

Evaluation of florpyrauxifen-benzyl to control three problematic submersed macrophytes in New Zealand

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INTRODUCTION

Exotic aquatic plant invasions continually threaten New Zealand's freshwaters, posing substantial ecologic, economic, and recreational risk. Of particular concern are invasive submersed macrophytes, which commonly develop as high biomass yielding monocultures (Wells et al. 1997, Hofstra et al. 2018). Once established, these aggressive submersed weeds have the potential to obstruct waterways, which can adversely affect water supply, power generation, and ecosystem services (Carpenter and Lodge 1986, Clayton 1996). The unique and diverse aquatic environments endemic to New Zealand require water resource managers to regularly deploy control tactics to combat submersed plant incursions.

Dozens of exotic macrophytes are documented in New Zealand (Champion et al. 2002, Ministry for Primary Industries 2020), though the submersed plants *Ceratophyllum demersum* L. (dicot), *Lagarosiphon major* (Ridley) Moss (monocot), and *Egeria densa* Planch. (monocot) remain the most historically problematic due to broad-invasion potential and dense growth form habits (Clayton and Champion 2006, Hofstra et al. 2018). Among invaded systems, populations of *C. demersum*, *L. major*, and *E. densa* are regularly monitored (e.g., LakeSPI; Clayton and Edwards 2006) and targeted for control using combinations of physical, biological, and chemical management approaches (Hussner et al. 2017). Mechanical control techniques can be successful when plant fragmentation and regeneration is of minimal concern (Clayton 1996, Redekop et al. 2016). Likewise, grass carp (*Ctenopharyngodon idella*) have been effective for invasive plant control (Champion 2018) but are nonselective grazers, which may cause concern for native submersed plant foraging. Additionally, the effectiveness of grass carp is constrained by the waterbody characteristics (e.g., the ability to contain the grass carp) (Hofstra et al. 2014). For targeted removal programs, large-scale plant suppression or eradication often succeeds when chemical control is deployed due to invasive plant morphology and costs

associated with problematic species (Howard-Williams et al. 1987, Bickel 2012, de Winton et al. 2013). New Zealand currently has just two herbicides registered for aquatic site applications, diquat dibromide (photosystem I inhibitor) and endothall dipotassium salt (protein phosphatase inhibitor). While both herbicides readily control *C. demersum* and *L. major* (Wells and Champion 2010, Wells et al. 2014), only diquat has shown effective control of *E. densa* (Hofstra and Clayton 2001, Skogerboe et al. 2006).

A limited aquatic herbicide portfolio narrows management opportunity and increases selection pressure among target plant populations. Richardson (2008) noted the implications of resistance management in aquatic weed control scenarios and how repetitive use patterns, or herbicide modes of action (MOA), can select for resistant populations among invasive plant biotypes (e.g., fluridone-resistant *Hydrilla verticillata* (L.f.) Royle in Florida; Michel et al. 2004). Other functional constraints, like potential irrigation and swimming restrictions or selectivity to native plants following herbicide application, can further limit or eliminate management opportunities when only two primary herbicides may be considered (Champion et al. 2019). Thus, there remains a need to evaluate additional MOA to expand management options for invasive aquatic plants and enhance stewardship of the current herbicides in New Zealand.

The recent development of a unique auxin-mimic compound, florpyrauxifen-benzyl (arylpicolinate subclass), in the United States provides water resource managers with an additional herbicide. Florpyrauxifen-benzyl has been classified as a reduced risk herbicide by the United States Environmental Protection Agency (U.S. EPA) and is registered in aquatic systems to target invasive weed species like *H. verticillata* and *Myriophyllum spicatum* L. (U.S. EPA 2017). Additionally, florpyrauxifen-benzyl is suspected to degrade primarily via aqueous photolysis under aquatic field dissipation scenarios (Meléndez et al. 2017). While monocots typically exhibit lower sensitivity than dicots to auxin-mimic herbicides (e.g., 2, 4-D and triclopyr), previous research demonstrated potential use for controlling several invasive macrophyte species including problematic submersed monocots (Richardson et al. 2016, Haug 2018). Literature also reports florpyrauxifen-benzyl effectively reduces total biomass of several Hydrocharitaceae and *Myriophyllum* spp. at lower use rates (e.g., <3 to 50 $\mu\text{g L}^{-1}$) than the existing herbicides available in New Zealand (Netherland and Richardson 2016, Richardson et al. 2016). A novel systemic herbicide chemistry with rapid plant

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absorption (Haug et al. 2021), like florpyrauxifen-benzyl, could provide an additional tool that water resource managers deploy to combat invasive submersed species in New Zealand. However, there are no published control data on the problem species *C. demersum*, *L. major*, or *E. densa* using florpyrauxifen-benzyl.

