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NOTE

Concentration–exposure time relationships for controlling fanwort (*Cabomba caroliniana*) with endothall amine salt and carfentrazone

TREVOR D HUNT, TONY M. DUGDALE, DANIEL CLEMENTS, AND MASHA FRIDMAN*

INTRODUCTION

Fanwort (*Cabomba caroliniana* Gray; also called camboda), a submersed, freshwater aquatic plant native to South America, is an invasive plant in Australia (Mackey and Swarbrick 1997) where it is listed as a Weed of National Significance. It is also invasive in northern United States, Canada, China, and Europe (Bultemeier et al. 2009). There are at least three confirmed phenotypes of fanwort (referred to as green, red, and aquarium); of which, the green phenotype is invasive and the most difficult to control (Bultemeier et al. 2009). It is unclear the degrees to which genetic versus environmental influences have in determining the three phenotypes (see Mackey and Swarbrick 1997, Wilson et al. 2007). The problematic populations found in Australia have bright green leaves and olive-green to reddish-brown stems (van Oosterhout 2009). However, we cannot assume that these populations are the same as the green phenotype referred to by Bultemeier et al. (2009) because genetic analysis indicates that most Australian populations are a hybrid of *C. caroliniana* Gray var. *caroliniana* and *C. caroliniana* Gray var. *pulcherrima* Harper (Schooler et al 2009). In the state of Victoria, Australia, fanwort is environmentally and aesthetically problematic in at least two important weir pools (Lake Benalla and Lake Nagambie/Goulburn Weir; (Dugdale et al. 2013). It also infests lakes and slow moving waterbodies in New South Wales, Queensland, and Northern Territory, Australia (Schooler et al. 2005). Before 2011, there were no aquatic herbicides registered to control fanwort in Australia, thus, making it very difficult to enact control programs; major problem infestations have been controlled by harvesting (e.g., Lake MacDonald, Queensland) and drawdown (e.g., Lake Benalla, Victoria; Dugdale et al. 2013). Although drawdown has a low financial cost (Dierberg and Williams 1989), it has a high impact on the aquatic environment and affects anthropogenic utility (Cooke 1980).

*First, second, third, and fourth authors: Department of Environment and Primary Industries, Biosciences Research Division, 5 Ring Road, La Trobe University, Bundoora, 3089, Victoria, Australia. Corresponding author's E-mail: trevor.hunt@depi.vic.gov.au. Received for publication May 8, 2013 and in revised form June 23, 2014

Controlling fanwort with herbicides is difficult. Many herbicides have been tested, including diquat; 2,4-DP; 2,4,5-T; 2,4-D; endothall amine salt; endothall dipotassium salt; terbutryn; fluridone; carfentrazone; and flumioxazin (Mackey and Swarbrick 1997, Wilson et al. 2007, Bultemeier 2008, Bultemeier et al. 2009, Dugdale et al. 2012,) with most failing to have any effect or only producing sublethal injury. An exception is endothall amine salt and flumioxazin, which have provided good results in previous research. Bultemeier et al. (2009) found that endothall amine salt, but not endothall dipotassium salt, was effective on green fanwort, whereas Dugdale et al. (2012) observed a similar response with the type of fanwort found in Victoria, Australia. Recently (2011), a herbicide containing carfentrazone-ethyl was registered to control fanwort in Australia, but reports of its efficacy have not been published.

Carfentrazone and endothall amine salt are toxic to some aquatic fauna (e.g., 50% lethal concentration [LC_{50}] for fish of 2 to 16 mg ae L^{-1} for carfentrazone [EPA 1998], and 0.079 to 0.41 mg ae L^{-1} for endothall amine salt [Compliance Services International 2001]) at concentrations that overlap with those used for weed control. In the United States, endothall amine salt is used in canals and flowing waters where fisheries are not an important resource and is rarely used in natural surface waters (Slade et al. 2008). Risk can be reduced by treating sections of a waterbody, limiting endothall amine salt concentration to 0.5 mg ae L^{-1}, and minimizing exposure time (Compliance Services International 2001).

