twofold label rate	Esteem® 600 g/kg	20 g/100 L	40
Metsulfuron-methyl at half label rate + surfactant	Esteem® 600 g/kg + Pulse®	5 g/100 L + 200 mL/100 L	10
Metsulfuron-methyl at label rate + surfactant	Esteem® 600 g/kg + Pulse®	10 g/100 L + 200 mL/100 L	20
Metsulfuron-methyl at twofold label rate + surfactant	Esteem® 600 g/kg + Pulse®	20 g/100 L + 200 mL/100 L	40
Glyphosate at label rate (isopropylamine salt)	Roundup Biactive® 360 g/L as isopropylamine salt	10 mL/L	1,206
Glyphosate at threefold label rate (isopropylamine salt)	Roundup Biactive® 360 g/L as isopropylamine salt	30 mL/L	3,618
Glyphosate at sixfold label rate (isopropylamine salt)	Roundup Biactive® 360 g/L as isopropylamine salt	60 mL/L	7,236
Dichlobenil at half label rate	Sierraron® G, 67.5 g/kg	1,150 g/100 m^2	7,762
Dichlobenil at label rate	Sierraron® G, 67.5 g/kg	2,300 g/100 m^2	15,525
Dichlobenil at twofold label rate	Sierraron® G, 67.5 g/kg	4,600 g/100 m^2	31,050
No herbicide	—	—	—

Table 2. Analysis of variance for the contained trial.

Source of Variation	Degrees of Freedom
Herbicide (control v dichlobenil v glyphosate v metsulfuron)	3
Herbicide rate within dichlobenil	2
Herbicide rate within glyphosate	2
Herbicide rate within metsulfuron	2
Presence of pulse surfactant within metsulfuron	1
Interaction of herbicide rate and presence of pulse surfactant within metsulfuron	2
Residual	52

for the three different herbicides, describing a main effect of herbicide rate is not biologically useful. In all analyses a container was the experimental unit. The variability of the angularly transformed viability of stem fragments was much greater in the control treatment than in other treatments, and therefore plots of these treatments were given statistical weights of 0.25 of those for other treatments. Missing values occurred because some measurements relied on stem fragments or viable stem fragments occurring in the appropriate container, and in one case an extreme observed value was excluded from analysis. In these cases, with the exception of average time of fragmentation of viable stem fragments, approximations described by Payne (2007) were used. Because there are many missing values for average time of fragmentation of viable stem fragments, an exact general linear model analysis was used. Average time of fragmentation was determined by calculating the mean time after treatment that fragments were collected, and average time of viable fragmentation was determined by calculating the mean time after treatment that subsequently viable fragments were collected. For the purpose of calculating an average time of fragmentation and an average time of viable fragmentation, fragmentation was deemed to occur at the time of stem collection.

Field Trial

For each of the 2007 and 2008 growing seasons, four patches of alligatorweed growing along the margins of lowland waterbodies in Melbourne, Australia, were selected (Table 3). 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 to prevent movement of alligatorweed stem fragments into and out of each patch.

In December 2007 a 1% glyphosate (3.6 g a.i. L^{-1}) solution was applied to two patches, and metsulfuron-methyl, without surfactant, was applied to another two at a tank rate of 0.06 g a.i. L^{-1}. Both herbicide solutions were applied with a pneumatic one-nozzle hand-wand applicator. Herbicide was applied from above to the foliage until runoff occurred. Two weeks after these applications, alligatorweed stem fragments were collected from within the barriers, and then at approximately weekly intervals for 5 to 7 weeks. On each collection date we gathered 5 to 20 fragments, depending on the number of fragments available. "True fragments" were those detached from the alligatorweed patch. When true fragments were not available, fragments were cut from the decaying alligatorweed patch, referred to as "cuttings."

The procedures performed in 2007 were repeated in November 2008 on four new patches. Alligatorweed stem fragments were collected from within each barrier 21, 28, and 43 d after treatment (DAT). On each collection date 10 to 15 true fragments were collected along with 5 to 10 cuttings. Fragments representing a range of sizes and apparent health were selected.