The objective of this study was to demonstrate an additional MOA for potential operational rates of florpyrauxifen-benzyl (30 and 50 $\mu\text{g L}^{-1}$) to control *C. demersum*, *L. major*, and *E. densa*, which have high biosecurity concern in New Zealand. Previous experiments suggest rapid herbicide activity and symptomology among sensitive plant species (e.g., *H. verticillata* and *Myriophyllum spicatum* L.). Therefore, target herbicide concentrations selected for this study used previous florpyrauxifen-benzyl dose response metrics as reference for Hydrocharitaceae (i.e., *L. major* and *E. densa*) control in this study (Netherland and Richardson 2016, Richardson et al. 2016). This experiment occurred simultaneously with the product label registration of florpyrauxifen-benzyl in the United States.

MATERIALS AND METHODS

Experiments occurred at the National Institute of Water and Atmospheric Research (NIWA) Ruakura, North Island, New Zealand mesocosm facility. Plants were field collected from Lake Karāpiro (*C. demersum* and *E. densa*) and Lake Rotoiti (*L. major*), then cultured in outdoor tanks filled with dechlorinated water. Individual *C. demersum*, *L. major*, and *E. densa* plants were established using 10 cm apical shoots placed in 300 ml pots filled with topsoil (72% dry matter; 1.3 g phosphorus and 0.13 g nitrogen kg^{-1} dry weight soil) and covered with washed river sand (~ 1 cm). Respective pots ($n = 14$ pots of individual species per tank) were then placed in species-specific 176 L treatment tanks to provide a minimum 8 wk of development prior to treatment. To minimize algae, help regulate water temperature, and reduce bleaching of surface biomass, 50% shade cloth was placed over each tank at establishment. Each tank remained at a consistent water level via addition of freshwater and contained one aeration stone (6 to 10 L min^{-1}) to suppress algae accumulation over the 8-wk study period.

Plants were treated 16 January 2018 with a 300 g L^{-1} suspension concentrate formulation (SLF-9522) of florpyrauxifen-benzyl¹ to target 30 or 50 $\mu\text{g L}^{-1}$ as in-water applications using a dilute solution of product, mixed with municipal-water as carrier, to achieve target treatment dose among each experimental unit. The 50 $\mu\text{g L}^{-1}$ florpyrauxifen-benzyl treatment closely reflects the maximum active ingredient (a.i.) concentration rate for in-water applications in a single application in the United States. Herbicide treatments (30 or 50 $\mu\text{g L}^{-1}$) were applied independently among each species treatment tank and replicated six times. Six untreated control tanks were also included per macrophyte species. At treatment, one untreated experimental unit representing each species was preharvested to obtain baseline shoot and root biomass (g dry weight) to evaluate untreated plant growth over the evaluation period. Water temperature averaged 21.7 C and 8.3 pH across tanks at treatment.

Throughout the 8-wk static exposure, observations of plant appearance (morphologic response to herbicide) and visual percent control estimates compared to untreated controls (0% = no control; 100% = complete necrosis) were recorded weekly. Among respective observation timepoints, photographs of each experimental unit were taken to serve as standards for visual percent control estimates during analysis. Shoot and root biomass from each tank was harvested following the completion of the study and dried at 60 C for 72 h to obtain constant dry mass (g dry weight). Only shoot biomass was collected for *C. demersum* since plants in the Ceratophyllaceae family lack true roots.

