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Efficacy of endothall dimethylalkylamine salt applied to static irrigation channels during winter to control aquatic weeds in temperate Australia

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ABSTRACT

Options for controlling aquatic weeds in irrigation channels are currently limited in Australia. A potential control option to enable efficient delivery of water is to apply endothall dimethylalkylamine salt to static irrigation channels ponded in temperate winter conditions, when long exposure times can be achieved. This article reports on a mesocosm dose-response experiment and field experiments to determine the efficacy of endothall dimethylalkylamine salt against five aquatic weed species (*Vallisneria australis*, *Potamogeton sulcatus*, *Elodea canadensis*, *Sagittaria platyphylla*, and *Myriophyllum papillosum*) that obstruct irrigation channels in Australia. Concentration-exposure time (CET) relationships were determined for each species in a mesocosm trial (~10 C water temperature). Effective control required long exposure times (7 to 21 d) and generally high concentrations (2.4 to 4.8 mg ae L^{-1}) for all species, except *E. canadensis*, which was not controlled at any rate. Field trials in irrigation channels (~10 C; 35 to 36°S) subsequently demonstrated effectiveness against *V. australis* (3.4 mg ae L^{-1}), *S. platyphylla*, and *M. papillosum* (5 mg ae L^{-1}) at exposure periods of 20 to 30 d. Estimated biomass reductions were 46, 91, and 98, 19 wk after treatment period, for *V. australis*, *S. platyphylla*, and *M. papillosum*, respectively. These biomass reductions resulted in improved operation of irrigation channels and delivery of irrigation water. We conclude that endothall dimethylalkylamine salt applied with long exposure times to static irrigation channels ponded during winter conditions provides an effective tool to reduce aquatic plant biomass and subsequently enables efficient delivery of irrigation water in temperate Australia. These results demonstrate a new use pattern for endothall dimethylalkylamine salt.

Key words: aquatic herbicide, aquatic vegetation, aquatic weed control, chemical control, irrigation, water delivery.

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INTRODUCTION

Excessive growth of aquatic plants in earthen irrigation systems reduces the systems' water-carrying capacity, promotes silting, and causes problems with automated regulators, thus compromising the reliability of water delivery to primary producers (Bill 1969, Bakry et al. 1992, Bentivegna and Fernandez 2005, Dugdale et al. 2013, Clements et al. 2015). The herbicide acrolein is currently used to control submerged aquatic weeds in irrigation channels in Australia and, although acrolein is effective on a wide range of species, it is very toxic to fauna, dangerous for people applying it, and only provides short-term control of most weeds (Bowmer et al. 1992). In some locations winter dewatering and/or mechanical excavation are also used, but these methods can be difficult to manage, costly, and may not achieve required aquatic weed management outcomes.

In the United States, the herbicide endothall dimethylalkylamine salt is used to control submerged macrophytes and algae in flowing irrigation channels; furthermore, it is used without an irrigation withholding period (Sprecher et al. 2002). Endothall is a protein phosphatase inhibitor; however, its exact herbicidal mechanism and mode of action is not yet clear (Tresch et al. 2011).

Research in Australia has shown that formulations of endothall provide effective control against key aquatic weed species (Clements et al. 2013, Clements et al. 2015, Dugdale et al. 2012, Hunt et al. 2015). However, in Australia, current irrigation withholding period restrictions imposed by the regulatory authority are incompatible with endothall use during the irrigation season. Currently, restrictions can only be complied with by applying endothall during the irrigation off season, when water is ponded during winter, and irrigation water is not supplied.

Controlling submerged aquatic weeds with herbicide is dependent on both the concentration of herbicide that the weed is exposed to (which is achieved by dosing the water column to a target concentration) and the duration of exposure, referred to as the concentration exposure time (CET) relationship. The minimum, or threshold, CET required for effective control differs for each weed species and under differing environmental conditions, such as temperature (Netherland et al. 1991, Netherland et al. 2000).

Table 1. Endothall (dimethylalkylamine salt) herbicide concentrations applied to tubs containing one pot of each of five aquatic weed species during winter. Achieved concentration was at 1, 7, and 21 d after herbicide application. Four replicate tubs per endothall concentration. Values in parentheses are one standard deviation with the use of between-tub variation.[1]

Nominal Target Concentration (mg ae L^{-1})	Endothall Application Rate (L/ML)	Achieved Endothall Concentration (mg ae L^{-1})			
		1 DAT (ELISA)	1 DAT (LC-MS)	7 DAT (LC-MS)	21 DAT (LC-MS)
No herbicide	0	ND	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
0.3	0.59	0.456 (0.015)	0.425 (0.014)	0.418 (0.016)	0.388 (0.017)
0.6	1.18	0.699 (0.040)	0.845 (0.032)	0.827 (0.040)	0.760 (0.032)
1.2	2.37	2.042 (0.150)	1.693 (0.132)	1.626 (0.096)	1.472 (0.079)
2.4	4.73	3.522 (0.135)	3.138 (0.152)	3.187 (0.067)	2.716 (0.321)
4.8	9.47	5.145 (0.497)	5.540 (0.753)	5.995 (0.267)	5.779 (0.066)

[1]Abbreviations: DAT = days after initial herbicide treatment; ELISA = enzyme-linked immunosorbent assay; LC-MS = liquid chromatography–mass spectrometry; ML = megalitre = 1,000,000 L; ND = not detected (detection limit of ELISA assay = 0.007 mg ae L^{-1}).

