J. Aquat. Plant Manage. 59: 27–34

Evaluation of flumioxazin on seven submersed macrophytes in New Zealand

DEBORAH HOFSTRA, DANIEL CLEMENTS, DENISE RENDLE, AND PAUL CHAMPION*

ABSTRACT

Several invasive submersed aquatic plant species have established in many waterways within New Zealand, causing substantial economic, recreational, and ecological impacts. The herbicides currently registered for use for the management of submersed aquatic plants do not control all aquatic weed species under field conditions, and additional control methods are sought. This study evaluates the effectiveness of flumioxazin against four target submersed species (*Ceratophyllum demersum*, *Egeria densa*, *Lagarosiphon major*, and *Elodea canadensis*) and three nontarget native species (*Myriophyllum triphyllum*, *Potamogeton ochreatus*, and *Nitella* sp. aff. *cristata*). Single applications of flumioxazin required high concentrations (400 µg ai L^{-1}) to reduce *C. demersum* and *L. major* biomass by at least 50% at pH 8.4, *E. densa* was not controlled effectively at any rate ($\geq$ pH 8.4). However, low-rate applications (25, 50, 75, 100 µg ai L^{-1}) followed by high-rate applications (100, 200, 300, 400 µg ai L^{-1}, respectively) provided additional control of all species exposed to flumioxazin. *M. triphyllum* and *P. ochreatus* biomass was reduced with increasing concentration, *N.* sp. aff. *cristata* showed no symptoms from the application of flumioxazin. The potential use of flumioxazin in New Zealand is restricted, because susceptible aquatic weeds are required to be growing in low pH (less than 8.5) waters to achieve effective control. To overcome this potential restriction a second application of flumioxazin within a short period (ca. 1 mo) following initial application to less dense vegetation could substantially improve efficacy under these conditions.

Key words: *Ceratophyllum demersum*, *Egeria densa*, *Elodea canadensis*, herbicide, *Lagarosiphon major*, protoporphyrinogen oxidase (PPO) inhibitor, sequential treatment.

INTRODUCTION

A primary goal in aquatic plant management is to develop methods that will reduce and control invasive species and their negative impacts, while retaining native biodiversity and minimizing off-target impacts. Mesocosm studies to determine effects on target as well as nontarget vegetation and the relative sensitivity of a species to a given herbicide, or herbicide rate, are regularly used to determine the effect of a particular control method, prior to larger-scale field trials (Glomski and Netherland 2013).

Several submersed invasive plant species have successfully invaded and are established in many waterways within New Zealand and are having substantial economic, recreational, and ecological impacts, which are of concern for waterway managers. These species include *Ceratophyllum demersum* L (coontail), *Egeria densa* Planch., *Lagarosiphon major* (Ridley) Moss, and *Elodea canadensis* Michx. (Coffey and Clayton 1988, de Winton et al. 2009). Currently, there are two herbicides, diquat (dibromide salt) and endothall (dipotassium salt), registered for use on submersed aquatic plants in New Zealand. However, these herbicides do not control all aquatic weed species under field conditions. For example, of these two registered herbicides only diquat is efficacious on *E. densa* (Hofstra et al. 2018). Given the limited number of management tools available for submersed weed control in New Zealand, additional approaches are sought. The purpose of this study was to investigate the potential use of the herbicide flumioxazin for the management of target submersed aquatic weeds should registration be pursued in New Zealand.

Flumioxazin is a protoporphyrinogen oxidase (an essential enzyme required by plants for chlorophyll biosynthesis) inhibiting contact herbicide that disrupts chlorophyll synthesis and damages cell membranes (Weed Science Society of America [WSSA] 2014). It has been approved for use in aquatic sites in the United States for the management of submersed plants since 2010 (Haller and Gettys 2013). In the United States, significant herbicide injury has been reported for the submersed weeds; coontail, curlyleaf pondweed (*Potamogeton crispus* L.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), fanwort (*Cabomba caroliniana* Gray), hydrilla (*Hydrilla verticillata* (L.f.) Royle), longbeak buttercup (*Ranunculus longirostris* Godr.), springtape (*Sagittaria kurziana* Gluck), variable watermilfoil (*Myriophyllum heterophyllum* Michx.), and waterstargrass (*Heteranthera dubia* (Jacq.) MacM.) (Glomski and Netherland 2013). Under static conditions, flumioxazin controlled hygrophila (*Hygrophila polysperma* (Roxb.) T. Anderson) at less than half the maximum label rate (400 µg ai L^{-1}) (Haller and Gettys 2013). Flumioxazin efficacy has also been demonstrated on emergent and floating species in the United States (Richardson et al. 2008).

