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# Imazamox control of invasive Japanese eelgrass (*Zostera japonica*): Efficacy and nontarget impacts

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## ABSTRACT

The nonnative eelgrass, Japanese eelgrass (*Zostera japonica* Asch. & Graebn) has infested several West Coast estuaries in North America. In Willapa Bay, WA, coverage has expanded enough to result in deleterious impacts on commercial shellfish production. Research on foliar and subsurface applications of the herbicide imazamox was conducted using replicated field trials to assess the efficacy for control of Japanese eelgrass and potential nontarget effects to the native eelgrass, (*Zostera marina* L.) and several macroalgae species. Foliar applications of imazamox controlled established Japanese eelgrass with or without surfactant, across a range of rates, from 0.03 to 0.84 kg ai ha$^{-1}$. Control of Japanese eelgrass seedlings was obtained with rates as low as 0.02 kg ai ha$^{-1}$ imazamox. Best efficacy was obtained when tidal waters fully drained off the site, and the eelgrass canopy was dry. When Japanese eelgrass had a thin, protective layer of tidal water over it, rates of imazamox as high as 0.56 kg ai ha$^{-1}$ were required for more consistent control. A foliar application of imazamox at 0.14 kg ai ha$^{-1}$ killed the native eelgrass, whereas a rate of 0.84 kg ai ha$^{-1}$ had no effect on macroalgae. Damage to native eelgrass was minimized when the canopy was protected in the water column. An in-water exposure of $< 90$ μg ai L$^{-1}$ imazamox for 2 to 3 h had no observed effect on native eelgrass. Movement of imazamox off-site in the water column during the receding or flood tides after treatment was minimal, with a resulting dose and exposure time below what was required to markedly affect nontarget eelgrass.

*Key words*: Estuary, Willapa Bay, *Zostera japonica*, *Zostera marina*.

## INTRODUCTION

Two seagrass congeners in the genus *Zostera* occur on the West Coast of North American estuaries, the native eelgrass (*Zostera marina* L.) and the nonnative Japanese eelgrass (*Zostera japonica* Asch. & Graebn.) (Ruesink et al. 2010, Shafer et al. 2014). The nonnative Japanese eelgrass sustains many of the ecosystem functions of the native eelgrass, including supporting diverse benthic assemblages, providing carbon to the estuarine food web, structural support for other primary producers, and habitat for juvenile salmonids and other fish species (Bulthuis 2013, Shafer et al. 2014). Japanese eelgrass, however, also has noted negative effects (Bando 2006, Tsai et al. 2010, Fisher et al. 2011). In Willapa Bay, WA, it has infested thousands of hectares of commercial Manila clam beds, where it reduces annual clam growth by 15 to 25% and results in a cumulative total net loss of approximately U.S. \$47,407 ha$^{-1}$ for each harvest cycle of Manila clams (Patten 2014). Because of its economic impact, the Washington State Noxious Weed Control Board declared Japanese eelgrass a Class C noxious weed (WA State Noxious Weed Control Board 2012). The California Department of Fish and Game has declared an eradication effort for Japanese eelgrass in Humboldt Bay, CA (CA Dept. of Fish and Game 2009).

There are limited nonchemical management options for Japanese eelgrass (Schlosser 2007, WA Dept. of Ecology 2014). The herbicides glyphosate (Bulthuis and Shaw 1993, Patten 2003, Major et al. 2004) and imazapyr (Patten 2003) were partially effective on Japanese eelgrass, but only when the canopy was dry. Both of these chemistries lack tolerance for residue levels on food, however, and would not be suitable for use on commercial clam beds. Imazamox,[1] a recently registered, aquatic herbicide with a use pattern that includes estuarine and marine sites (EPA 2009), is exempt from all uses of food-residue tolerance requirements, including shellfish (WA Dept. of Ecology 2014). Because of its suitability for potential use, imazamox was assessed for the management of Japanese eelgrass across a range of tidal estuary conditions. In addition, studies were conducted to assess nontarget impacts to native eelgrass and macroalgae and to develop environmental concentration data for use in risk assessments under estuarine conditions.