Long exposure times can be achieved when applying endothall to cold water during winter months (Clements et al. 2013, Wells and Champion 2010, Clements et al. 2015). This is because the primary mode of endothall decay is by microbial activity (Reinert et al. 1986), which is reduced at lower temperatures. Further, in southeast Australia (35 to 36°S), delivery of irrigation water ceases for about 6 wk each winter (June through August), and irrigation channels hold standing water. This provides an opportunity for herbicide treatment with longer exposure times, because of ponded water coupled with slow decay, compared with undertaking control during the irrigation season (Clements et al. 2015).

This article determines CET relationships for applications of endothall dimethylalkylamine salt[1] in static water ponded conditions during winter, in both mesocosm and irrigation channel field experiments, against key aquatic weeds present in temperate Australia.

MATERIALS AND METHODS

Experiment 1: Mesocosm experiment

The effectiveness of endothall dimethylalkylamine salt at 18 combinations of herbicide concentration (0, 0.3, 0.6, 1.2, 2.4, 4.8 mg ae L^{-1}) and exposure time (1, 7, and 21 d) (Table 1) were tested on five aquatic weed species during winter (average water temperature: 9.5 C) (Table 2). To achieve this, aquatic weed species were cultured in pots within 1,200-L troughs filled with water. After an establishment period, pots were removed from the culture troughs and transferred to 100-L tubs (within the same shade house) that were dosed with appropriate concentrations of endothall dimethylalkylamine salt. After the designated exposure time, pots containing plants were removed from the herbicide dosing tubs, rinsed in clean water, and then transferred to recovery

Table 2. Aquatic plant species evaluated in the mesocosm dose–response experiment and plant material utilized.

Species	Location of Plant Collection	Plant Stock			Plant Material Prior to Herbicide Treatment		
		Description	Plant Length (cm)	Number of Plants/Propagules (per pot)	Description	Maximum Plant/Stem Length (cm) Ave. (SD)[1]	Number of Propagules (per pot) Ave. (SD)
Ribbon weed (*Vallisneria australis* S.W.L. Jacobs & Les)	Boort, Victoria (35°59′59.64″ S; 143°56′3.70″ E)	Individual plants with no stolons or peduncles/ flowers	25–30	2	Plants surface reaching or near the water surface. No new ramets.	38 (2.0)	2 (0)
Floating pondweed (*Potamogeton sulcatus* A. Benn.)	Boort, Victoria (36°10′2.24″ S; 143°50′28.80″ E)	Individual length of stem material, with a length of rhizome containing one node	25	3	Many new stems erupting from sediment, near surface-reaching. No plants with floating leaves.	35 (3.5)	13 (2.4)
Elodea (*Elodea canadensis* Michx.)	Numurkah, Victoria (36°3′36.12″ S; 145°23′32.03″ E)	Branched stem (two stems with 2–4 apical growing tips present	25	5	New stems but relatively prostrate growth.	24 (4.7)	-
Sagittaria (*Sagittaria platyphylla* (Engelm) J.G. Sm.)	Numurkah, Victoria (36°3′42.22″ S; 145°23′6.12″ E)	Individual plants with no stolons or peduncles/ flowers	35–40	2	New ramets produced, emergent plant form.	44 (4.4)	4 (1)
Common water milfoil (*Myriophyllum papillosum* Orchard)	Boort, Victoria (35°59′56.56″ S; 143°56′5.63″ E)	Individual crowns with a single stem and roots.	25	3	Few new daughter plants, large emergent stems on all plants.	64 (8.7)	3 (0.5)

[1]Ave. = average, SD = standard deviation between pots.

tubs (containing clean water). Plants remained in recovery tubs for 8 wk, after which time biomass was harvested and measured, and dry weight was determined. A detailed methodology for conducting the experiment is described below.

Experimental design. The experimental treatments consisted of a 6 by 3 factorial plus a baseline control (plants that were harvested prior to herbicide application). The factorial consisted of six concentrations of endothall dimethylalkylamine salt (0, 0.3, 0.6, 1.2, 2.4, 4.8 mg ae L^{-1}) at each of three exposure times (1, 7, and 21 d) (Table 1). The experiment was arranged as a four-replicate randomized block with a dosing and recovery tub containing one pot of each species, giving a total of 76 pots per species. Blocking was associated with initial size of plants prior to entering dosing tubs and location of dosing and recovery tubs in the shade house.

Plant material and culture conditions. Municipal water that had been stored in troughs and continuously aerated for a minimum of 2 wk was used in all culture troughs and dosing and recovery tubs. All plant material was collected for culture in early March 2015 from irrigation channels in southeast Australia (Table 2). Plant stock for each species (described in Table 2) were inserted into 100, 3.1-L plastic squat pots filled with screened topsoil and topped with a layer of washed sand. In early March 2015, pots of individual species were positioned into 1,200-L culture troughs filled with municipal water at a depth of 47.5 cm and left to establish for 15 wk. For each species plants were positioned into two troughs that were adjacent to one another in a shade house (30% shade) in Victoria, Australia (38°6′56.32″ S; 145°10′12.33″ E). Troughs were aerated continuously during culture and were not covered with additional shade cloth.