Due to rapid shoot terminal abscission (ca. 5 to 7 days after treatment) among *L. major* and *E. densa* to both florpyrauxifen-benzyl rates tested (30 or 50 $\mu\text{g L}^{-1}$), a nested experiment occurred to observe the potential regrowth of abscised shoots. Occurring 4 WAT (weeks after treatment), three 10-cm-long apical shoots from each experimental unit ($n = 18$ shoots treatment^{-1} species^{-1}) were manually cut and weighed (fresh weight), then placed in respective 11 L mesocosms filled with freshwater ($n = 9$ tanks; 3 tanks species^{-1} at the three exposures tested: untreated control and 30 and 50 $\mu\text{g L}^{-1}$ florpyrauxifen-benzyl) for viability monitoring. Mesocosms remained at a constant water level, covered with 50% shade cloth, and provided with aeration during the 4-wk evaluation period. Stem lengths (mean of $n = 3$ randomly selected stems) and visual observations of symptomology were noted at time 0 and 2- and 4-wk evaluations to examine potential plant fragment viability (growth or decline in plant structure) compared to the untreated controls. Shoot biomass and mean three-stem lengths were collected at the end of the observation period to gauge fragment viability or decline based on initial herbicide exposure to individual species response of simulated allofragmentation.

Data were analyzed using RStudio² “base,” “agricolae,” and “dplyr” packages to provide statistical and visual representation of herbicide dose by species response and to examine normality (de Mendiburu 2020, R Core Team 2020, Wickham et al. 2021). Plant dry weights were evaluated using ANOVA (analysis of variance) and separated using Fisher’s protected least significant difference (LSD) test at the $P < 0.05$ significance level. Visual control estimates were subjected to Student’s *t* test to separate treatment means at the $P < 0.05$ significance level. Local polynomial regression models were then utilized to calculate critical control values (CC₈₀: weeks after treatment to reach 80% visual control). The untreated control was not included for visual control analyses since it was a constant value of zero. *Ceratophyllum demersum* CC₈₀ values are not reported since 80% visual control did not occur during the 8-wk study duration.

RESULTS AND DISCUSSION

Both *E. densa* and *L. major* untreated control plants increased in biomass ($\mu = 2.64$ to 4.96 times, respectively) compared with the pretreatment harvest, suggesting conditions for the Hydrocharitaceae were favorable. *Lagarosiphon major* and *E. densa* displayed herbicide symptomology within 24 to 48 h after treatment (HAT), with apical bending and

TABLE 1. SHOOT AND ROOT TREATMENT RESPONSE METRICS FOR THREE PROBLEMATIC SUBMERSED MACROPHYTES FOLLOWING AN 8-WK STATIC EXPOSURE TO FLORPYRAUXIFEN-BENZYL.

Treatment	<i>Egeria densa</i>		<i>Ceratophyllum demersum</i>		<i>Lagarosiphon major</i>	
	Shoot	Root	Shoot	Shoot	Root	
Dry biomass ^{1,2}						
Untreated control	14.08 ± 2.61 (a)	1.87 ± 0.80 (a)	15.24 ± 2.95 (a)	27.38 ± 3.37 (a)	10.6 ± 5.48 (a)	
florpyrauxifen-benzyl 30 µg L ⁻¹	4.85 ± 1.91 (b)	0.41 ± 0.13 (b)	9.45 ± 0.58 (b)	3.10 ± 3.10 (b)	0.01 ± 0.01 (b)	
florpyrauxifen-benzyl 50 µg L ⁻¹	7.25 ± 1.00 (b)	0.74 ± 0.19 (ab)	7.90 ± 1.94 (b)	0.01 ± 0.01 (b)	0.01 ± 0.01 (b)	
% Reduction ^{3,4}						
florpyrauxifen-benzyl 30 µg L ⁻¹	65.60 ± 13.47	78.10 ± 6.70	38.00 ± 3.78	88.70 ± 11.31	99.99 ± 0.07	
florpyrauxifen-benzyl 50 µg L ⁻¹	48.51 ± 7.10	60.60 ± 10.37	48.10 ± 12.70	100.00 ± 0.00	99.99 ± 0.07	

¹Biomass means (g dry weight ± standard error [SE]) within columns with the same letter do not significantly differ according to Fisher's protected least significant difference test ($P < 0.05$).

²Due to *C. demersum* morphology, root biomass metrics are not provided.

³Percent reduction means (1 - treatment mean/untreated control mean) with ± SE depict biomass reduction compared to untreated control group.