Collected true fragments and cuttings were placed in plastic bags and within 3 h transferred to culture. Culture conditions and fragment assessments were the same as described earlier in Container Trial. True fragments and cuttings were kept separate for the 2008 trial only. The fragments were left for 12 to 16 weeks. In 2007, a subsample of 20 fragments were potted into soil and grown to confirm they were capable of growing into new plants.

Table 3. Characteristics of patches of alligatorweed used in the field study, together with type of herbicide applied, number of stem fragments tested for viability, and viability of those fragments.

Patch	Site	Year	Area (m^2)	Herbicide applied	Number of fragments collected[a]	Percent of fragments viable
1	Edgar's Creek	2007	25	Glyphosate	79	61
2	Eumemmmerring Wetland	2007	2	Metsulfuron-methyl	20	0
3	Frog Hollow	2007	3.1	Metsulfuron-methyl	115	7
4	Patterson River	2007	7.5	Glyphosate	120	64
5	Patterson River	2008	21.7	Metsulfuron-methyl	55	76
6	Patterson River	2008	8.9	Glyphosate	50	80
7	Patterson River	2008	18.8	Glyphosate	49	82
8	Patterson River	2008	6.0	Metsulfuron-methyl	51	80

[a]and used in the regression analysis.

Field Trial Statistics

In 2008 the percent of viable stem fragments was compared to the percent of viable cuttings using a paired t-test analysis, with the pairs corresponding to patch.

The percent viability of true fragments, for each patch treated in 2008, was calculated as the percentage of true fragments collected that were viable more than 18 DAT. More than 18 DAT was used because prior to that time frame few fragments were present, and some may have already detached from the patch prior to herbicide application. However, at each site treated in 2007, the percent of viable fragments was calculated as the percentage of true fragments and cuttings collected that were viable more than 18 DAT.

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. The unit of analysis was a single patch. The effect of herbicide was examined by using a model that adds an extra herbicide type factor to the parsimonious general linear model.

RESULTS AND DISCUSSION

Contained Trial

At the time of herbicide treatment, alligatorweed plants growing in the containers had a moderately dense growth habit (62 of the 65 containers had >75% cover) and were growing relatively prostrate (<0.1 m). Within the culture facilities, new shoot and root growth was observed arising from all of the stem cuttings taken from the controls, indicating that the culture facilities were suitable for maintaining the alligatorweed fragments. Most stem fragments placed into culture either grew (regenerated new roots and/or shoots) or rotted. 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. Of the subsample of 26 stem fragments, all developed into mature plants, confirming their potential to colonize if given suitable conditions.

All three herbicides induced large amounts of fragmentation compared to the untreated plants, with the greatest fragmentation occurring with glyphosate (Table 4). The viability of the metsulfuron-methyl-treated plants was similar to the viability of the untreated plants; however, few stem fragments were produced from untreated plants (10 produced 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 not treated with herbicide (Table 4). The viability of stem fragments of dichlobenil and glyphosate-treated plants averaged 1 and 2%, respectively. While the difference in number of viable stem fragments from glyphosate and untreated plants did not reach traditional statistical significance levels ($P > 0.1$), we estimated that about three times as many viable fragments occurred with glyphosate-treated plants than with untreated plants. The average time of fragmentation of all, and of viable fragments, differed by more than a week between different herbicides (including no herbicide; Table 4).

There was no evidence of any effect of herbicide rate within glyphosate or herbicide rate within metsulfuron-methyl ($P > 0.05$) and no effect of herbicide rate within dichlobenil on fragmentation rate ($P = 0.80$; Table 5). However, there was an effect of herbicide rate within dichlobenil ($P = 0.012$), with no viable plants being observed when the dichlobenil was applied at the label rate or higher (Table 5). The overall viable fragmentation rate at half the dichlobenil label rate was similar to the glyphosate viable fragmentation rate, but less than the metsulfuron-methyl viable fragmentation rate. There was no evidence of any effect of the use of surfactant within metsulfuron-methyl on any measurement ($P > 0.05$).