The efficacy of flumioxazin is known to be dependent on water pH, with higher efficacy when applied to low–neutral pH waters ($<$ 8.0). The product rapidly degrades by hydrolysis at pH $>$ 8.5 (Mudge and Haller 2010). A pH-mediated degradation of flumioxazin via hydrolysis will typically result in short exposure periods in higher-pH

*First, second, third, and fourth authors: Programme Leader, Scientist, Research Technician, and Principal Scientist, respectively, National Institute of Water and Atmospheric Research, P.O. Box 11 1115, Hillcrest, Hamilton, New Zealand. Corresponding author's E-mail: deborah.hofstra@niwa.co.nz. Received for publication May 1, 2020 and in revised form June 27, 2020.

waters. Flumioxazin is degraded by hydrolysis in approximately 4 d, 16 h, and 17 min at pH 5.0, 7.0, and 9.0, respectively (Katagi 2003). The half-life of flumioxazin in low-, medium-, and high-pH water has been reported as 39.0, 18.6, and 1.7 h, respectively (Mudge et al. 2010). In neutral pH conditions, Mudge and Haller (2010) reported effective concentration 50% (EC_{50}) values for coontail of 34 μg ai L^{-1} and hydrilla of 77 μg ai L^{-1}, whereas the EC_{50} values determined for American eelgrass (*Vallisneria americana* Michx.) (1,244 μg ai L^{-1}), and egeria (3,285 μg ai L^{-1}) were above the maximum label rate. Coontail was reported as the only submersed species to be controlled in high-pH conditions (> 9) at the maximum label concentration of 400 μg ai L^{-1} (Mudge and Haller 2010). Additionally, > 90% hydrilla control in high-pH treatments (> 9) has been shown, demonstrating rapid flumioxazin uptake in hydrilla, inferring that pH does not directly influence flumioxazin activity. Field trials with flumioxazin have shown variable control of hydrilla, with regrowth at or near the water surface less than 8 wk after treatment (WAT) of water at pH 8.0 (Mudge 2007, cited in Mudge 2013). A similar situation has been reported for fanwort, where no evidence of an effect of pH or shading was found, and fanwort was highly sensitive to flumioxazin (> 96% control with > 5 μg ai L^{-1}) (Bickel et al. 2018).

Species reported to be not significantly affected in the United States include American pondweed (*Potamogeton nodosus* Poir.), egeria, elodea, Illinois pondweed (*Potamogeton illinoensis* Morong), sago pondweed (*Stuckenia pectinatus* (L.) Boerner), southern naiad (*Najas guadalupensis* (Spreng.) Magnus), and American eelgrass (Glomski and Netherland 2013). In further studies, flumioxazin (50 μg ai L^{-1}) did not reduce shoot biomass of American pondweed or Illinois pondweed 8 WAT (Mudge 2013) and *Vallisneria nana* R. Br. was not visually affected at low rates (< 10 μg ai L^{-1}) (Bickel et al. 2018).

Amongst the research literature, efficacy has been reported for one of the target species of interest in the present study, *C. demersum*, whilst *E. densa* and *E. canadensis* are reportedly not significantly affected (Mudge and Haller 2010, Glomski and Netherland 2013). There are no known studies reporting efficacy results for *L. major*. Hence the approach taken in the present study was to assess flumioxazin efficacy broadly at a range of concentrations up to maximum label rate. An additional focus of the study was to evaluate whether a sequential, split treatment would improve efficacy results.

Sequential herbicide treatments are used for improving efficacy and potentially reducing the environmental load of herbicide in both terrestrial and aquatic systems (e.g., Jensen 1992, Fox et al. 1996, Nelson 1997, Lockhart and Howatt 2004, Netherland 2014). Specific aquatic examples include the use of sequential treatments for difficult-to-control species such as *Butomus umbellatus* (Turnage et al. 2019) and *Alternanthera philoxeroides* (Hofstra and Champion 2010). Sequential treatments are also used to extend exposure periods, typically for herbicides that have low use rates (Netherland 2014).

The aim of the experiments presented in this study was to assess the efficacy of flumioxazin as a potential herbicide for the control of target weeds in New Zealand. Efficacy was assessed against four invasive submersed weed species, *C. demersum*, *E. densa*, *L. major*, and *E. canadensis,* as well as three nontarget native macrophytes *Myriophyllum triphyllum* Orchard, *Potamogeton ochreatus* Raoul and *Nitella* sp. aff. *cristata* (referred to as *N. cristata* hereafter). Experiment 1 determined the effect of flumioxazin on target invasive species. Experiment 2 evaluated sequential flumioxazin applications, specifically initial low-rate (25 to 100 μg ai L^{-1}) applications, followed by high-rate (100 to 400 μg ai L^{-1}) applications to improve efficacy on the target submersed species. Experiment 3 assessed the susceptibility of nontarget desirable native submersed species to flumioxazin.