Controlling submersed aquatic weeds with herbicides is dependent on both the concentration of the herbicide that the weed is exposed to (which is achieved by dosing the water column to a target concentration) and the duration of the exposure, referred to as the *concentration–exposure time* (CET) relationship. The minimum, or threshold, CET relationship required for effective control differs for each weed species (Netherland et al. 1991). Comparable control of particular species has been reported with a range of different combinations of endothall concentration and exposure times (e.g., Netherland et al. 1991, Sprecher et al. 2002, Getsinger et al. 2011, Mudge and Theel 2011). A way to simplify this concept is to present the combination of concentration (*C*) and exposure time (*ET*) as their product

(here and elsewhere in this article we use *product* in the mathematical context), so similar control should be achieved with 5 ppm of endothall for 3 h or 1 ppm for 15 h, both having a product of 15. Knowing herbicide CET relationships allows (1) the herbicide to be used efficiently; (2) broad-spectrum herbicides to be used with a degree of selectivity, according to the relative susceptibility of the local plant community (Getsinger et al. 2008, 2011); and (3) for strategies to minimize off-target ecotoxicity by reducing the amount of herbicide used to achieve effective control (Getsinger et al. 2011). Thus, it may be possible to use endothall amine salt to control fanwort in waterbodies without damaging native plants or fauna if the optimal CET combination required to control fanwort is lower than the CET that injures nontarget organisms. If so, the use of endothall against fanwort in natural water bodies at low rates may be acceptable and a useful supplemental herbicide for fanwort control in Australia.

The aim of this research was to investigate whether fanwort could be controlled at reduced concentrations of endothall amine salt for prolonged exposure times and to relate that to the efficacy of carfentrazone, at the manufacturers recommended rate, over a range of exposure times.

MATERIALS AND METHODS

Overview

To test the effectiveness of endothall amine salt and carfentrazone to control fanwort, we conducted an experiment in which fanwort was cultured in small pots within larger tubs filled with water. After an establishment period of 7 wk, the pots were removed from the culture tubs and immersed in a tub that contained one of the herbicides (or no herbicide for the controls) for a range of exposure times. After the designated exposure time, the pots were removed from the herbicide tub, rinsed in clean water, and then transferred back to the culture tub, where they remained for 6 wk; at which time, the biomass was harvested to determine herbicide effectiveness.

Plant material

Plants used in this study were collected from Lake Benalla, Victoria, Australia (36° 33′ 07″S; 145° 58′ 49″E). This population has bright green leaves and olive-green to reddish-brown stems, and a specimen has been deposited in National Herbarium of Victoria (MEL 2369353A).

Culture conditions

Forty 100-L capacity polyethylene tubs were filled to 80 L (water depth = 35 cm) with municipal water and left for 7 wk to equilibrate. Each tub was constantly aerated and covered with two layers of 90% shade cloth, which remained in place for the duration of the experiment, to prevent algal growth. Light was reduced to 98% of ambient beneath the shade cloth (Apogee LQS-QM Quantum meter[2]).

Seventeen pots of fanwort were grown in each of 20 of these tubs (hereafter referred to as *culture tubs*). Pots[3] were filled to 75% capacity with topsoil, augmented with fertilizer (Osmocote,[4] 2 kg m^{-3}), then topped with washed sand. Two fanwort sprigs (15 cm long with apical meristems) were planted to a depth of 8 cm in each pot. Plants were left to grow until they were reaching the water surface (7 wk). To determine the start-point biomass before treatment, one pot from each culture tub was removed and all aboveground material excised and dried. To record temperature, two data loggers[5] were placed into randomly selected tubs. The water pH was not monitored.