⁴No significant difference detected between treatments relative to the untreated control group using Student's *t* test ($P < 0.05$).

node swelling appearing as first symptoms. *Ceratophyllum demersum* untreated control biomass did not increase over the study period ($\mu = 1.03$ times) respective to the pretreatment harvest.

Of the three species tested in the 8-wk study, *L. major* had the greatest sensitivity to florpyrauxifen-benzyl (Table 1; Figure 1). Within 72 HAT, *L. major* proximal leaves were observed abscising from the stem, with regular shoot fragmentation occurring 5 to 7 days after treatment (DAT) among tanks treated with florpyrauxifen-benzyl 30 and 50 µg L⁻¹ rates (Figure 2A). Within 9 DAT, all *L. major* treated plants experienced comparable leaf shattering and stem abscission, internode lengthening, and some stem necrosis, with complete plant canopy collapse occurring within 2 WAT. In general, *L. major* abscised stem fragments floated at the water surface (prompted fragmentation viability inves-

tigation). Significant reduction to *L. major* shoot and root biomass occurred among plants treated with 30 µg L⁻¹ florpyrauxifen-benzyl ($P < 0.05$), with complete necrosis of all plant material when exposed to 50 µg L⁻¹ (Table 1). Within 3 WAT, 50 µg L⁻¹ florpyrauxifen-benzyl achieved CC₈₀ of *L. major*, with CC₈₀ occurring among 30 µg L⁻¹ treated plants 4 WAT. However, there was no difference between the 30 and 50 µg L⁻¹ treatment rates for *L. major* shoot and root reduction (Student's *t* test, $P = 0.364$ and 1, respectively). Following the 4 WAT evaluations, continued necrosis occurred among *L. major* plants, with almost no viable plant material discovered 8 WAT for plants treated at the low rate.

Upon initial observations, *E. densa* did not appear as sensitive to florpyrauxifen-benzyl as *L. major*, though terminal epinasty and internode twisting began within 3