High fragmentation occurred for all herbicides and irrespective of rate of application, suggesting that aquatic alligatorweed responds to stress from herbicide application by auto-abscission of stem fragments. The differences between herbicides in the average time of fragmentation of stems, and of viable stems, indicates that different herbicides work through different physiological mechanisms of the plant, influencing the time taken by the alligatorweed to react. This implies that the detail of strategies to minimize viable stem fragmentation could differ depending on herbicide.

Langeland (1986) noted that a large number of axillary buds on glyphosate-treated alligatorweed subsequently grew without symptoms of the herbicide. To our knowledge, however, the viability of herbicide-treated alligatorweed stem fragments or other aquatic weeds has not been previously quantified. The roles of auto- and allofragments as important vectors for dispersal of aquatic plant and in aquatic plant colonization have long been recognized (Boedeltje et al. 2003). Regeneration and colonization of aquatic plant fragments have been investigated and quantified by other authors (e.g., Barrat-Segretain et al. 1998, Boedeltje et al. 2003, Liu and Yu 2009, Riis et al. 2009). Although Riis (2008) reports 650 to 6950 stems day^{-1} of aquatic plants dispersing in a vegetated stream, as far as we are aware, our study is the first to quantify the number of fragments produced per area of plants (with or without herbicide application).

This study confirms that herbicide application dramatically increases stem fragmentation of alligatorweed in an aquatic environment and that a proportion of these stem fragments are viable. The actual number of fragments, viability rates of those fragments, and number of viable fragments differed greatly with herbicide. In some cases, for example metsulfuron-methyl, the number of viable fragments was many times greater than would be obtained with an untreated plant. In most other cases, over an 11 week period of herbicide treatment, the number of viable fragments obtained was at least as large as untreated alligatorweed samples.

TABLE 4. EFFECTS OF HERBICIDE ON STEM FRAGMENTATION MEASUREMENTS IN THE CONTAINED STUDY.

Measurement	Herbicide means				Standard error of difference between herbicide means				P Value
	Control (C)	Dichlobenil (D)	Glyphosate (G)	Metsulfuron-methyl (M)	C vs. D or C vs. G	C vs. M	D vs. G	D vs. M or G vs. M	
Log_{10}(Overall fragmentation Rate +10)	1.22	2.14	2.59	2.24	0.085	0.079	0.060	0.052	$\mathbf{2.3 \times 10^{-20}}$
Back transformed (stem fragments m^{-2})	7	129	382	165					
Angularly transformed overall viability %	29	5	8	40	7.7	7.6	3.0	2.6	$\mathbf{1.6 \times 10^{-19}}$
Back transformed (%)	23	1	2	41					
Log_{10}(Overall viable fragment production rate +10)	1.12	1.10	1.28	1.88	0.118	0.110	0.083	0.072	$\mathbf{1.9 \times 10^{-15}}$
Back transformed (viable stem fragments m^{-2})	3	3	9	66					
Average time of fragmentation (DAT)[a]	47	44	42	38	2.5	2.4	1.8	1.5	$\mathbf{8.2 \times 10^{-5}}$
Average time of viable fragmentation (DAT)	41	52	49	40	7.4-8.5	6.8	6.3	3.7-5.7	**0.033**

[a]Deleted tub 64 which was a control with very low value from few fragments.

TABLE 5. EFFECT OF DICHLOBENIL APPLICATION RATE ON STEM FRAGMENTATION MEASUREMENTS IN THE CONTAINED STUDY.

Measurement	Transformation	Transformed			SED[1]	Backtransformed			P Value
		Half label	Label	Twice Label		Half label	Label	Twice Label	
Overall fragmentation rate (stem fragments m^{-2})	Log (y +10)	2.16	2.17	2.10	0.104	134	137	116	0.80
Viability of fragments (%)	Angular	14	0	0	5.2	6	0	0	**0.012**
Overall viable fragmention rate (stem fragments m^{-2})	Log (y +10)	1.30	1.00	1.00	0.146	10	0	0	0.065
Average time of fragmentation (DAT)	None	45	44	44	3.1	—	—	—	0.94

[1]=Standard error of difference between means.