Experimental design

The 40 tubs were arranged in pairs: one tub for culture and the other tub for herbicide exposure. Herbicide treatments were randomized across exposure tubs, whereas exposure time was randomized across rows of pots within tubs, which was the experimental unit (Table 1). Each exposure tub was dosed with its allocated herbicide treatment, and all pots in culture tubs were transferred into their paired exposure tubs. At the appropriate interval, a single row of four pots was transferred out of the exposure tub, into a 1,200-L trough of clean water to rinse off residual herbicide, and back into its paired culture tub. Rinse troughs were allocated to an individual herbicide or no herbicide.

For endothall amine salt treatments, exposure times were calculated to provide CET combinations that provided four nominal products of $C \times ET$ (6, 12, 24, and 36) across the three concentrations of 3.0, 1.5, and 0.5 mg ae L^{-1}. This was achieved with exposure times ranging from 2 to 72 h (Table 1; e.g., 3 mg $L^{-1} \times 2$ h = 6; 1.5 mg $L^{-1} \times 4$ h = 6; 0.5 mg $L^{-1} \times 12 = 6$). The exposure times used for carfentrazone and no herbicide treatments were 2, 12, 24, and 72 h.

At 1 and 24 h after treatment, water samples were collected from endothall exposure tubs to determine actual endothall concentration. Following the final transfers, water samples were also taken from culture tubs to detect endothall contamination. All samples were analyzed by us using an enzyme–linked immunoabsorbent assay (ELISA).[6]

Plant health was assessed by observation at 2-wk intervals up to 6 wk after treatment (WAT); at which time, all aboveground material was harvested and dried at 60 C to a constant weight.

Statistical analyses

Because a range of nonoverlapping exposure times were used for each herbicide and endothall amine salt was used at multiple concentrations, the fanwort response with differing exposure times was modeled to allow comparisons to be made at (1) common exposure periods, and (2) common levels of fanwort damage. Although plants in some treatments were exposed for up to 72 h, preliminary statistical analysis of the data showed there

Table 1. Herbicides, concentrations, and exposure times applied to fanwort in this study; each replicated four times CET = concentration–exposure time.

Herbicide[1]	Concentration (C)[2]	Exposure Time (ET)	CET Product ($C \times ET$)
No herbicide	0[3]	2	0 (0)
		12	0 (0)
		24	0 (0)
		72	0 (0)
Endothall amine salt (mg ae L^{-1})	0.5 (0.61)	12	6 (7.3)
		24	12 (14.6)
		48	24 (29.3)
		72	36 (43.9)
	1.5 (1.82)	4	6 (7.3)
		8	12 (14.6)
		16	24 (29.1)
		24 (21)	36 (38.2)
	3.0 (3.39)	2	6 (6.8)
		4	12 (13.6)
		8	24 (27.1)
		12	36 (40.7)
Carfentrazone (mg ai L^{-1})	2.0[3]	2	4
		12	24
		24	48
		72	144

[1]Carfentrazone: Shark (Australia), X,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanic acid; endothall amine salt: Teton (USA), 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid, mono(*N*,*N*-dimethylalkylamine) salt.
[2]Numbers not in parentheses are nominal values of concentration, exposure time, or concentration × exposure time; those in parentheses are actual values (where different to nominal).
[3]Not measured.