MATERIALS AND METHODS

General design

Three experiments were undertaken over two consecutive summer growing seasons (2017 to 2018) at NIWA's (National Institute of Water and Atmospheric Research) experimental research facility in Hamilton, New Zealand (37°46.510′S, 175° 18.752′E). General design elements across all studies, except where specifically indicated, included the field collection sites of macrophytes, which were then cultivated under a common garden setting in outdoor tanks filled (1.1 m deep) with dechlorinated water, at ambient temperature and under 80% shade cloth. Water in the tanks was gently mixed through a central air stone. Water temperature[1] was on average 22°C (± 1.9 [SD, standard deviation between tanks]). The pH[2] values ranged from 6.5 (morning) to a maximum of 8.8 (midafternoon) based on weekly monitoring (twice daily).

All species were propagated from apical shoots (200 to 250 mm in length), except *N. cristata*, which was grown from small (20-mm diameter) clumps of shoots with rhizoids. Plants were inserted into pots containing soil (1.3 g phosphorus, 3.6 g nitrogen per kg dry weight of soil) and augmented with fertilizer[3] (N–P–K; 15.3–1.96–12.6; 5 g/L soil). A thin layer (ca. 10 mm) of sand was added to cap the soil once plant shoots had been inserted. Plant numbers, pot and tank dimensions, treatment rates, and monitoring periods are provided below under each specific experiment.

After 6 to 8 wk in culture, when invasive species were surface reaching, flumioxazin[4] was applied to the water column at dawn (6:00 A.M.) at specified herbicide rates. Herbicide treatments were applied to native plants after a 12-wk growth period, although not all plants were surface reaching. Herbicide treatments were repeated across at least four tanks with static exposure, and there was a minimum of four untreated control tanks.

Plant condition and response to flumioxazin was monitored weekly during the experimental period. Observations included plant color, or a change in foliage color, leaf drop, fragmentation of stems, and loss of stem turgor. The onset of plant recovery or new growth by plants in treatment tanks signaled the timing for destructive harvest (4 to 6 WAT). All viable shoot material was harvested from each of the tanks to determine herbicide efficacy. Although species were harvested separately, individual plants of the same

species often became entangled over the course of the experiment and were not separated within each tank. Harvested plants were washed and dried at 60 C in a drying oven until constant dry weight was achieved. Harvested dry-weight data are reported as species biomass per tank for each treatment. The dry-weight data are reported and analyzed separately for each species in each experiment. Before statistical analysis biomass data were square-root transformed to meet the assumptions of homoscedasticity. To examine the response of each species to flumioxazin rate, data were analyzed using ANOVA (analysis of variance). Where there were significant effects Student's *t* post hoc tests were used to separate the means. All mention of statistical significance refers to $P < 0.05$. Nontransformed values are presented in plots as well as regression analysis.

Experiment 1: Invasive submersed species

Five shoots of each species (*C. demersum*, *E. densa*, and *L. major*) were planted into individual pots (2 L, 160 mm in height). A sample of 15 shoots per species was used to establish an estimate of initial shoot dry weight (0.18 ± 0.09 g for *C. demersum*, 0.18 ± 0.07 g for *E. densa*, 0.28 ± 0.09 g for *L. major*). Six pots were prepared for each species and placed in a single tank (1,500 L), where pots of the same species were grouped together. There were 20 tanks in total. The treatment rates were 100, 200, 300, 400 μg ai L^{-1} flumioxazin. On the day of treatment, average water temperature was 22 C (SD ± 2) and pH averaged 8.2 (SD ± 0.6) at 5:45 A.M. Plant condition and response to flumioxazin was monitored weekly during the experimental period and plants were destructively harvested at 4 WAT.

Experiment 2: Retreatment and regrowth of invasive submersed species

Plants, pots, and tanks were prepared as for Experiment 1, with the addition of another species (*E. canadensis*). Initial herbicide treatment rates were 25, 50, 75, 100 μg ai L^{-1} flumioxazin. Plant condition was monitored for 3 WAT, by which time new shoot development for some species signaled the time frame for retreatment. Tanks were retreated with flumioxazin 3 WAT at higher rates (100, 200, 300, and 400 μg ai L^{-1}), aimed at achieving a greater level of biomass reduction than obtained in Experiment 1. Retreatment rates were replicated across four tanks, corresponding to the prior flumioxazin treatment (i.e., the lowest rate was applied to tanks that had previously received the lowest rate), control tanks remained untreated. At the time of initial herbicide application (low rates), water temperature across tanks was 22 C (SD ± 0.15) and pH ranged from 7.9 (SD ± 0.3) in the morning of treatment to 8.3 (SD ± 0.3) by midday. On the day of retreatment (6:00 A.M.), water temperature across tanks ranged from 20 C (SD ± 0.6) and pH ranged from 7.5 (SD ± 0.4) in the morning of treatment to 8.2 (SD ± 0.1) by midday. Tanks were sampled for flumioxazin within 3 and 6 h after the initial treatment (HAT) and after retreatment, and water samples analyzed (solvent extraction, SPE clean-up, and LC-MS/MS, detection limit < 10 ppb[5]) to determine flumioxazin concentration. Plants were monitored for a further 6 wk after retreatment (6 WArT), then destructively harvested. An assessment of the regrowth potential of a subsample of fragments produced from treatment tanks was set up during the destructive harvest of Experiment 2. Containers (2 L) were filled with water and located adjacent to each other in the same greenhouse as the original experimental tanks. Five fragments (ca. 150 mm long) of each species that were considered likely to be viable (e.g., turgid), were removed from each tank during the harvest, except for *C. demersum* and *E. canadensis* in the 400 μg ai L^{-1} treatment tanks, of which there were insufficient viable fragments remaining. Blotted fresh weights (free water removed) of the fragments were recorded before they were placed into their respective containers to determine viability, keeping species and treatments separate. Water in each of the containers was aerated through a central air stone. Plant fragments were assessed monthly until new shoots had developed, or the fragments were no longer viable. After 4 mo, all growing plant material was harvested, and fresh weights obtained. The fresh-weight data are graphically presented.