After 11 to 13 wk of culture, 76 of the most uniform potted plants of each species were selected from the 1,200-L troughs and one pot of each species was placed into each of 76 individual tubs (100-L capacity polyethylene tubs, filled to 80 L with municipal water: water depth 38 cm) that were adjacent to one another in the shade house. Prior to any herbicide application (after 15 wk plant culture during autumn) plants had increased in size substantially and were healthy (Table 2).

In mid-June 2015, herbicide treatments (excluding the baseline plants) were randomly assigned within blocks (blocked by position within the shade house) and applied to the 100-L dosing tubs. Herbicide was applied by pouring the required volume of endothall dimethylalkylamine salt stock solution into each tub, based on the measured tub volume (80 L).[2] After the designated exposure time within each dosing tub, all the pots in each tub were removed and immediately immersed in a trough of clean water to rinse off residual herbicide (one 1200-L rinse trough was used for each herbicide concentration) and then transferred to 72 recovery tubs (100 L) that contained clean water. All plants remained in their associated recovery tub (one pot of each species per 100-L tub) for 8 wk after their exposure period (8 WAT).

Plant response to herbicide. A destructive harvest of all aboveground plant material occurred at 8 wk after the associated exposure periods (1, 7, and 21 d). Eight weeks was chosen because, at that time, most damaged plant material had decayed and new growth was initiating on some plants. Plant material was harvested from each pot, and washed and dried at 75 C until constant dry weight was achieved. Plants from the baseline control were harvested prior to herbicide application.

Water sampling. To verify endothall concentration in the dosing tubs, water samples (60 ml) were collected prior to plants being transferred into the recovery tubs at 1, 7, and 21 d after herbicide application for each of the 21-d exposure dosing tubs. Water samples, collected from recovery tubs, verified that they were not contaminated with residual herbicide. Two drops of 20% v/v hydrochloric acid were added to each sample to prevent microbial growth and the samples placed into a refrigerator for preservation. Water samples were evaluated with the use of an enzyme-linked immunosorbent essay (ELISA[3]), for the 1-DAT samples. Liquid chromatography–mass spectrometry (LC-MS) subsequently confirmed the 1-DAT ELISA results. The 7- and 21-d exposure samples were analyzed by LC-MS to determine endothall decay over the exposure period. Chromatographic separation of endothall was achieved with the use of an Eclipse XDB-C8 column (150 × 2.1 mm, 3.5 µm, Agilent Technologies) on a Vanquish UPLC system (Thermo Scientific). The mobile phase was composed of 0.5% formic acid (A) and acetonitrile containing 0.1% of formic acid (B). The flow rate was 0.25 ml/min with a gradient elution of 5 to 60% B over 10 min. The injection volume was 5 µl. Endothall was detected with the use of LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific) operated in electrospray ionization (ESI) negative Fourier-transform mode. The heated capillary was maintained at 350 C with a source heater temperature of 300 C and the sheath, auxiliary, and sweep gases were at 40, 15, and 5 units, respectively. The source voltage was set to 3.2 kV and the resolution was set to 60,000. Endothall was quantified with the use of an external calibration curve.

Water temperature was monitored over the trial period with the use of temperature loggers[4] that were placed randomly into two troughs (during the culture period) and tubs (during herbicide exposure and recovery). Water temperature during the exposure period averaged 9.5 C (SD 2.1) within dosing tubs.

Statistical analyses. Each species was analyzed separately. Biomass was analyzed with the use of a randomized block analysis of variance with treatment structure presented in Table 3. The treatment structure used in the analyses of variance differed from a standard factorial as it added a baseline control (plants harvested prior to herbicide application), to emphasize the result that there was no effect of number of days in dosing tubs or actual date of harvest for the controls. Biomasses of *V. australis*, *P. sulcatus*, and *S. platyphylla* were logarithmically transformed, prior to statistical analysis, so that the residual variation did not change appreciably as the mean increased.

Response curves were calculated with the use of a linear response to the logarithm of the achieved herbicide concentration (obtained by ELISA at 1 d after herbicide application; Table 1) for each exposure time when herbicide

Table 3. *P* values of analyses of variance for examining endothall (dimethylalkylamine salt) concentration and exposure time effects on final biomass in the winter mesocosm experiment. All *P* values are based on *F* tests with 54 residual degrees of freedom. Statistically significant effects ($P < 0.05$) are shown in bold. Effects used in the predicted responses of Figure 1 are indicated.