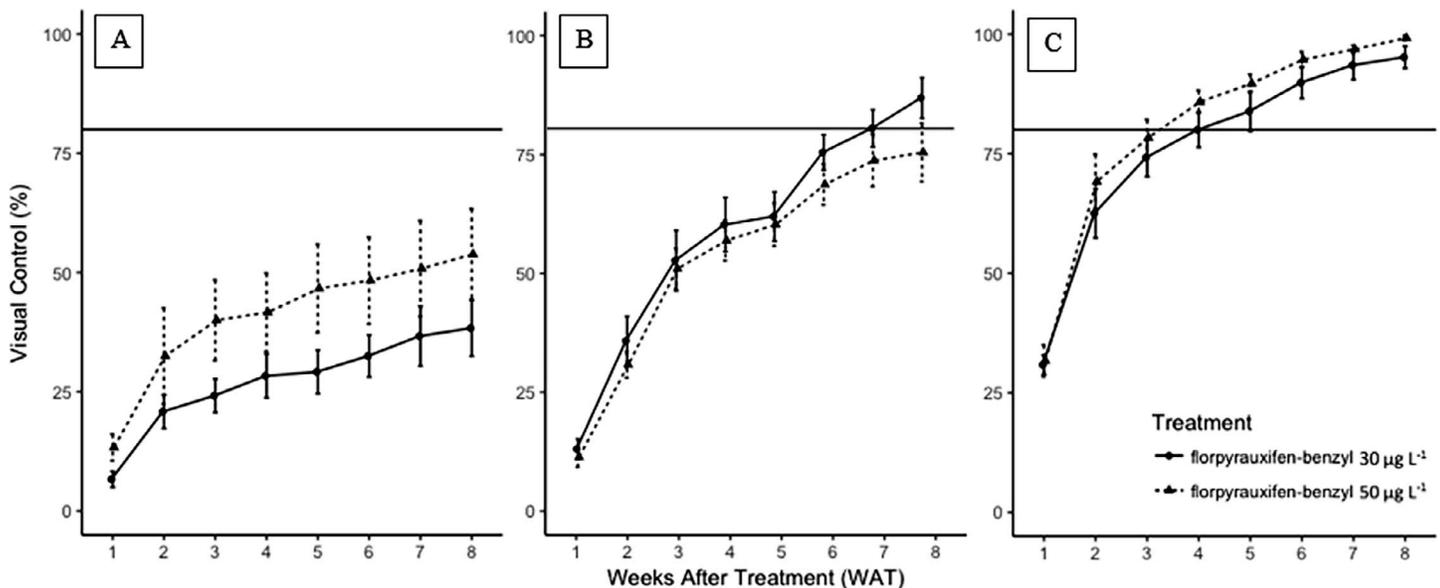


Figure 1. Week after treatment (WAT) visual control estimates of (A) *Ceratophyllum demersum*, (B) *Egeria densa*, and (C) *Lagarosiphon major* treated with two rates of florpyrauxifen-benzyl. Visual control estimates expressed as a percentage control of treated plants compared to untreated control (0% = no control; 100% = complete necrosis). Discrete points with bars represent the mean ± SE; ($n = 6$). Critical control values derived from local polynomial regressions are represented by the horizontal line set to CC₈₀ (weeks after treatment [WAT] to reach 80% control): *C. demersum* = NA, *E. densa* = (30 µg L⁻¹: 7 WAT; 50 µg L⁻¹: NA), and *L. major* = (30 µg L⁻¹: 4 WAT; 50 µg L⁻¹: 3 WAT). No significant difference detected between treatments using Student's *t* test ($P < 0.05$).