Experiment 3: Native nontarget submersed species

Shoots of *M. triphyllum* and *P. ochreatus* were planted in small (750 ml, 100 mm in height) pots and grown for 12 wk in a 0.8-m-deep culture tank, along with the *N. cristata* (2-L pots). A sample of 15 shoots per species was used to establish an estimate of initial shoot dry weight (0.043 ± 0.020 g for *M. triphyllum*, 0.065 ± 0.030 g for *P. ochreatus*, 0.100 ± 0.050 g for *N. cristata*). Plants (five pots of each species) were moved into each of the treatment tanks and acclimated for 2 wk before herbicide was applied. All plants exceeded 0.6 m in height and *N. cristata* covered at least 75% of the pot surface at the time of treatment. The treatment rates were 100, 200, 300, 400 μg ai L^{-1} flumioxazin. Water temperature was 21.0 C (SD ± 0.2) and pH was 7.80 (SD ± 0.07) at the time of treatment (6:00 A.M.). Plant condition and response to flumioxazin was monitored weekly for 4 wk, after which time a destructive harvest was undertaken to determine dry-weight biomass.

RESULTS

Experiment 1: Invasive submersed species

All three species, *C. demersum*, *L. major*, and *E. densa*, continued to grow over the experimental period in untreated control tanks. Plants of *E. densa* also developed flowers as the shoots grew across the water surface. In contrast, within the first week posttreatment, herbicide symptoms were observed on *C. demersum* plants. In particular, at the two highest treatment rates (300 and 400 μg ai L^{-1}) *C. demersum* was initially discolored (bleached), with some fragmentation occurring within 10 d to 2 WAT. Within 3 to 4 WAT plants of *C. demersum* had collapsed, although a large portion of the plant biomass was turgid and remained potentially viable. Both *L. major* and *E. densa* appeared to be less susceptible to flumioxazin than *C. demersum*, with few symptoms observed on plants in the low-

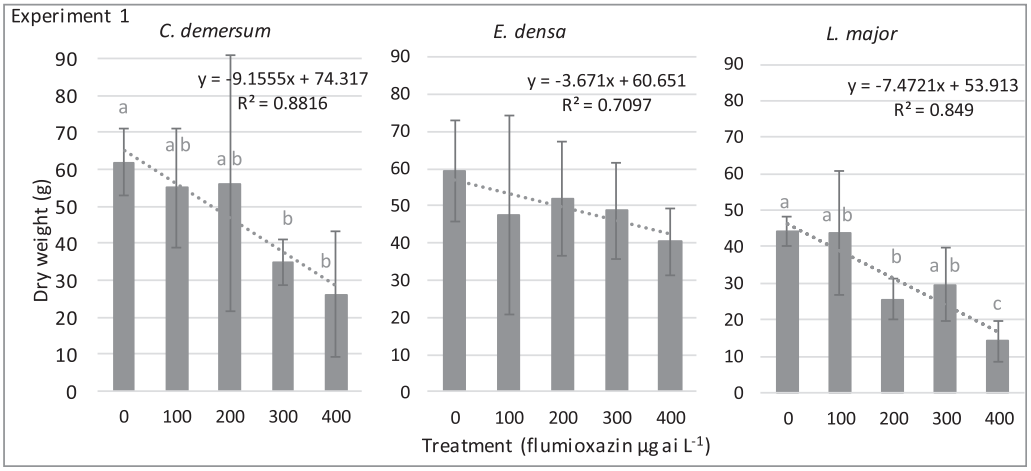

Figure 1. Effect of flumioxazin concentration on biomass of submersed invasive plants 4 wk after treatment in Experiment 1. Solid bars represent average dry weight (g) from replicate tanks ($n = 4$); error bars represent one standard deviation. Letters denote significant differences between treatments (Student's t tests, $P \leq 0.05$) for *Ceratophyllum demersum* (analysis of variance [ANOVA] $P = 0.050$) and *Lagarosiphon major* (ANOVA $P = 0.001$). ANOVA was not significant for *Egeria densa* ($P = 0.687$). For each species linear regression is represented by the dotted line, equation, and R^2 values.