Treatment Effects	Numerator Degrees of Freedom	*V. australis* (log transformed)	*P. sulcatus* (log transformed)	*E. canadensis*	*S. platyphylla* (log transformed)	*M. papillosum*
No herbicide vs. herbicide applied (i.e., 0 concentration vs. concentrations > 0)[1]	1	**1.4×10^{-7}**	**0.000037**	**0.0081**	**9.7×10^{-6}**	**0.012**
Days to harvest when no herbicide applied (baseline, 1, 7, or 21 d exposure)	3	0.41	0.76	0.46	0.53	0.83
Herbicide concentration when herbicide applied	4	**6.6×10^{-17}**	0.32	0.10	**2.4×10^{-9}**	**6.6×10^{-6}**
Linear response to logarithm of achieved concentration[1,2]	1	**3.0×10^{-18}**	0.051	**0.013**	**5.3×10^{-11}**	**4.6×10^{-7}**
Any further effect of herbicide concentration	3	**0.0014**	0.84	0.70	0.26	0.16
Exposure time when herbicide applied[1]	2	**0.0032**	**0.000093**	0.15	**0.000014**	**0.030**
Interaction of herbicide concentration and exposure time when herbicide applied	8	**0.0060**	0.79	0.63	0.13	**0.038**
Linear response to logarithm of achieved concentration differs with exposure time[1]	2	**0.00050**	0.22	0.15	0.11	**0.0069**
Any further herbicide concentration by exposure time interaction	6	0.31	0.95	0.89	0.21	0.33

[1]Component effects used in the predicted response of Figure 1 and Table 5.
[2]A single value of achieved concentration is used for each target concentration.

was applied and a single value (ignoring exposure time) when no herbicide was applied. Most of the other treatment effects (not part of these response curves) were not statistically significant ($P > 0.05$). An exception was the treatment effect "Any further effect of achieved concentration" with *V. australis* (Table 3). Even with this exception, the deviation from the response curve could be considered relatively small and the response curves can be considered to provide a reasonable summary of the treatment effects present in the experiment. The component effects of the analyses of variance that were used in calculating these response curves are indicated in Table 3. Response curves were exponentially backtransformed to the original measurement scale and graphed. Exponentially backtransformed means (geometric means) of every treatment combination are also presented.

The statistical analyses were calculated with the use of the ANOVA and PREDICT directives, and the FITINDIVIDUALLY, and ORTHPOLYNOMIAL procedures in GenStat 16 (Payne 2013). The ORTHPOLYNOMIAL procedure was used to calculate the contrast for the linear response to the logarithm of the achieved concentration when herbicide was applied, which was then used to construct the effect for the linear response to the logarithm of achieved concentration in the analyses of variance.

Experiment 2: Field experiment in irrigation channels

To verify the effectiveness of endothall dimethylalkylamine salt on *V. australis*, *S. platyphylla*, and *M. papillosum* (three out of the five species used in the mesocosm experiment), three trials were conducted (one for each species; Table 4). Each trial consisted of separate irrigation channel pools that were divided by flow gates or the construction of earthen bund walls with the use of an excavator. The *V. australis* and *M. papillosum* trials consisted of three pools receiving a single herbicide treatment and one reference pool receiving no herbicide. The *S. platyphylla* trial consisted of three irrigation channels each with paired pools, with one reference pool and one herbicide treatment pool per channel. At all sites, reference pools (no herbicide) were upstream of pools receiving herbicide. Herbicide concentrations and exposure times (Table 4) were chosen to be similar to the concentration and exposure times that achieved effective control in experiment one.

Herbicide application and water sampling. Endothall dimethylalkylamine salt was applied to the treatment pools, filled with standing water, in winter 2014. The volume of each pool was calculated and then an appropriate volume of herbicide was diluted with water and applied to the water surface with the use of a truck-mounted boom sprayer (*V. australis* and *M. papillosum* trials) with nozzle output

Table 4. Details of winter 2014 endothall (dimethylalkylamine salt) field experiments against aquatic weeds in irrigation channels in temperate Australia. All channels are 0.5–1-m depth. All herbicide treatments occurred in July 2014.[1]

Species	Site Description	Herbicide Concentration	Exposure Time[2]	Water Temperature	Site Experimental Design
Vallisneria australis	One irrigation channel: Boort, Vic 7,000 m (L) by 10 m (W) (36°9′45.61″ S; 143°50′25.73″ E)	Initial 3.4 mg ae L^{-1}; average over exposure period 3 mg ae L^{-1}	31 d	8.75 C	One reference pool and three downstream treatment pools, divided by gravity fed flow gates.
Sagittaria platyphylla	Three irrigation channels: 1. Katunga, Vic 780 m (L) by 10 m (W) (35°56′51.87″ S;145°24′22.09″ E) Cobram, Vic 800 m (L) by 10 m (W) (35°56′17.35″ S;145°37′37.34″ E) Strathmerton, Vic 800 m by 10 m (W) (35°53′57.57″ S;145°28′51.92″ E)	Initial ~5.0 mg ae L^{-1}; average over exposure period ~4.0 mg ae L^{-1}	20–28 d	~9.3 C	Three reference pools and three endothall treated pools. Reference and endothall pools paired in same channel, divided by flow gates.
Myriophyllum papillosum	One irrigation channel: Boort, Vic 600 m (L) by 8 m (W) (35°59′48.32″ S; 143°56′11.41″ E)	Initial 5.4 mg ae L^{-1}; average over exposure period 4.3 mg ae L^{-1}	29 d	<10 C	One reference pool and three downstream herbicide pools in the same channel; bund walls constructed between each pool.