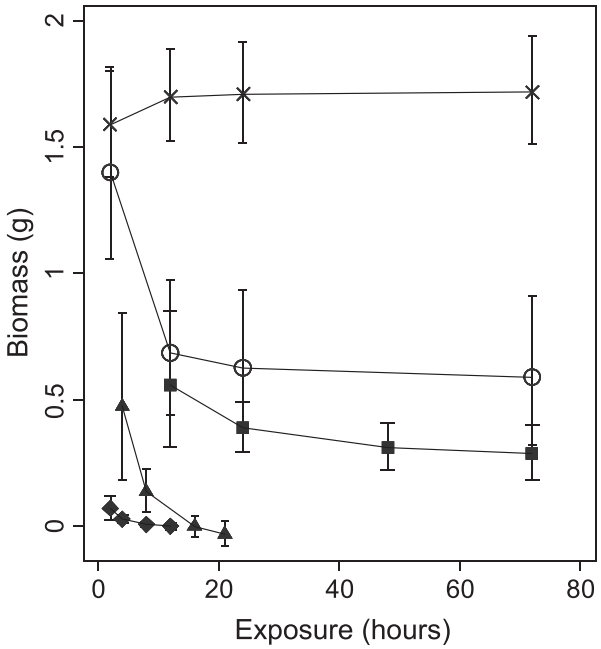

Figure 1. Impact of endothall concentration (mg ae L^{-1}) and exposure time and carfentrazone exposure time on fanwort biomass. x = no herbicide; ■ = endothall at 0.5 mg ae L^{-1};▲ = endothall at 1.5 mg ae L^{-1}; ◆ endothall at 3 mg ae L^{-1}; ○ = carfentrazone. Nominal endothall concentrations used. Biomass values are predicted estimates obtained from an ordinary least squares multiple regression model (see "Methods"). Capped bars represent 95% confidence intervals.

was no meaningful reduction in biomass beyond 50 h of exposure in all treatments; hence, the first outcome measure was predicted biomass (g) after 50 h exposure to the herbicide treatment (hereafter referred to as biomass). The second outcome measure, ET_{50} (h), is an estimate of exposure time in hours required for a decline in biomass by 50%, relative to control biomass, which indicated the speed of herbicide activity on fanwort (Bultemeier et al. 2009, Mudge et al. 2012). A greater level of fanwort biomass reduction, such as 80%, could not be used for this measure because some treatments did not achieve that level of biomass reduction. The estimate of 50% biomass (0.85 g) was obtained from pooled data for control (no herbicide) plants.

Ordinary least squares (OLS) multiple regression modeling of biomass was used to compare the effects of treatments. Predicted estimates of biomass and ET_{50} were obtained from the following OLS model:

$$\ln_e(Biomass + 1) = \beta_0 + \beta_1\left(\frac{1}{Exposure}\right) + \beta_{2-5}(Treatment) + \beta_{6-9}\left(Treatment \times \frac{1}{Exposure}\right). \quad (1)$$

F tests were used for comparisons of predicted biomass and ET_{50} estimates.

Differences in biomass between endothall amine salt treatments with $C \times ET$ combinations that produced an equivalent product were estimated using ANOVA. The level of significance was set at $P = 0.05$. All statistical analysis was conducted using Stata/SE 12.1.[7]

RESULTS AND DISCUSSION

Experimental conditions

Plants in control tubs continued to grow throughout the trial. When assessed before treatment, plants in all tubs were healthy and reaching the water surface; stems varied in length from 30 to 50 cm. Plants with multiple branching and new leaves were observed, whereas many stems had produced adventitious roots at the lower node(s). Flowers were not present. Average temperature throughout the trial was 20.5 C (12.3 to 35.3 C). Actual concentrations of endothall amine salt determined by ELISA were 13 to 21% greater than targeted (Table 1), whereas concentrations in all associated culture tubs was below the level of detection (7 μg L^{-1}), which indicates herbicide carryover was not an issue when plants were washed after the exposure period concluded.

Fanwort response to herbicide

All herbicide treatments were associated with plant injury (Figure 1). Visual assessment showed the level of plant injury to be consistent with herbicide concentration, although with some variability within exposure intervals. Canopy collapse was observed in endothall amine salt

Table 2. Main and interaction effects in an ordinary least squares regression model of transformed fanwort biomass.