rate tanks. Plants of *E. densa* and *L. major* in the high rate (300 and 400 µg ai L^{-1}) tanks were discolored with bleached foliage, and reddened apical tips (top 2 to 5 cm). The reddened shoot tips later abscised (1 WAT), with further leaf drop and minor fragmentation of stems following (2 WAT). Even at these higher rates, new shoots were emerging on the treated plants by 3 WAT, and to a greater extent, on plants treated at the lower rates.

Biomass data supported the monitoring observations with greater levels of biomass reduction for *C. demersum* and *L. major* than for *E. densa*, when compared with untreated plant biomass of the same species (Figure 1). For example, although biomass values were highly variable for all species between tanks, a reduction of over 50% in plant dry weight was achieved for *L. major* when treated at the highest rate (400 µg ai L^{-1}), and for *C. demersum* at the two highest rates (300 and 400 µg ai L^{-1}).

Experiment 2: Retreatment and regrowth of invasive submersed species

Flumioxazin concentrations determined from water samples from treated tanks in Experiment 2 reduced rapidly posttreatment. Within 3 h following the initial low-rate applications, concentrations were below 12 µg ai L^{-1} for the two target rates of 25 and 50 µg ai L^{-1}. For the midtarget rate of 75 µg ai L^{-1}, in-tank concentrations dropped from 25 to 10 µg ai L^{-1} by 3 and 6 HAT, respectively. For the highest-target rate of 100 µg ai L^{-1} in tank concentrations dropped from 48 to 16 µg ai L^{-1} by 3 and 6 HAT, respectively. Similarly, after the sequential retreatment at higher rates (100, 200, 300, 400 µg ai L^{-1}) the target concentrations had reduced in the treated tanks by more than 75% by 6 HAT (i.e., 23, 40, 68, 95 µg ai L^{-1}, respectively).

Plants in the untreated control tanks exhibited healthy growth over the experimental period, with surface reaching and branching stems, and for *E. densa* the production of flowers. The initial low-dose treatment had a visible impact of color loss on all species, but within 3 WAT *E. densa*, *L. major*, and *E. canadensis* had developed new shoots. In contrast, the canopy of *C. demersum* had collapsed within the water column in all tanks except the untreated controls. Following retreatment at higher rates (100 to 400 µg ai L^{-1}) *C. demersum* was visibly impacted, with leaf drop and stem fragmentation at all treatment rates. By 6 WArT there was little *C. demersum* biomass that appeared to be viable in any of the treatment tanks and none at the highest rate. *Elodea canadensis* was similarly impacted at the highest rates (300, 400 µg ai L^{-1}) with few plant fragments remaining, and in the lower rates (100 and 200 µg ai L^{-1}) stems were very brittle and fragmentation had occurred. In contrast, for both *L. major* and *E. densa*, plants had new buds developing at the nodes, although stems were denuded of leaves and fragmentation was extensive. New bud development was particularly evident for plants in tanks treated at lower rates, and by 6 WArT some *E. densa* plants treated at the highest rate had developed new shoots.

For all species, there was greater plant biomass in the untreated control tanks than the treated tanks (Figure 2). In addition, all species exhibited a dose response to flumioxazin with greater reduction in plant biomass achieved at higher concentrations (300, 400 µg ai L^{-1}) compared to the lowest concentration (100 µg ai L^{-1}) (Figure 2). *Ceratophyllum demersum* was the most susceptible species, with 88% reduction in biomass (compared with untreated control plants), at the lowest treatment rate (100 µg ai L^{-1}). In contrast, all of the other species had about 65 to 70% biomass reduction at the lowest treatment rate. *Egeria densa* had more biomass remaining at 6 WArT than the other species examined. Further, *E. densa* and *L. major* were the only two species of which fragments survived and grew during the fragment regrowth study (Figure 3).