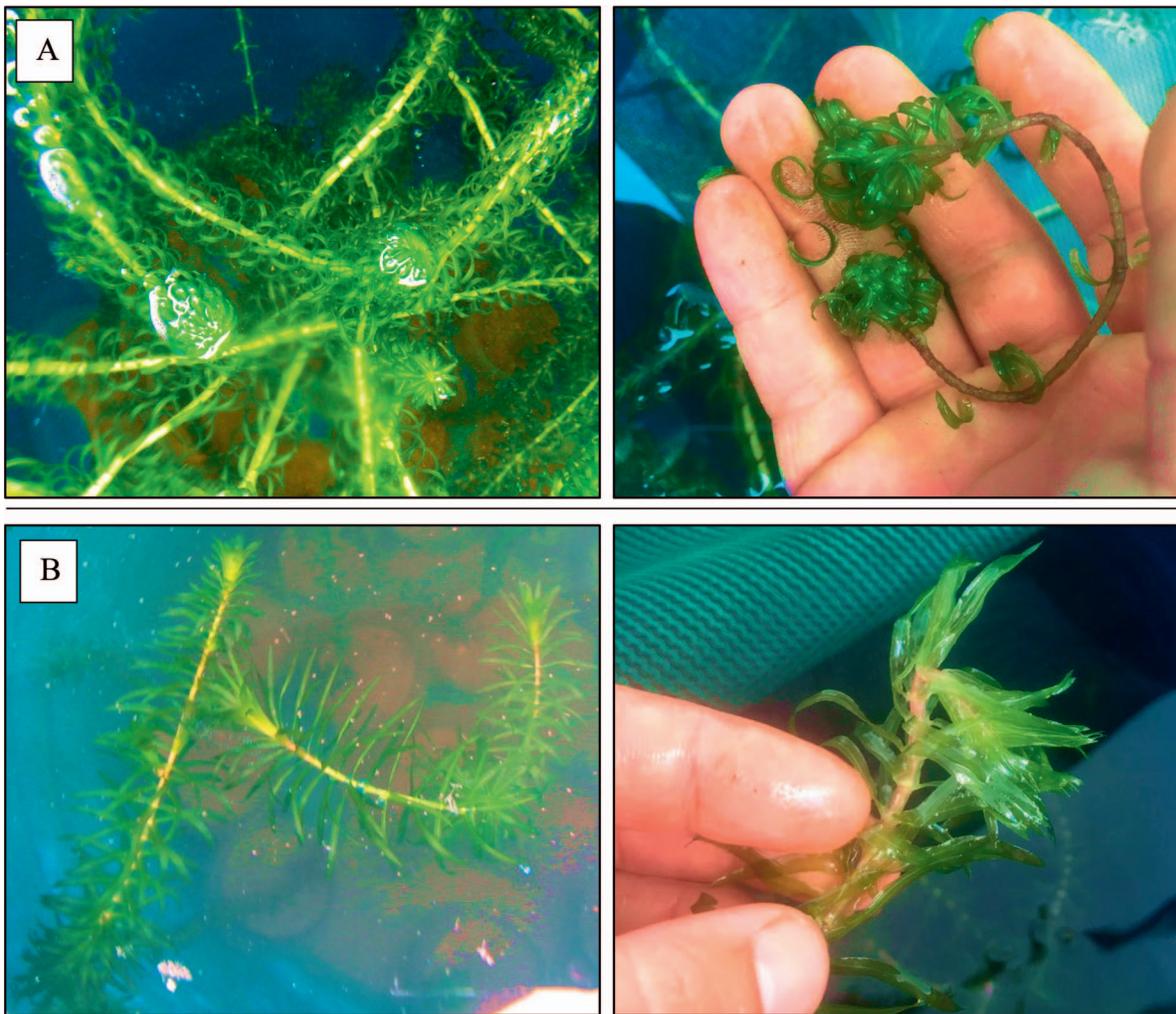


Figure 2. Leaf shattering response of *Lagarosiphon major* (A), and apical epinasty response of *Egeria densa* (B), ca. 7 days after treatment following static florpyrauxifen-benzyl $50 \mu\text{g L}^{-1}$ exposure (A and B; left: untreated control; right: treated tank).

to 5 DAT at both the low and high florpyrauxifen-benzyl rates (Figure 2B). Within 9 DAT, treated *E. densa* plants exhibited epinasty and increased internode length between both treatment rates, with visual observation of nodes turning bright orange/brown at the $50 \mu\text{g L}^{-1}$ rate. *Egeria densa* began showing signs of chlorosis and node expansion 2 WAT at both rates tested. At this time, stems became brittle, and fragmentation was common when the water was agitated. Florpyrauxifen-benzyl did decrease *E. densa* shoot and root biomass ($\mu = 1.9$ to 2.9 times that of the untreated control group) at both rates tested ($P < 0.05$; Table 1). While *E. densa* was not completely controlled 8 WAT, florpyrauxifen-benzyl $30 \mu\text{g L}^{-1}$ treatments did provide CC_{80} 7 WAT (Figure 1). Still, there was no difference among treatment rates in reducing *E. densa* shoot or root biomass (Student's *t* test, $P = 0.417$ and 0.272 , respectively). Variation in herbicide sensitivity between species belonging to the same family can be expected, and our findings are consistent with a previous small-scale study, which noted lower absorption and translocation potential of florpyrauxifen-benzyl for *E. densa* than another Hydrocharitaceae species, *H. verticillata*

(Haug et al. 2017). An interesting phenomenon did occur among *E. densa* $50 \mu\text{g L}^{-1}$ florpyrauxifen-benzyl treatments, as two experimental units exhibited flowering 3 and 6 WAT. Flowering in these tanks may be linked to plant stress induced from the synthetic auxin characteristics of florpyrauxifen-benzyl, as plant growth regulators (i.e., auxin mimic herbicides) are involved in ethylene simulation and signaling pathways that control flower initiation (Bielach et al. 2017). A similar flower stimulation response was reported for the submersed species, *Myriophyllum sibiricum* Komarov, to the auxin mimic herbicide, 2-4,D, when exposed at a $10 \mu\text{g L}^{-1}$ rate (Forsyth et al. 2007). Although some treated *E. densa* biomass remained viable 8 WAT, a longer exposure may have resulted in greater observed control since much of the plant material collected at harvest showed signs of chlorosis and necrosis at the nodes (some stem fragmentation ensued when agitated).

While not as sensitive as the other species tested, *C. demersum* did show signs of chlorosis and terminal epinasty at the florpyrauxifen-benzyl $30 \mu\text{g L}^{-1}$ rate, with increased stem brittleness discovered at the $50 \mu\text{g L}^{-1}$ rate 9 DAT. At

the 2 WAT evaluations, all *C. demersum* tanks treated with florpyrauxifen-benzyl 50 $\mu\text{g L}^{-1}$ experienced canopy collapse, with analogous plant structure decline beginning among the 30 $\mu\text{g L}^{-1}$ plant treatments; this plant canopy response was not observed for the *C. demersum* untreated control tanks. Final collapse of the *C. demersum* plant canopies occurred 5 to 6 WAT for tanks treated with florpyrauxifen-benzyl at 30 $\mu\text{g L}^{-1}$.