Main and Interaction Effects	R^2	*df*	*F*	*P* Value
Full model	0.859	9, 70	156	< 0.001
Treatment	0.843	4, 75	262	< 0.001
Exposure time (1/h)	0.137	1, 78	1	0.302
Treatment × exposure time interaction	0.074	4, 70	6	< 0.001

treatments > 3 mg ae L^{-1} and 2 h exposure, 1.5 mg ae L^{-1} and 8 h exposure, or 0.5 mg ae L^{-1}, and 48 h exposure. At 6 WAT, very few stems remained across all four endothall amine salt 3.0 mg ae L^{-1} exposure intervals, whereas plants treated with endothall amine salt at 0.5 mg ae L^{-1} and carfentrazone retained a large proportion of original stem material, many reaching the water surface. Stems in the endothall amine salt at 0.5 mg ae L^{-1} treatment were predominantly leafless, whereas the carfentrazone-treated plants were quite variable with many stems retaining leaves. At the end of the trial, regrowth was observed on plants in all herbicide treatments.

The regression model of fanwort response to herbicide accounted for 85.9% of the variability in biomass (Table 2). Estimates of biomass reduction in the presence of endothall amine salt were 83, 100, and 100% at respective rates of 0.5, 1.5, and 3 mg ae L^{-1}, with statistical differences among all rates (Table 3A). In addition, higher concentrations of endothall amine salt were associated with reduced exposure times to achieve 50% biomass reduction: ET_{50} estimates of 6.9, 2.8, and 0.3 h were obtained for endothall amine salt concentrations of 0.5, 1.5, and 3.0 mg ae L^{-1}, respectively (Table 3), with endothall at 3.0 mg ae L^{-1} statistically different from all other rates (Table 3B).

These results confirm the findings of Dugdale et al. (2012), who showed that fanwort was very sensitive to endothall amine salt, with biomass reduced by 100% after a 6-h exposure period at 5 mg ae L^{-1}. Bultemeier et al. (2009) found endothall amine salt reduced photosynthesis of green fanwort by 96% after a 24-h exposure at 2.3 mg ai L^{-1} (approximately 1.85 mg ae L^{-1}), although photosynthesis recovered by 144 h after treatment. In the current experiment, because biomass was reduced so effectively at low endothall amine salt CET products ($\leq$ 36) it may be possible to use endothall amine salt as a selective herbicide against fanwort in Australia in open-water systems. Although CET relationships are known for endothall dipotassium salt against many submersed aquatic plants (e.g., Skogerboe and Getsinger 2001, Skogerboe and Getsinger 2002, Getsinger and Poovey 2010), equivalent data for endothall amine salt is less common (e.g., Slade et al. 2008). There are only two Australian native, submersed aquatic plants that have been tested with endothall amine salt, to our knowledge: ribbon weed (*Vallisneria australis* S.W.L Jacobs & Les) and floating pondweed (*Potamogeton sulcatus* A. Benn). Both were susceptible to endothall amine salt, when tested at 5 mg ae L^{-1} in a screening trial, but biomass reduction was less than it is with fanwort (Dugdale et al. 2012). Therefore, with careful dosing, it is likely that control of fanwort could be achieved in natural waterbodies without substantial damage occurring to at least two commonly found native, submersed plant species. If so, this would provide native communities a competitive edge over fanwort and improve the long-term effectiveness of endothall amine salt. However, before this can be tested, CET relationships for ribbon weed and floating pondweed, at least within the range likely to be used against fanwort, need to be determined.

All CET products of 14 and above resulted in > 80% biomass reduction (Figure 2). We consider this a threshold dose that can be used to guide control of fanwort with endothall amine salt. However, equivalent CET combinations of endothall amine salt (i.e., those that had similar $C \times ET$ products) that were $\geq$ 14 did not produce equivalent biomass reduction, with less control observed for CET combinations derived from 0.5 mg ae L^{-1} than for those from 1.5 and 3 mg ae L^{-1} ($P < 0.05$) This suggests a minimum threshold of initial endothall amine salt concentration is critical to achieve control, independent of exposure time.