A single application of flumioxazin at 300 to 400 µg ai L^{-1} reduced *C. demersum* biomass by about 40 to 60% at pH 8.4 (Experiment 1). With a prior treatment of 75 to 100 µg ai L^{-1}, biomass was reduced by 99% at pH 7.5 (Experiment 2). Although *E. canadensis*, *E. densa*, and *L. major* displayed a lesser response to flumioxazin compared to *C. demersum,* the

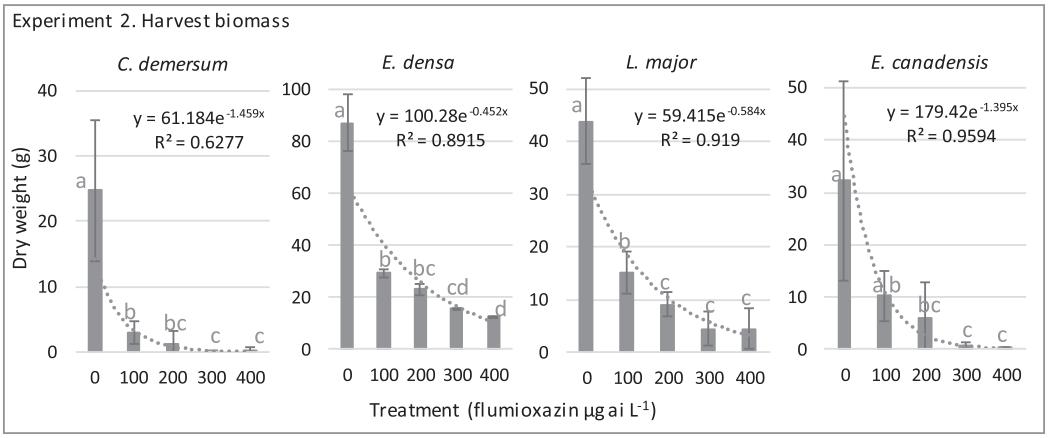

Figure 2. Harvested biomass of invasive submersed plants after two sequential treatments with flumioxazin in Experiment 2. Values are mean dry weights from treatment tanks ($n = 4$) at 6 wk after retreatment, with standard deviations shown by the error bars. Letters denote significant differences between treatments (Student's t tests, $P \leq 0.05$) for *Ceratophyllum demersum* (ANOVA $P = 0.0001$), *Egeria densa* (analysis of variance [ANOVA] $P = 0.007$), *Lagarosiphon major* (ANOVA $P = 0.001$) and *Elodea canadensis* (ANOVA $P = 0.0004$). Regression analysis is represented by the gray dotted lines, equations, and R^2 values.

second application also provided additional control of these species (Figure 2). The sequential treatment approach, while using more herbicide overall, provided a level of biomass reduction that is promising for scaling up to field application.

Experiment 3: Native nontarget submersed species

Growth of native nontarget species was highly variable both between individual plants in pots and between tanks, including the untreated control tanks. Hence at 4 WAT, there was also a lot of variation in plant biomass. In tanks treated with flumioxazin, there was variation between individuals of *M. triphyllum* and *P. ochreatus* in the severity of the herbicidal symptoms. The high level of variation is reflected in the biomass data (Figure 4). At all flumioxazin concentrations tested, *M. triphyllum* and *P. ochreatus* exhibited herbicidal symptoms including leaf loss and some fragmentation. In particular, *P. ochreatus* had very brittle stems and a large portion of the leaves were notably brown and necrotic at the higher herbicide rates (300 and 400 µg ai L^{-1}). The biomass data illustrate a trend of reduced *M. triphyllum* and *P. ochreatus* biomass with increasing flumioxazin concentration. In contrast, *N. cristata* showed no symptoms from the application of flumioxazin (Figure 4).

Amongst all three experiments variation was observed in flumioxazin efficacy between potted plants within treatment tanks where plants towards the outer edges of tanks appeared to be more impacted by flumioxazin than plants nearer to the center of tanks. This variation, particularly evident for *C. demersum*, may have occurred because of the aeration in the center of the tanks, dispersing concentrations of flumioxazin around the outer edges of the tanks that had not mixed effectively prior to uptake or hydrolysis. To our knowledge, this issue of the water-dispersible

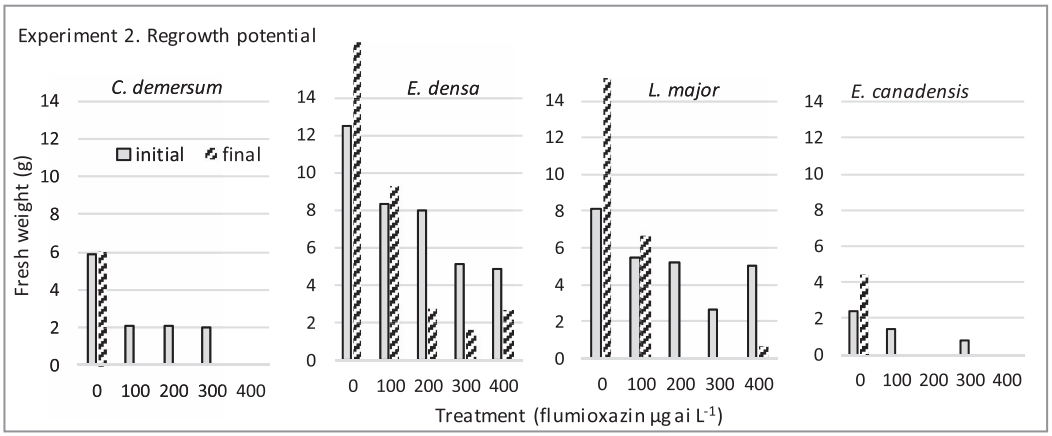

Figure 3. Regrowth potential of harvested fragments of invasive submersed plants after two sequential treatments with flumioxazin in Experiment 2. Bars represent the recovery of a subsample ($n = 5$) of the harvest fragments. Solid bars represent initial fresh weight of fragments 6 wk after retreatment and dashed bars represent final fragment fresh weight after 4 wk of culture.