Ceratophyllum demersum biomass was reduced at both florpyrauxifen-benzyl rates ($P < 0.05$, Table 1). Plant suppression (collapsed plant canopy) occurred simultaneously for both florpyrauxifen-benzyl 30 and 50 $\mu\text{g L}^{-1}$ rates 4 WAT, although complete control did not occur 8 WAT (Table 1; Figure 1). While *C. demersum* plants treated with 50 $\mu\text{g L}^{-1}$ florpyrauxifen-benzyl had greater biomass reduction than did the 30 $\mu\text{g L}^{-1}$ rates, no significant decrease occurred between rates tested (Student's *t* test, $P = 0.534$). Necrosis was not observed among any *C. demersum* tanks, and CC_{80} was not achieved using either florpyrauxifen-benzyl rate tested.

Redekop et al. (2016) reported the dispersion potential of *E. densa* and *L. major* following simulated allofragmentation in an untreated flowing system and noted stem regeneration four weeks after fragmentation ($n = 11$ to 50% not regenerated). To evaluate the potential viability of abscised (herbicide induced) apical shoots forming new plant populations from the treated plants in this study, we observed individual species response to simulated allofragmentation (10 cm long plant stems) over a 4-wk period (concurrent with 8-wk tank study). All *C. demersum*, *E. densa*, and *L. major* fragments collected from tanks dosed with florpyrauxifen-benzyl were sunken at the time 0 and 2- and 4-wk evaluations, while untreated fragments remained buoyant. Further, no growth (no increased shoot weight or length) occurred for any species tested at the 2- or 4-wk evaluations at either florpyrauxifen-benzyl rate. For *L. major*, complete necrosis occurred (no observed biomass) among the 50 $\mu\text{g L}^{-1}$ treatments at the 4-wk evaluation, while the untreated control shoot length had doubled ($\mu = 2.03$ times) at the end of the observation period. Similarly, *E. densa* displayed no fragment viability compared to the untreated controls, having shoot length reductions ($\mu = 2$ and 3 cm shorter, respectively) at the 2- and 4-wk evaluations (10 cm initial stem length). *Ceratophyllum demersum* stem fragments from the 30 and 50 $\mu\text{g L}^{-1}$ treatments displayed no increase or decrease in stem length at the end of the study period; however, the average stem length within the untreated control group increased 40% (untreated control $\mu = 14$ cm stem length). Likewise, *C. demersum* stems from treated tanks were brittle with twisted internodes at the 4-wk evaluation. Though observational, it is hypothesized induced allofragmentation following an application of florpyrauxifen-benzyl at 30 or 50 $\mu\text{g L}^{-1}$ treatment rates would not likely increase stem fragment viability among the study species. However, operational situations would benefit from future studies comparing herbicide dose response with the potential regrowth of *C. demersum*, *E. densa*, and *L. major* following shoot dislocation of treated plants within hours, or days, after herbicide exposure to test fragment viability.

In conclusion, florpyrauxifen-benzyl has potential for managing these three problematic submersed macrophytes in New Zealand, particularly *L. major*, which was the most sensitive species evaluated. Further, there was no difference between rates tested among any of the species studied (Table 1). Therefore, the low rate (30 $\mu\text{g L}^{-1}$) florpyrauxifen-benzyl showed effectiveness in controlling *L. major* and *E. densa*, providing another chemical control option for *E. densa* management other than the sole chemistry, diquat dibromide (Hofstra and Clayton 2001). While herbicide response at both tested rates did not provide complete control of *C. demersum*, florpyrauxifen-benzyl did reduce the plant canopy stature, which may be applicable when plant canopy suppression is the primary intention. For complete control of *C. demersum*, future research should evaluate combinations of available herbicides in New Zealand with application rates tested in this trial and the influence of water chemistry on florpyrauxifen-benzyl efficacy (e.g., water pH of application site and degradation rates). Future research should also evaluate the operational rates tested in the study with likely sensitive native species (e.g., *Myriophyllum triphyllum* Orchard) and assess how concentration and exposure times could influence use patterns for *C. demersum*, *L. major*, and *E. densa* management (e.g., Beets et al. 2019).

SOURCES OF MATERIALS

¹ProcellaCOR SC, SePRO Corporation, Carmel, IN 46032.

²R version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria.

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