## MATERIALS AND METHODS

### Study location

Research was conducted on the tideflats of Willapa Bay, WA, at a tidal height zone of 0.75 to 1.5 m between the years 2006 and 2013. Willapa Bay is a large, shallow bar–built estuary with 347 km$^2$ in surface area at mean higher high water (MHHW) and 191 km$^2$ at mean lower low water (MLLW). The tidal range between MHHW and MLLW is 2.4 to 3.4 m. More than half of the estuary's surface area and volume is drained at low tide (Hickey and Banas 2003). Approximately 20% of the intertidal area is used for commercial aquaculture of Pacific oysters (*Crassostrea gigas*

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TABLE 1. EXPERIMENTAL CONDITIONS FOR JAPANESE EELGRASS EFFICACY AND NONTARGET IMPACT STUDIES.

| Site | Date of Application | Plot Size (m) | No. of Replications | Vegetation Type | Amount of Water Covering Plant Canopy | Time Before Tidal Coverage (h) |
|---|---|---|---|---|---|---|
| 1 | 20 September 2006 | 4 × 7 | 3 | Japanese eelgrass | Thin layer of water | 3.5 |
| 2 | 15 April 2007 | 3 × 11 | 3 | Japanese eelgrass | Canopy moist; no water cover | 4.0 |
| 3 | 3 March 2007 | 4 × 7 | 3 | Japanese eelgrass | Thin layer of water | 3.5 |
| 4 | 27 April 2009 | 4 × 7 | 3 | Japanese eelgrass | Canopy dry | 3.5 |
| 5 | 10 June 2009 | 2 × 4 | 3 | Japanese eelgrass | Canopy dry | 3.5 |
| 6 | 1 January 2007 | 3 × 7 | 3 | Mixed Japanese eelgrass and native eelgrass | Dry to submerged | 2.5 |
| 7 | 28 May 2008 | 3 × 4 | 4 | Mixed Japanese eelgrass and macroalgae | Canopies dry | 2.5 |
| 8 | 7 June 2011 | 2 × 2 | 15 | Mixed Japanese eelgrass and native eelgrass | 0.5–1 cm | 2.5 |
| 9 | 7 July 2011 | 5 × 6 | 12 | Mixed Japanese eelgrass and native eelgrass | Canopies moist, no water cover | 2 |
| 10 | 17 June 2013 | 2 × 2 | 4 | Japanese eelgrass seedlings | Dry | 2 |
| 11 | 17 June 2013 | 2 × 2 | 4 | Japanese eelgrass seedlings | Dry | 3 |
| 12 | 17 June 2013 | 2 × 2 | 4 | Japanese eelgrass seedlings | Dry | 1 |
| 13 | 27 May 2010 | 33 × 33 | 4 | Native eelgrass | 0–15 cm | 2.5 |
| 14 | 7 May 2012 | 30 × 70 | 1 | Native eelgrass | 0–30 cm | 3 |
| 15 | 23 May 2013 | 9 × 33 | 1 | Japanese eelgrass | Dry | 1.5 |

Thunberg) and Manila clams (*Ruditapes philippinarum* Adams and Reeve) (Feldman et al. 2000).

## Trials for efficacy rate and nontarget impacts

Established stands of pure Japanese eelgrass, mixed Japanese eelgrass and native eelgrass, or mixed species of macroalgae were directly oversprayed with imazamox in a series of replicated experiments, using a randomized complete-block design, between the spring and late summer of 2006 and 2013. Depending on the experiment, treatments were applied with a carbon dioxide ($CO_2$)-powered or hand-powered backpack sprayer, using a boom with varying length equipped with TeeJet 11025 spray nozzles.[2] Imazamox rates ranged from 0.022 to 0.84 kg ai $ha^{-1}$, with or without the surfactant Competitor,[3] at 2.8 L $ha^{-1}$. Estuarine water was used as the carrier, and the spray volume was 230 L $ha^{-1}$. Treatments were applied to Japanese eelgrass once the tidal water had completely drained off the site. For sites with native eelgrass, treatments were made after water had receded off the site, but when there was still a thin (approximately 0.25 to 0.5 cm) layer of water over the top of the canopy. Sites with macroalgae were dry, with no protective water film over the algae. The three algae species present—*Ulva intestinalis* L., *Ulva flexuosa* Wulfen, and *Polysiphonia hendryi* var. *deliquescens* Hollenberg—were affixed in approximately equal amounts to surface gravel at the site. Plot size, replication number, site conditions, and dry time before tidal coverage for each trial are detailed in Table 1 (Sites 1 to 12). Plots were evaluated for efficacy or for nontarget impacts at 1 to 9 mo after treatment (MAT) based on a visual rating of the percentage of cover, or the percentage of change in Japanese eelgrass seedling density or native eelgrass shoot length before and after treatment.