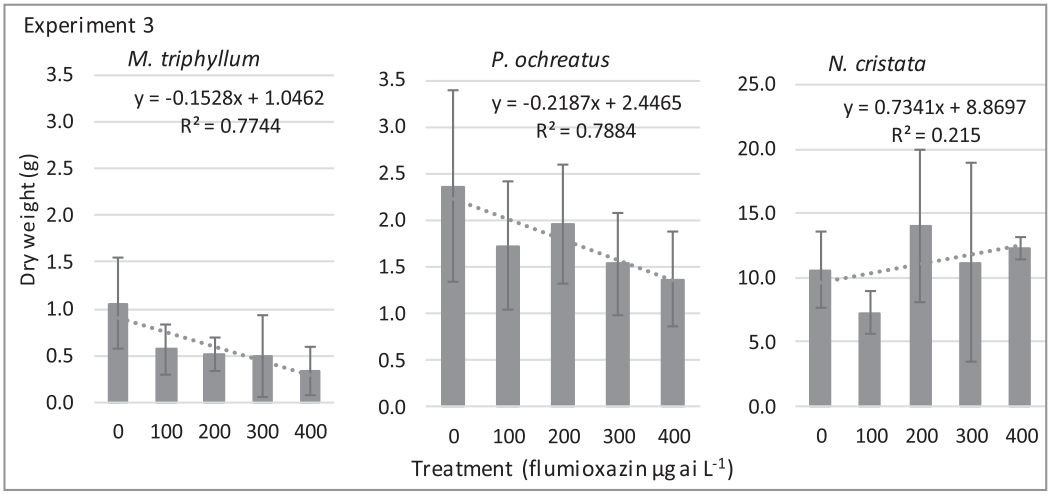

Figure 4. Effect of flumioxazin concentration on biomass of submersed native plants 4 wk after treatment in Experiment 3. Values are mean ($n = 4$) dry weights from treatment tanks. Error bars represent one standard deviation. Analysis of variance (ANOVA) was not significant for *Myriophyllum triphyllum* ($P = 0.073$), *Potamogeton ochreatus* ($P = 0.376$), or *Nitella cristata* ($P = 0.349$). Linear regression is represented by the dotted lines, equations, and R^2 values for each species.

granule not mixing effectively has not been described previously.

DISCUSSION

Significant reductions in biomass for all target species were achieved in the present study following flumioxazin treatment. Single applications at high rate reduced *C. demersum* and *L. major* biomass. Sequential applications effectively reduced biomass of all four target species *C. demersum*, *L. major*, *E. densa*, and *E. canadensis*. This compares to previous studies on *C. demersum* from the United States when exposed to single applications of flumioxazin where a concentration of 34 µg L^{-1} reduced dry-weight biomass by 50% (EC_{50}) at pH 7.0 (Mudge and Haller 2010) and 81.5% reduction was achieved with 50 µg L^{-1} at pH 6.9 to 8.1 (Mudge 2013). Although the visual symptoms described in these previous studies align with those determined in the present study for *C. demersum*, with bleaching and plant collapse posttreatment, the outcome on *C. demersum* from the United States, was achieved at lower concentrations (34 µg L^{-1}) by an order of magnitude compared to the present study (300 to 400 µg L^{-1}). The sequential treatment experiment (Experiment 2) did indicate the collapse of *C. demersum* at the lower rates (< 100 µg L^{-1}), but plants remained turgid, indicating the stems were still viable prior to the second flumioxazin application. It is possible that had the plants been monitored for longer before harvest (Experiment 1) or before retreatment (Experiment 2) that the residual biomass would have declined and decayed. Decomposition of the small sample of shoots that were available to assess recovery, indicates that further biomass decline was likely to occur over time. However, it is also possible that differences in *C. demersum* biotypes/phenotypes may contribute to the observed differences in *C. demersum* susceptibility to flumioxazin. In a European-based study, *C. demersum* from New Zealand was reported to have higher phenotypic plasticity than the European population (Hyldgaard and Brix 2012) and differences between plant biotypes and their responses to herbicides is well documented (Bultemeier et al. 2009, Berger et al. 2012, LaRue et al. 2013, Benoit and Les 2013).