[1]Vic = Victoria, Australia; (L) by (W) = length by width.
[2]Endothall exposure time determined by irrigation channel operations, such that when channels were returned to normal operation herbicide-treated water was flushed out of all pools.

calibrated to deliver a set volume commensurate with speed, or a hand gun (*S. platyphylla* trial). Water samples were collected, at approximately weekly intervals post herbicide application, to determine herbicide concentration and degradation. Refer to Experiment 1 for water sample preparation and ELISA method. Endothall concentration in the endothall-treated pools are shown in Table 4.

Plant sampling. Before herbicide was applied, in the *V. australis* and *M. papillosum* trials, six sampling transects were established in each pool, in areas where the target weed was present. These were randomly assigned for biomass to be harvested before herbicide was applied or 19 wk after the exposure time had elapsed (19 WAT), to give three transects for each sampling date. The procedure was similar in the *S. platyphylla* trial, except that three transects were selected per pool, because all transects in the reference pools and herbicide treatment pools were sampled at 19 WAT. On each date, three samples were collected from each of the three sampling transects, to give nine samples per date per pool. Biomass was sampled for each species by removing all vegetative material above the sediment surface. For all species, except *S. platyphylla*, a modified rake sampler (0.102 m^2; Marshall and Lee, 1994) harvested biomass. For *S. platyphylla*, all vegetative material inside a 0.09 m^2 quadrat was harvested. Removed biomass from each sample was weighed wet to determine wet weight biomass.

Statistical analyses. Before herbicide application in the *V. australis* and *M. papillosum* trials, average biomass was calculated for each transect in each pool to verify variability between pools. The distribution of biomass, prior to herbicide application, between samples in the untreated reference pools for *V. australis* and *M. papillosum* averaged 792 g (SD 309, $n = 9$) and 670 g (SD 317, $n = 9$), respectively. This was similar to the distribution of biomass between transects in the three endothall treated pools of the corresponding trial. For *V. australis* and *M. papillosum* biomass averaged 813 g (SD 395, $n = 27$) and 856 g (SD 705, $n = 27$), respectively. This provides some confidence that placing the untreated reference pools upstream of the herbicide pools, which was necessary to prevent herbicide contamination, did not unduly create bias from systematic differences in initial biomass.

At 19 WAT, average biomass was calculated for each pool in each trial. For the *V. australis* and *M. papillosum* trials plant biomass in endothall treatments was compared to the biomass in the untreated reference pool using an unequal replication *t*-test on the logarithm (base 10) of the pool averages, with pool being the unit of analysis. For *S. platyphylla*, plant biomass in the endothall treatment was compared to that from the untreated reference pools using a paired comparison *t*-test on the logarithm (base 10) of the pool averages, with pool being the unit of analysis. All analyses had 2 degrees of freedom. Results are presented as the percent decline in biomass with endothall treatment, as compared to the untreated reference pools, by calculating estimates of treatment difference on the logarithmic scale and backtransforming with the use of a $100 \times (1 - 10^{y})$ transformation. Similarly 95% confidence limits are calculated on the logarithmic scale, and then back transforming the limits using a $100 \times (1 - 10^{y})$ transformation.

RESULTS AND DISCUSSION

Experiment 1: Mesocosm experiment

The lack of any statistically significant effect of "Days to harvest when no herbicide applied (baseline, 1, 7, or 21 d exposure)" (Table 3) indicates that, when no herbicide is applied, the exposure time, date of harvesting, and whether plants were placed into dosing tubs at all (baseline control) had little effect on the result. This is evidence that the necessary experimental differences in exposure time and harvesting date for different herbicide treatments contributed negligible bias to the results.

With the exception of *V. australis*, when herbicide was applied (0.45 to 5.15 mg ae L^{-1}) the response for each species and exposure time could be explained as a linear response to the logarithm of achieved concentration (Table 3). This indicates that, under the conditions of this experiment, biomass changes with endothall concentration and exposure time are predictable. For *V. australis* the response curves underpredicted the biomass remaining at an achieved endothall concentration of 2.0 mg ae L^{-1} (Figure 1). This appeared to be associated with a relatively high threshold concentration of endothall being required to reduce biomass (between 2 and 3 mg ae L^{-1}) compared to no herbicide being applied. For *P. sulcatus* there appeared to be a large reduction in biomass compared to no herbicide, associated with the lowest endothall concentration of 0.45 mg ae L^{-1}, with little change in biomass at higher concentrations, compared to the lower concentrations. In other species there appeared to be little difference in biomass between 0 and 0.45 mg ae L^{-1} (Figure 1). These observations indicate that threshold concentrations of endothall exist before any appreciable reduction in biomass is achieved and these thresholds differ between species.

Endothall dimethylalkylamine salt, applied above 0.45 mg ae L^{-1}, reduced biomass of all species relative to the untreated reference, with generally greater biomass reduction at higher endothall concentration and longer exposure times (Figure 1). Maximum biomass reductions of 60 to 70% were achieved for all species, except *P. sulcatus* and *E. canadensis* (~55%).