Additional nontarget assessments were made for native eelgrass on large sites treated with 0.14 kg ai $ha^{-1}$ imazamox. Sites contained both eelgrass species located on well-drained gently sloping ground, in shallow, isolated pools containing 5 to 15 cm of static water and in shallow tidal-drainage swales that started on-site and moved off-site with water draining off the treated area. At the first location, Site 13 (Table 1) native eelgrass shoot growth was measured in the static pools at 0, 1, and 2 MAT as a function of the depth of water (0, 5, 10, and 15 cm). Plants were marked within each pool to allow for repeated measures of eelgrass shoot length. The mean number of shoots measured per plot was 25. There were four replicated pools per water depth. The calculated in-water exposure concentrations before tidal flooding for the 5, 10, and 15 cm depth pools were 278, 139, and 93 $\mu$g ai $L^{-1}$, respectively. At the second site (Site 14, Table 1) the percentage of reduction in native eelgrass coverage was measured at 21 d after treatment (DAT) in static pools (20 to 30 cm deep, $n = 4$) and in shallow drainage swales at the bottom edge of the treated zone ($<$ 2 cm deep, $n = 7$; and 5 to 10 cm deep, $n = 10$).

## Imazamox concentrations in water, sediment, and eelgrass

To assess water concentration of imazamox that could result from a typical treatment, water samples from Site 14 were obtained from a tidal pool, the tidal swale within the treated area, as it drained off the site during the ebb tide, and on the flood/shore side of the plot during the first and second flood tides after treatment. On-site samples were collected immediately after treatment. For the swale that drained the treated area, samples were collected 30 to 45 min after treatment in the middle of the swale at 0, 30, 60, and 120 m from the edge of the treated zone. Sample locations for the first flood tide after treatment were collected at five locations along transects that radiated out from the treatment zone (3 m inside the treatment zone, and 3, 30, 60, and 120 m outside the treatment zone). The transects were laid to run along the middle and outer two edges of the flood water as it moved over and beyond the treated zone. Samples along transects were collected as soon as the incoming flood water reached the 8-cm depth. All other samples were collected from the middle of the water column for that location. Water samples were collected in 60 ml Nalgene amber HDPE bottles.[4] Samples were held on ice in a dark cooler and shipped to the laboratory within 24 h. Samples were analyzed by SePRO Lab Services[5] using a Shimadzu LC-20[6] high-performance liquid chromatography

Table 2. Average Percentage of Cover of established Japanese Eelgrass as a Function of Imazamox Rate with Surfactant.

| Imazamox (kg ai ha$^{-1}$) | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| | Cover (%)$^{1,2}$ | | |
| 0 | 100 b | 88 b | 100 d |
| 0.07 | — | — | 30 c |
| 0.14 | 75 ab | 10 a | 16 bc |
| 0.28 | 70 ab | 7 a | 9 ab |
| 0.56 | 53 a | — | — |

[1] Sites 1, 2, and 3 were visually rated 9, 2, and 4 mo after treatment, respectively, for percentage of cover.

[2] Means within a column followed by same letter do not significantly differ (Waller-Duncan, $\alpha = 0.05$).