Carfentrazone at 2 mg ai L^{-1} (twice the maximum concentration registered in the United States) was less effective than endothall amine salt in controlling fanwort. After the 50 h exposure period, biomass reduction was only 65%, less than with any of the endothall amine salt concentrations (Table 3). Estimated ET_{50} was 5.2 h, which

Table 3. Predicted effects of herbicide treatments; two measures of treatment effects were estimated: (A) predicted biomass at 50-h herbicide exposure,[1] and (B) ET_{50} (exposure time required to reduce biomass (BM) by 50%).[2]

	(A) Predicted BM at 50 h of Herbicide Exposure[3]				
Herbicide Treatments	Predicted BM (g) (95% CI)	Difference in BM (g) Relative to "Control" (*P* Value)	Difference in BM (g) Relative to E-AS at 0.5 mg ae L^{-1} (*P* Value)	Difference in BM (g) Relative to E-AS at 1.5 mg ae L^{-1} (*P* Value)	Difference in BM (g) Relative to E-AS at 3.0 mg ae L^{-1} (*P* Value)
No herbicide	1.7 (1.5, 1.9)	•			
E-AS at 0.5 mg ae L^{-1}	0.3 (0.2, 0.4)	−1.4 (<0.001)	•		
E-AS at 1.5 mg ae L^{-1}	−0.1 (−0.2, 0.0)	−1.8 (<0.001)	−0.4 (<0.001)	•	
E-AS at 3.0 mg ae L^{-1}	0.0 (0.0, 0.0)	−1.7 (<0.001)	−0.3 (<0.001)	0.1 (0.049)	•
Carfentrazone at 2.0 mg ae L^{-1}	0.6 (0.3, 0.9)	−1.1 (<0.001)	0.3 (0.041)	0.7 (<0.001)	0.6 (<0.001)

[1]Comparisons of predicted biomass estimates after 50 h of exposure to herbicide treatments.
[2]ET_{50} = ½(mean control BM at all exposures) = ½(1.69) = 0.85 g. There was no significant difference among control BM at different exposure times (*P* value = 0.286; ANOVA)
***50% biomass reduction not achieved.
[3]• = reference value; E-AS = endothall amine salt.

was not different to that of endothall amine salt at 0.5 and 1.5 mg ae L^{-1}. Bultemeier et al. (2009) also found endothall amine salt to have a greater effect than carfentrazone had, which reduced photosynthesis of green fanwort by only 31% after a 144 h exposure at 0.4 mg ai L^{-1}. Despite the limited activity on green fanwort, Bultemeier et al. (2009) demonstrated carfentrazone was much more effective against the red and aquarium fanwort phenotypes.

These poor results for carfentrazone contrast with anecdotal observations from Australia, which indicate that carfentrazone provides effective control of fanwort in the field. Possible explanations for this poor control relate to pH and shade. Firstly, carfentrazone half-life varies from 3.6 h at pH 9 to 8.6 days at pH 7 (EPA 1998), so we would expect short-duration exposure, and subsequently poor control, in alkaline water. We did not measure the pH in this study, but fanwort does not grow well in alkaline water (Mackey and Swarbrick 1997, Wilson et al. 2007); therefore, given the observed vigor of the plants at treatment time, it is unlikely the pH was high and inhibited carfentrazone efficacy. Secondly, carfentrazone requires sunlight for activity (Hess 2000), although it is not clear what intensity is required. It is possible that carfentrazone did not provide effective control because the fanwort was treated under shade, and so our results may not hold for fanwort grown in environments with more light. Regardless, fanwort is problematic in waterbodies in Victoria that are turbid (Lakes Benalla and Nagambie have 25th and 75th percentiles of turbidity of 19 to 37 and 12 to 25 NTU, respectively) and have neutral pH (6.9 to 7.5 and 6.9 to 7.3, respectively; Waterwatch Victoria 2014). Further, fanwort is often associated with Mexican waterlilly (*Nymphaea mexicana* Zucc.), where it grows under the lily canopy in heavy shade (pers. obs.). So, for carfentrazone to be a useful herbicide in Victoria, it needs to be effective in low light conditions and in neutral pH.