Field Trial

The patches of alligatorweed in the field were tall (0.3 to 0.8 m), dense (7 of the 8 patches had >95% cover), and luxuriant prior to herbicide application. They collapsed after herbicide application and stem fragments became abundant. After several weeks the patch had broken up so that there was very little stem material left attached. This implies that almost all of the stems present in a patch become stem fragments. We observed that very few stem fragments were produced in adjacent untreated patches during the periods that the study was carried out. Stem fragments had an average of eight nodes per stem upon collection. Observations of the alligatorweed stem fragments in culture were the same as those reported for the container trial; 69 of 70 cuttings collected immediately prior to treatment with herbicide were viable. Of the subsample of 20 stem fragments considered viable and grown, all developed into mature plants, confirming their potential to colonize if given suitable conditions.

Fragments collected in 2008 are estimated to have 4% lower viability than cuttings collected in 2008, but this difference was not statistically significant (P = 0.42). A 95% confidence interval ranges from true fragments being 18 percentage units less viable than cuttings to 10 percent more viable than cuttings. This shows that the viability of a stem fragment does not change appreciably if the process of abscission is completed (i.e., detached) or not. Thus the data from 2007 that contain both true fragments and cuttings should not be appreciably biased.

The parsimonious model for the viability percentage of fragments can be written as (Table 6):

$$\text{Viability\%} = \alpha + \beta \times \text{AreaGT5}$$

where Viability% indicates the viability of cuttings and fragments in 2007 and the viability of true fragments in 2008, α is a parameter that differs between 2007 and 2008, β is a constant parameter, and AreaGT5 is an indicator variable that takes the value 0 when the site area is >5 m^2 and takes the value 1 when the site area is <5 m^2.

The percentage variance accounted for by this model is 99.1 and the residual standard deviation is 3.3%. For any specific year and patch area there was no significant difference in viability between herbicides (P = 0.35; Table 6) or site (P = 031; Table 6). Patches treated with glyphosate were estimated to have fragments with about 3 (95% confidence interval = (-6, 12)) percentage units greater viability than fragments

TABLE 6. TESTS FOR INCLUDING AND EXCLUDING TERMS FROM PARSIMONIOUS MODEL IN THE FIELD STUDY.

Terms	F-Ratio	d.f.	P Value
Terms Included			
Indicator that patch area is <5m^2	27.19	1, 5	**9.5×10^{-6}**
Year	36.12	1, 5	**0.0018**
Terms Excluded			
Sites	1.80	2, 3	0.31
Linear response of area when area <5m^2	0.46	1, 4	0.46
Linear response of area when area >5m^2	3.42	1, 4	0.14
Herbicide type	2.11	1, 4	0.35

from sites treated with metsulfuron-methyl, and thus we can be confident of that any difference between the two herbicides is small.

In 2007, plants from sites <5 m^2 had about 5% viability while plants from sites >5 m^2 had about 60% viability (Table 7). In 2008, plants from sites >5 m^2 had about 80% viability. No sites treated in 2008 were <5 m^2.

These field results confirm anecdotal observations that a high proportion of stem fragments produced as a result of herbicide application are viable. These fragments, when lodged in a suitable habitat, are likely able to colonize, making eradication programs using herbicides ineffective unless the problem of viable fragmentation is addressed.

The study also shows that the viability of fragments derived from small patches of aquatic alligatorweed is much lower than those from larger patches. Furthermore, it shows that a high proportion of stem fragments are viable regardless of whether glyphosate or metsulfuron-methyl was used. This is in contrast to the container trial that found differences in the viability of alligatorweed stem fragments with herbicide. A further difference between the results in the contained 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 (Table 7) but only 2% (inclusive of all treatment rates) in the contained trial (Table 4). It is clear that, in the field, other factors have a larger impact on fragment viability than type of herbicide used.