Table 3. Average Percentage of Cover of established Japanese Eelgrass as a Function of Imazamox Rate without Surfactant.

| Imazamox (kg ai ha$^{-1}$) | Site 4 | Site 5 |
|---|---|---|
| | Cover (%)$^{1,2}$ | |
| 0 | 100 c | 100 c |
| 0.035 | 2 b | 8 b |
| 0.07 | 0 a | 2 ab |
| 0.105 | 0 a | 0 a |
| 0.14 | 0 a | 0 a |
| 0.21 | 0 a | 0 a |

[1] Sites were visually rated 1 mo after treatment for percentage of cover.

[2] Means within a column followed by same letter do not significantly differ (Waller-Duncan, $\alpha = 0.05$).

(HPLC), method ISO 17025,[7] within 48 h of their collection. The limit of herbicide detection was 1 μg ai L$^{-1}$.

To assess imazamox concentrations in sediment and eelgrass, a sandy sediment location, Site 15 (Table 1), was treated with 0.14 kg ai ha$^{-1}$ imazamox. Samples were collected 24 h after treatment. Sediment samples, 0 to 5 cm deep, were obtained using a 7-cm coring device, from six locations across the site, and placed in sample bags. Eelgrass samples were collected from three locations at the site. Samples were triple rinsed in off-site estuarine water to remove any contaminated sediment and placed in sample bags. Sediment and eelgrass samples were immediately placed on ice in a dark cooler after collection, shipped on ice within 2 h, and chemically stabilized in the laboratory within 24 h. Samples were analyzed within 48 h of collection by Pacific Agricultural Laboratory,[8] using U.S. Environmental Protection Agency–approved HPLC methods. The limits of detection were 0.5 and 100 μg ai L$^{-1}$ for sediment and vegetation, respectively.

## Statistical analysis

Herbicide efficacy and nontarget plant data were analyzed by one-way ANOVA using SigmaPlot 12 software.[9] For data with homogeneity of variance, mean separation was accomplished by Waller-Duncan $t$ test ($\alpha = 0.05$). Nonparametric data was analyzed by Kruskal-Wallis one-way ANOVA on ranks, and mean separation were analyzed by protected Fisher's Protected LSD test ($\alpha = 0.05$).

# RESULTS AND DISCUSSION

## Efficacy

Imazamox controlled established Japanese eelgrass, with or without surfactant, across a range of rates, from 0.035 to 0.84 kg ai ha$^{-1}$ (Tables 2–4). A fall application was less effective than that in spring or early summer, even at very high rates (Table 2). At most sites, a rate of 0.14 kg ai ha$^{-1}$ was adequate for good control, but one site required 0.28 kg ai ha$^{-1}$ (Table 5), and another required 0.56 kg ai ha$^{-1}$ (Table 2). A layer of water over Japanese eelgrass at application decreased efficacy (Table 5). Seedlings were controlled with rates as low as 0.022 kg ai ha$^{-1}$ (Table 5).

These results indicate that control of established Japanese eelgrass or seedlings with imazamox under ideal tidal conditions, when the canopy was completely exposed and dry during low tide, can be obtained with $\leq$ 0.035 kg ai ha$^{-1}$ imazamox without the need for a surfactant. Under tidal conditions less than ideal, where Japanese eelgrass still had a protective water layer over it, rates of imazamox as high as 0.56 kg ai ha$^{-1}$ imazamox were required for more consistent control.

## Nontarget eelgrass and macroalgae effects

The effect of imazamox on native eelgrass was dependent on the conditions at the time of application. Imazamox applied over the top of a fully exposed canopy killed native eelgrass (Sites 6 and 9; Tables 4 and 5). Damage to native eelgrass from imazamox was reduced or minimized with an in-water exposure. This occurred when treatments were made at a site where there was a thin protective layer of slowly flowing water over native eelgrass canopy (Site 8, Table 4). In this situation, only the 0.28 kg ai ha$^{-1}$ rate had a significant effect on the canopy.