CONCLUSIONS

This research demonstrated CET relationships for endothall amine salt on fanwort that indicate that it could be an effective tool for fanwort control, even at low concentration (0.5 mg ae L^{-1}) provided contact times can be maintained for at least 50 h. Field verification is now required to confirm the findings of this research. Control of fanwort with carfentrazone was not as effective as endothall amine salt was, but its performance may have been inhibited by the heavy shading employed to reduce algal growth.

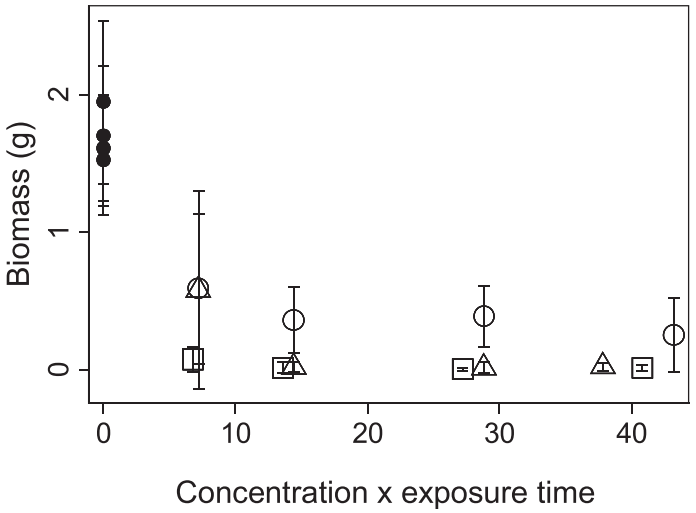

Figure 2. Impact of increasing endothall concentration exposure time combinations on fanwort biomass. • = no herbicide; ○ = endothall at 0.6 mg ae L^{-1}; Δ = endothall at 1.8 mg ae L^{-1}; □ = endothall at 3.4 mg ae L^{-1}. Actual endothall concentrations used in calculating concentration–exposure time combination values. Capped vertical bars indicate 95% confidence intervals for each treatment group.

SOURCES OF MATERIALS

[1]Shark herbicide, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

[2]LQS-QM Quantum meter, Apogee Instruments, 721 West 1800 North, Logan, UT 84321.

[3]68-mm^2 pot, code T68S, and 2 × 10-cell tray, code K10Z, Garden City Plastics, 89 Camms Road, Monbulk, VIC 3793, Australia.

[4]Osmocote fertilizer, Scotts Miracle-Gro Company, 14111 Scottslawn Road, Marysville, OH 43041.

Table 3. Extended.

(A) Predicted BM at 50 h of Herbicide Exposure[3]	(B) ET_{50} Estimates			
Reduction in BM (%)	Predicted ET_{50} (h) (95% CI)	Difference in ET_{50} Relative to E-AS at 0.5 mg ae L^{-1} (P Value)	Difference in ET_{50} Relative to E-AS at 1.5 mg ae L^{-1} (P Value)	Difference in ET_{50} Relative to E-AS at 3.0 mg ae L^{-1} (P Value)
0%	***			
83%	6.9 (0.8, 13.0)	•		
100%	2.8 (1.5, 4.1)	−4.1 (0.196)	•	
100%	0.3 (0.1, 0.4)	−6.6 (0.034)	−2.5 (<0.001)	•
65%	5.2 (1.1, 9.3)	−1.7 (0.652)	2.4 (0.260)	5.0 (0.017)

[5]HOBO U20 data logger, Onset, 470 MacArthur Blvd, Bourne, MA 02532.

[6]RaPID Assay Endothall Test Kit 7007000, Strategic Diagnostics Incorporated (sdix), 111 Pencader Drive, Newark, DE 19702-3322.

[7]Stata/SE 12.1 statistical software, Stata, 4905 Lakeway Drive, College Station, TX 77845-4512.

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