The importance of concentration and exposure time varied according to species. *P. sulcatus* was affected only by endothall exposure time (above 0.45 mg ae L^{-1}), requiring 21 d for notable biomass reduction. In contrast, *E. canadensis* was mainly affected by endothall concentration (Table 3; Figure 1). *V. australis*, *S. platyphylla*, and *M. papillosum* biomass were affected by both concentration and exposure time, with 7 d exposure time required for effective control of *V. australis*. For *S. platyphylla* and *M. papillosum*, 21 d exposure time provided the greatest level of control (Table 3, Figure 1).

The endothall concentrations at which 50% biomass reduction (EC_{50}) was achieved for each species and exposure time are presented in Table 5, along with the equations describing the relationships between endothall concentration and biomass. Aquatic plant responses to endothall are known to be species specific, with a wide range of sensitivities reported for endothall dipotassium salt (Skogerboe and Getsinger 2001, Skogerboe and Getsinger 2002); however, limited data are available for endothall dimethylalkylamine salt (Slade et al. 2008). The range of EC_{50} values reported here provide further evidence of species selectivity for endothall dimethylalkylamine salt, along with the difference in threshold concentrations discussed above.

Experiment 2: Field experiment in irrigation channels

Aquatic weed control was achieved in the field with estimated biomass reductions of 46%, 91%, and 98% at 19 WAT, for *V. australis*, *S. platyphylla,* and *M. papillosum*, respectively ($P = 0.07$, 0.05, 0.007; Table 6). Anecdotal observations, from the water authority operating the irrigation system, indicated that control of *V. australis* and *M. papillosum* lasted 2 yr, which restored channel function over this period. In contrast, *S. platyphylla* control lasted approximately 6 mo (R. Talbot, Goulburn-Murray Water, pers. comm.). Previously, excellent *S. platyphylla* control (for a similar duration) was achieved in winter field trials (average water temperature 10.9 C) with endothall dimethylalkylamine salt and dipotassium salt of endothall at rates of 6 to 7 mg ae L^{-1} and 80 d exposure time (Clements et al. 2013). Together this information provides evidence that endothall dimethylalkylamine salt applied to static irrigation channels ponded during winter will provide a useful tool to reduce biomass of a range of aquatic weeds in irrigation channels in temperate Australia, when high concentrations and exposure times are used.

Overall, these results indicate that winter-applied endothall dimethylalkylamine salt is reasonably effective in reducing biomass of aquatic weeds that infest irrigation channels, 19 WAT. The plant biomass reductions achieved increased water delivery efficiency for up to 2 yr and therefore provided a mechanism for irrigation authorities to restore function of irrigation channels.

Implications. Despite the mesocosm and field experiments being conducted during winter in cool conditions, which is generally associated with poor endothall efficacy likely because of low metabolic activity in the target plants (Netherland et al. 2000, Sprecher et al. 2002), effective aquatic weed control was achieved when concentration and exposure time were both at relatively high levels. For example, 55 to 70% biomass reduction was achieved for all aquatic weed species tested at 5.2 mg ae L^{-1} and 21 d exposure time. The extended exposure time achieved is key to obtaining effective control during cold-water winter conditions. The exposure times tested are much longer than the several hours exposure time typically considered necessary for endothall to provide effective control against aquatic weeds, when plants are actively growing in warmer water (Netherland et al. 2000, Sprecher et al. 2002, Dugdale et al. 2012, Mudge et al. 2015). We suggest that achieving a long exposure time can compensate for the reduced plant metabolic activity during winter and enables effective control and a new use pattern for endothall.

To our knowledge there are no other studies that report on CET relationships for the dimethylalkylamine salt formulation of endothall, during winter, on *V. australis*, *P. sulcatus*, *E. canadensis*, *S. platyphylla*, or *M. papillosum*, so we