Native eelgrass located in well-drained sections of upper intertidal zones would likely have an exposed canopy during a typical low tide and be killed by an application of imazamox. The biological significance of native eelgrass removal in this tidal range is likely to be minor. Native eelgrass doesn't normally occur in this upper tidal range because it lacks tolerance to desiccation. Its existence in these zones is only due to that fact that Japanese eelgrass slows tidal drainage and facilitates the establishment of native eelgrass in higher, normally drier, tidal zones (Ruesink et al. 2010). Without Japanese eelgrass, these sites dewater enough during summer low tides to normally desiccate native eelgrass.

The greatest ecological risk to native eelgrass from imazamox is from short-term, unintentional in-water exposure at locations where it is naturally found. This could occur when the concentration and exposure time (CET) to imazamox in on-site static pools and swales and off-site drainage swales became high enough to cause damage. At Site 13, 1 MAT, after an overspray of static pools 5, 10, and 15 cm deep, corresponding to calculated doses of 280, 140, and 90 μg L$^{-1}$ for 2.5 h, respectively, native eelgrass had 50%, −21%, and 8% changes in mean shoot growth, respectively (Table 6). After 2 MAT, native eelgrass had begun to recover, and there was no statistical difference between water depths. At Site 14, an overspray of static

Table 4. Average Percentage of Control of Established Japanese Eelgrass and Nontarget Species as a Function of Imazamox Rate with Surfactant.

| Imazamox (kg ai $ha^{-1}$) | Site 6 Japanese Eelgrass | Site 6 Native Eelgrass | Site 7 Japanese Eelgrass | Site 7 Macroalgae | Site 8 Japanese Eelgrass | Site 8 Native Eelgrass | Site 9 Japanese Eelgrass | Site 9 Native Eelgrass |
|---|---|---|---|---|---|---|---|---|
| | Cover (%)[1,2] | | | | | | | |
| 0 | 90 b | 90 b | 58 b | 100 | 53 b | 53 b | 100 b | 100 b |
| 0.14 | 4 a | 7 a | — | — | 53 b | 21 b | — | — |
| 0.21 | — | — | 0 a | 100 | — | — | — | — |
| 0.28 | 2 a | — | — | — | 29 a | 6 a | 0 a | 0 a |
| 0.42 | — | — | 0 a | 100 | — | — | — | — |
| 0.84 | — | — | 0 a | 100 | — | — | — | — |

[1] Site 6, 7, 8, and 9 were visually rated 3, 5, 2, and 3 mo after treatment, respectively, for percentage of change in cover.

[2] Means within a column followed by same letter do not significantly differ (Waller Duncan, $\alpha = 0.05$).

pools with 20 to 30 cm of standing water (100 to 200 mg ai $L^{-1}$ nominal concentration, 181 mg ai $L^{-1}$ measured concentration) resulted in no observed reduction in the percentage of native eelgrass cover after 3 h exposure. In shallow drainage swales, at the bottom edge of the treated zone, < 2 cm and 5 to 10 cm deep (541 mg ai $L^{-1}$ measured concentration), there was a mean ± standard error (SE) 65% ± 5% and 96% ± 2% reductions, respectively, in the percentage of native eelgrass cover. Based on observed and nominal concentrations of imazamox in water, a CET for native eelgrass can be inferred from the above field data. For 2 to 3 h exposure, there is minimal damage at 90 μg ai $L^{-1}$, suppressed growth at 140 to 280 μg ai $L^{-1}$, and death at > 400 μg ai $L^{-1}$ or from a direct canopy application.

Native eelgrass provides valuable ecological services and is a protected species (Shafer et al. 2104). Regulatory agencies have expressed concerns over nontarget impacts to native eelgrass that could occur from using an herbicide to control Japanese eelgrass (Bulthuis 2013, Shafer et al. 2014, WA Dept. Ecology 2014). Overall, these results indicate that the nontarget impact of imazamox to native eelgrass could occur if it was directly sprayed, or if water moving off treated areas concentrated imazamox to high enough levels to exceed the dose–exposure threshold. By treating early enough in the season to ensure minimal water on-site during treatments, by not directly spraying pools or drainage swales on-site, and by leaving a 10-m buffer around lower edges of treated sites, nontarget damage to native eelgrass is likely to be negligible. The National Pollutant Discharge Elimination System permit issued for this use contains these precautions and extends the 10-m buffer around the entire treated site (WA State Dept. of Ecology 2014).