Compared with *C. demersum*, the Hydrocharitaceae target species in the present study were all less susceptible to flumioxazin. There are, however, few literature examples to compare results for these species, with no known examples for *L. major*. *Egeria densa* and *E. canadensis* are reported as not sensitive to flumioxazin (Mudge and Haller 2010, Glomski and Netherland 2013). Mudge and Haller (2010) report EC_{50} values for *E. densa* (3,285 µg L^{-1} at pH 7 and 3,747 µg L^{-1} at pH 9) which exceeds the maximum label rate of flumioxazin. The study by Glomski and Netherlands (2013), which used cell leakage as an indicator of herbicide injury, report that *E. canadensis* was not sensitive at the rates of 200 and 400 µg L^{-1}. The sequential treatment approach (Experiment 2) effectively reduced the biomass of all three Hydrocharitaceae target species, and indicates that *E. canadensis* is susceptible to this treatment approach, in contrast to previous predictions (Glomski and Netherland 2013).

The threshold concentration and exposure times (CET) required to achieve effective control are often species specific and may differ under varying environmental conditions, such as pH and temperature (Netherland et al. 2000, Mudge et al. 2010, Clements et al. 2018). Flumioxazin is known to be rapidly absorbed by some target plants and breaks down quickly in water with pH greater than 8.5 (Katagi 2003, Mudge et al. 2010). Based on the flumioxazin concentrations that were determined up to 6 HAT during Experiment 2, and the similar pH range in tanks on the day of treatment (below 8.5) across the three experiments, it was expected that flumioxazin exposure time would have been sufficient to enable uptake by susceptible species. Although there is limited information published on plant uptake rates for flumioxazin, time frames of minutes rather than hours

have been suggested for susceptible species (Bickel et al 2018).

With injury symptoms observed for both the native *M. triphyllum* and *P. ochreatus* following single applications of flumioxazin (> 100 μg L^{-1}), it is likely that any treatment regime targeting the invasive species, and in particular the more efficacious sequential treatment approach, will also have negative impacts on these native species, although not on the native charophyte (*N. cristata*). However, native plants such as *M. triphyllum* and *P. ochreatus* can recover from their seedbanks (Hofstra et al. 2018).

Any potential use pattern for flumioxazin must consider treatment timing relative to water pH, to maximize plant uptake and minimize herbicide use. The pH of water surrounding dense submersed vegetation can exceed 8.5, because of photosynthetic processes. Early morning treatments (when pH is naturally reduced) of flumioxazin to actively growing submersed aquatic weeds with high light penetration into the water column are recommended to overcome potential ineffective control, by maximizing exposure times. However, pH can still be high at these times if dense infestations are present, negatively impacting flumioxazin efficacy. Therefore, the use pattern of flumioxazin is likely restricted to situations where the target biomass is less dense (i.e., early-season growth or following previous management actions), and pH is not likely to rise as steeply as it would in more dense weed beds. In addition, for effective aquatic plant control in dense weed beds or high pH conditions, a second application may overcome these restrictions and increase the outcome of weed control operations. However, it is noted that the label for the aquatic use of flumioxazin (Clipper®, Valent 2012-CLP-0001) requires a stand-down period of 28 d before retreatment.

In summary, single applications of flumioxazin required high concentrations (400 μg ai L^{-1}) to reduce target weed biomass of *C. demersum* and *L. major* by at least 50% at pH 8.4, whereas *E. densa* was not controlled effectively at any rate ($pH \geq 8.4$). However, an initial low-rate application followed by a high-rate application provided additional control of all target species exposed to flumioxazin, but two of the three native nontarget species were also impacted. Although contained studies provide an initial indication of potential herbicide use patterns, field-based studies at larger scale are required to confirm weed control and environmental outcomes.

SOURCES OF MATERIALS

[1]Hobo pendant temp/light logger (UA-002-64), Onset, 470 MacArthur Blvd., Bourne, MA 02532.

[2]EXO1 Multiparameter Sonde (SKU: 599501), 1700/1725 Brannum Lane, Yellow Springs, OH 45387-1107.

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

[4]Valor 500 WG Herbicide, Sumitomo Chemical Co., Ltd., 27-1, Shinkawa 2-chome, Chuo-ku, Tokyo 104-8260, Japan.

[5]Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204, New Zealand.

[6]Licor LI-192 underwater quantum sensor coupled to a LI-1500 light sensor logger. Licor Biosciences, Lincoln, NE 68504.

ACKNOWLEDGEMENTS

This research was funded through NIWA's Strategic Science Investment Fund. The authors would like to thank Giorgia Kershaw, Susie Elcock, and Glenys Croker for assistance with planting and harvesting the experiments. Flumioxazin was provided by Mr. Paton (Sumitomo). The contributions of anonymous reviewers are very much appreciated.

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