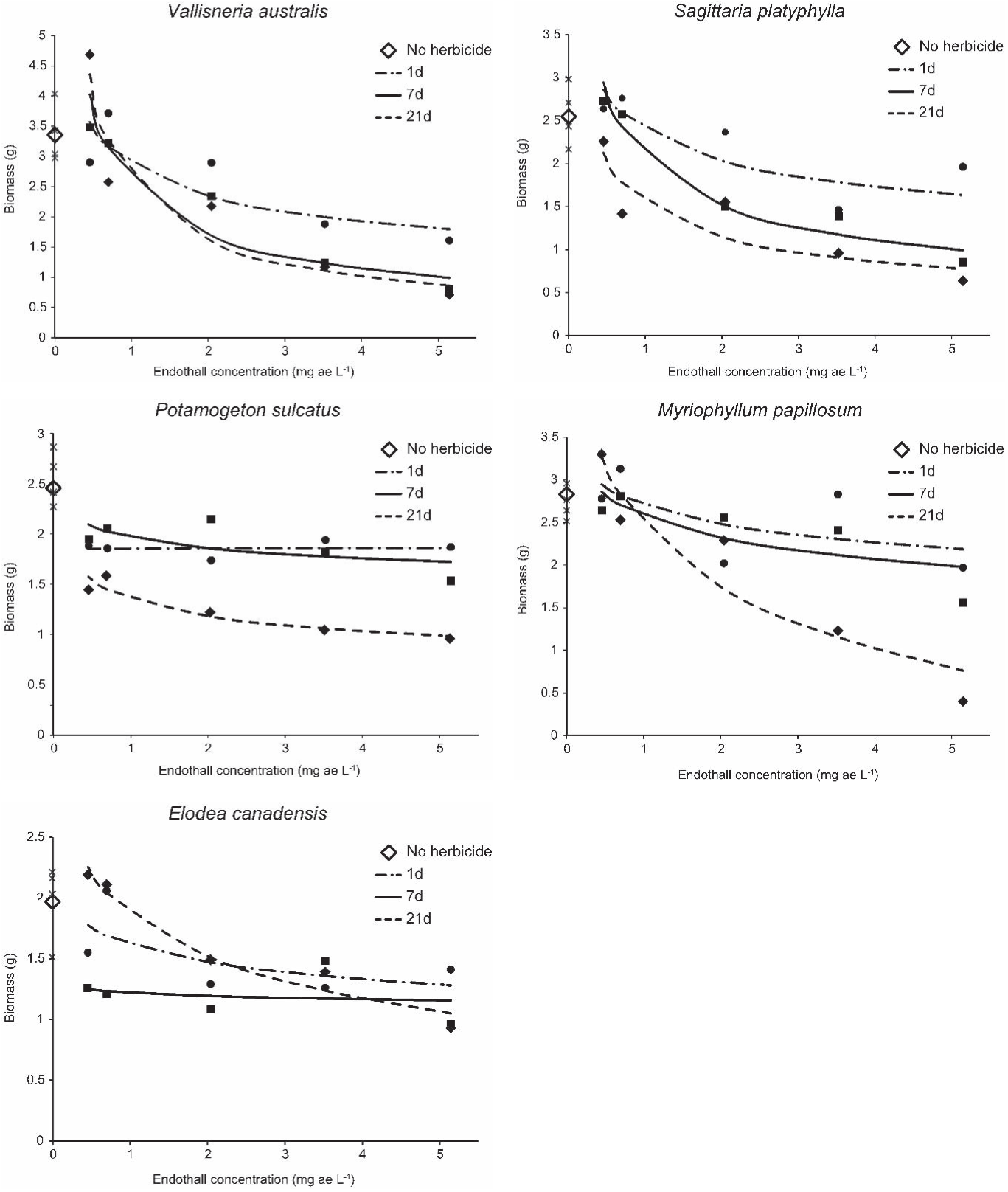

Figure 1. Effect of endothall (dimethylalkylamine salt) concentration on biomass (g dry weight) for each aquatic weed species, 8 wk after herbicide treatment, in the winter mesocosm experiment for 1-, 7-, and 21-d exposure time. Each solid symbol represents the geometric mean of four pots with the same combination of herbicide concentration and exposure time (crosses, circles, squares, and diamonds = 0-, 1-, 7-, and 21-d exposure times, respectively). The open symbol (diamond) represents the geometric mean of baseline (preherbicide application) and untreated reference pots, not exposed to herbicide ($n = 16$ pots; "no herbicide"). Lines are response curves from Table 5.

Table 5. Equations relating biomass (BM, in g/pot) to achieved endothall (dimethylalkylamine salt) concentration (conc, in mg ae L^{-1}) and exposure time in the winter mesocosm experiment. These equations describe the curves in Figure 1. SE = standard error calculated using the residual mean square value from the ANOVA (Table 3) as an estimate of residual variance. EC_{50} = effective concentration for 50% biomass reduction, 8 wk after herbicide treatment. Note: equations are valid within the concentration range of 0.45 to 5.15 mg ae L^{-1}.

	Equation		
Species	1 d Exposure	7 d Exposure	21 d Exposure
Vallisneria australis			
Equation	$Log_{10}(BM) = 0.46 - 0.28$ (SE = 0.068) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.55 - 0.58$ (SE = 0.068) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.41 - 0.67$ (SE = 0.068) $\times \log_{10}(conc)$
BM no herbicide[1]	3.44	3.44	3.44
Calculated EC_{50}	–[2]	2.1	1.9
Potamogeton sulcatus			
Equation	$Log_{10}(BM) = 0.29 + 0.00$ (SE = 0.144) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.29 - 0.08$ (SE = 0.144) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.13 - 0.19$ (SE = 0.144) $\times \log_{10}(conc)$
BM no herbicide	2.47	2.47	2.47
Calculated EC_{50}	–[2]	–[2]	1.5
Elodea canadensis			
Equation	$BM = 1.57 - 0.47$ (SE = 0.382) $\times \log_{10}(conc)$	$BM = 1.17 - 0.09$ (SE = 0.382) $\times \log_{10}(conc)$	$BM = 1.82 - 1.15$ (SE = 0.382) $\times \log_{10}(conc)$
BM no herbicide	1.98	1.98	1.98
Calculated EC_{50}	–[2]	–[2]	5.3
Sagittaria platyphylla			
Equation	$Log_{10}(BM) = 0.38 - 0.23$ (SE = 0.078) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.32 - 0.45$ (SE = 0.078) $\times \log_{10}(conc)$	$Log_{10}(BM) = 0.19 - 0.42$ (SE = 0.078) $\times \log_{10}(conc)$
BM no herbicide	2.55	2.55	2.55
Calculated EC_{50}	–[2]	2.9	1.6
Myriophyllum papillosum			
Equation	$BM = 2.70 - 0.72$ (SE = 0.398) $\times \log_{10}(conc)$	$BM = 2.58 - 0.84$ (SE = 0.398) $\times \log_{10}(conc)$	$BM = 2.46 - 2.39$ (SE = 0.398) $\times \log_{10}(conc)$
BM no herbicide	2.87	2.87	2.87
Calculated EC_{50}	–[2]	–[2]	2.8