A possible explanation for the differences in these results is incomplete herbicide coverage on the plant due to differences in the density and architecture of the plants between field infestations and contained trial plants. Alligatorweed plants growing in containers had a moderately dense growth habit (62 of the 65 containers had >75% cover) and were relatively prostrate (<0.1 m). This is unlike aquatic alligatorweed in the field, which is denser (7 of the 8 patches had >95% cover), more upright (0.3 to 0.8 m tall), and more luxuriant. Hence, a more even and thorough coverage of herbicide can be achieved on the prostrate container 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, smaller sites are expected to receive a more complete coverage of herbicide. We have observed that when herbicide is applied to large, dense patches of aquatic alligatorweed, very little reaches the interior of the patch; therefore, a portion of the aerial biomass is protected by the overlying canopy and not effectively treated. 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. These stems may contribute to the pool of viable fragments but, because they were present in all patches and containers, are likely to have a similar effect across all treatments. Langeland (1986), when noting growth of axillary buds on glyphosate treated alligatorweed, speculated that much of the glyphosate is either not translocated into the buds or metabolized before reaching them. This is supported by the studies of Funderburk and Lawrence (1963) and Earle et al. (1951) who found that downward translocation of a number of foliar-applied herbicides was poor. Given these results, it is apparent that complete coverage of the stems is required to obtain good herbicidal activity. Another factor that may have reduced the efficacy of the herbicide in our study is that metsulfuron-methyl was used without a surfactant, resulting in poor coverage on the plant; however, we discount this because our container trial showed that the presence of a surfactant had no effect on the fragmentation rate and fragment viability of alligatorweed.

Table 7. Predicted viability of stem fragments for different combinations of year and patch in the field study. There were no patches <5m^2 in 2008.

Year	Patch area (m^2)	Viability (%	95% confidence interval
2007	<5	4	(-4, 11)
	>5	63	(55, 70)
2008	>5	80	(75,85)

Bowmer et al. (1993) have shown that alligatorweed leaves readily absorb glyphosate, but only about 7% of the glyphosate is translocated to the rhizomes. This lack of translocation results in sublethal concentrations of glyphosate in rhizome tissues. Tucker et al. (1994) report that <0.1% of glyphosate and imazapyr was present in the lower stems of alligatorweed (despite 0.9 and 15.1% of the glyphosate and imazapyr, respectively, being found in the roots). A similar process could be occurring in the dense alligatorweed patches treated with glyphosate during this study, where not enough herbicide reaches the inner, protected stems of the dense patches via translocation, exacerbating the poor herbicide coverage of these protected stems. Although none of the studies cited above were conducted with metsulfuron, we assume that translocation of metsulfuron is also poor in alligatorweed. 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 (Gonzalez-Carranza et al. 1998). Given the rapid shedding of leaves and stems that occurs post-herbicide application in alligatorweed, 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.

An alternative explanation for the higher production of viable fragments from larger patches may be related to the physiological age of the alligatorweed, where larger patches are older than small ones, but we have no supporting evidence. Langeland (1986) observed greater control of alligatorweed with glyphosate when herbicide was applied later in the season, possibly because the plants treated early in the season were physiologically younger and had large numbers of dormant buds that had not initiated. Translocation to these dormant buds would have been restricted, protecting them from the herbicide. Clearly, more research is required

to determine how stems remain viable and if improved herbicide application techniques can reduce the production of viable fragments.

Intuitively, it is reasonable to assume that the more viable alligatorweed stem fragments produced after herbicide application, the greater the potential to inadvertently increase the spread. However, research by Riis (2008) has shown that colonization of aquatic plant stem fragments in newly created lowland streams is not constrained by the number of propagules; there was no relationship between the number of drifting propagules and primary colonization. Rather, colonisation of such propagules is the main constraint (i.e., retention of the propagule at a suitable micro-site followed by root regeneration and then attachment to the sediment. Riis (2008) found 1% of all dispersed *Ranunculus* stem propagules were retained, and 0.034% were retained and colonized (per 100 m stream reach). We do not know the colonization rate of alligatorweed around Melbourne, where our study was carried out. The banks of the waterbodies where it occurs are generally well vegetated and thus provide a much greater opportunity for retention of propagules compared to the stream studied by Riis (2008). However, such vegetation also provides competition for newly retained propagules that should reduce the colonization success. Until the colonization rate by alligatorweed is better understood, the number of propagules entering the water needs to be restricted. Use of barriers to prevent fragment escape, as used by Prichard (2002) in New South Wales, Australia, should be used where there is a high risk of dispersal to downstream areas where alligatorweed is absent or sparse.

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