[1]BM no herbicide = mean of four treatments not exposed to endothall, including untreated reference pots (1, 7, and 21 d exposure) plus a baseline control (preherbicide application).
[2]Estimated EC_{50} beyond the bounds of the data (>6 mg ae L^{-1}).

cannot otherwise determine their susceptibility to the herbicide. However, there is screening trial data on endothall dimethylalkylamine salt against *V. australis*, *P. sulcatus*, and *S. platyphylla* (Dugdale et al. 2012) and full CET relationships for elodea (Mudge et al. 2015) all in summer conditions, where effective control of all species was achieved with 6 h exposure time at 2 to 5 mg L^{-1}, in contrast to the 7 to 21 d required in the current study.

Typically, endothall dissipates from water, usually within 1 to 4 d for dipotassium salt and within 21 d for dimethylalkylamine salt (Sprecher et al. 2002). However, endothall residue monitoring during our mesocosm experiment showed that there was very minimal decay over the 21-d exposure period, at any concentration (Table 1). Further, our field trials showed that there was also minimal decay over the 1 to 31–d exposure periods achieved (Table 4). Because endothall dimethylalkylamine salt was applied to the entire volume of each tub (mesocosm experiment) or closed irrigation channel pool (field trials), there was no dissipation or dilution from water inflow. Together these two factors resulted in long effective exposure times. Previously, long exposure of endothall has been achieved during cold water conditions (Wells and Champion 2010, Clements et al. 2013, Clements et al. 2015), and we attribute this to the low temperatures inhibiting microbial activity, which is the primary driver of endothall decay (Reinert et al. 1986).

Endothall dimethylalkylamine salt was chosen for these experiments because it is more effective on a wider range of weed species than the endothall dipotassium salt. In the

Table 6. Biomass reduction achieved 19 wk after endothall (dimethylalkylamine salt) exposure for each aquatic weed species in pooled irrigation channels in winter (8–10 C). Statistically significant effects ($P < 0.05$) are shown in bold. Note that negative declines are percentage increase.

				Biomass Reduction (%; relative to untreated reference pools)		
Species	Endothall Application Concentration[1] (mg ae L^{-1})	Exposure Period (d)	*P* Value	Estimated Reduction	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Vallisneria australis	3.4	31	0.066	46.3	−10.5	73.9
Sagittaria platyphylla	5.0	20–28	0.050	90.7	−1.2	99.1
Myriophyllum papillosum	5.4	29	**0.0073**	97.9	91.1	99.5

[1]Endothall dimethylalkylamine salt concentration evaluated with the use of an enzyme-linked immunosorbent assay.

United States, endothall dimethylalkylamine salt is used to control submerged macrophytes and algae and is more effective and faster acting than endothall dipotassium salt (Sprecher et al. 2002, MacDonald et al. 2003, Slade et al. 2008). It would be desirable to utilize the endothall dipotassium salt formulation during winter, which is 200 to 400 times less toxic to fish than the endothall dimethylalkylamine salt formulation and has very low toxicity to aquatic vertebrates (Keckemet 1969, Sprecher et al. 2002). A previous winter field trial has been conducted with the endothall dipotassium salt formulation (Clements et al. 2015) and determined the dose–response relationship for sagittaria in an irrigation channel. That trial showed that at 5 mg ae L^{-1} for a 3-wk exposure period, 69% biomass reduction could be achieved, 6 WAT. This is very similar to the 70% biomass reduction (8 WAT) obtained in the current dose–response trial for sagittaria exposed to endothall dimethylalkylamine salt during winter at 4.8 mg ae L^{-1} for 21 d.

In summary, effective control of aquatic weeds during winter (~10 C water temperature) with the use of endothall dimethylalkylamine salt requires long herbicide exposure (7 to 21 d) and high concentrations (2.4 to 4.8 mg ae L^{-1}). These results demonstrate a new use pattern for endothall dimethylalkylamine salt, where achieving long exposure times in static irrigation channels ponded during winter conditions provides effective control of aquatic weeds.

SOURCES OF MATERIALS

[1]Teton®, United Phosphorus Inc., 630 Freedom Business Center Drive, King of Prussia, PA 19406.

[2]Gardena flow meter®, Art. 8188-20, Ulm, Germany.

[3]RaPID Assay® Endothall Test Kit, Strategic Diagnostics Inc., 128 Sandy Drive, Newark, DE 19713.

[4]HOBO U20® Water Level Data Logger, Onset Computer Corporation, 470 MacArthur Boulevard, Bourne, MA 02532.

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