Unlike native eelgrass, the risk to microalgae from a direct application of imazamox appears minimal (Table 4). There was no observed effect on *U. intestinalis*, *U. flexuosa*, or *P. hendryi* var. *deliquescens* at rates up to 6-fold beyond the recommended 0.14 kg ai $ha^{-1}$ rate. Similar studies on red algae (*Griffithsia pacifica* Kylin) and marine diatom (*Skeletonema costatum* (Greville) Cleve) (ENVIRON 2012) failed to generate an effect at the anticipated environmental exposure concentrations.

## Imazamox concentrations in water, sediment, and eelgrass

Median water concentration in the first on-site flood water was 61 μg ai $L^{-1}$. After the flood water left the site, the median concentrations at the 3-, 30-, 60-, and 120-m locations were 44, 7, 0, and 0 μg ai $L^{-1}$, respectively (Table 7). The imazamox concentration in water in the second flood tide to cover the site was 6.0 μg ai $L^{-1}$. Water sampled within the treated zone from two shallow pools had posttreatment imazamox concentrations of 181 and 541 μg ai $L^{-1}$. Water moving off the site in a drainage swale had imazamox concentrations of 32, 7.6 and < 1 μg ai $L^{-1}$ at 30, 60, and 120 m. Means ± SE imazamox concentrations in sediment and Japanese eelgrass, 24 h after treatment, following two tidal flushes, were 5.9 ± 2.14 μg ai $L^{-1}$ and 1,016 ± 256 μg ai $L^{-1}$, respectively.

These data were used to determine the environmental exposure in vegetation, sediment, and water for the risk

Table 5. Average Percentage of Reduction in Japanese Eelgrass Seedling Density as a Function of Imazamox Rate without Surfactant.

| Imazamox (kg ai $ha^{-1}$) | Site 10 | Site 11 | Site 12 |
|---|---|---|---|
| | Reduction in seedling density (%) 1 mo after treatement[1,2] | | |
| 0 | 0 b | 0 b | 0 b |
| 0.022 | 96 a | 100 a | 100 a |
| 0.044 | 94 a | 100 a | 100 a |
| 0.066 | 96 a | 100 a | 100 a |
| 0.088 | 96 a | 97 a | 100 a |

[1] Percentage of change in seedlings per plot between 0 and 1 mo after treatment.

[2] Means within a column followed by same letter do not significantly differ (Waller-Duncan, $\alpha = 0.05$).

Table 6. Percentage of Increase in Shoot Growth of Native Eelgrass in Tide Pools, 1 and 2 Months after Treatment, with Imazamox as a Function of Pool Water Depth.

| | Months after Treatment | |
|---|---|---|
| | 1 | 2 |
| Depth of water (cm) | % increase in mean shoot length[1,2] | |
| 0 | Dead a | Dead a |
| 5 | −50 bc | 20 b |
| 10 | −21 cd | 24 b |
| 15 | 8 d | 41 b |

[1] Repeated measures of the same shoots 0, 1, and 2 mo after treatment.

[2] Treatment difference was analyzed by Kruskal-Wallis one-way ANOVA on ranks, and means within a column followed by same letter do not significantly differ according to Fisher's Protected LSD test ($\alpha = 0.05$).

Table 7. Imazamox Concentration in the Leading Edge of the Incoming Tidal Water following a Treatment of a 30- by 70-Meter Japanese Eelgrass Site on Sandy Sediment, Site 14.

| | Imazamox (μg ai $L^{-1}$ ) | | |
|---|---|---|---|
| Sample Locations | Maximum | Minimum | Median |
| 3 m inside plot upper edge | 82 | 24 | 61 |
| 3 m outside plot upper edge | 79 | 35 | 44 |
| 30 m outside plot upper edge | 83 | 5 | 7 |
| 60 m outside plot upper edge | 18 | < 1 | 0 |
| 120 m outside plot upper edge | 6 | 0 | 0 |

assessment on imazamox during estuarine use (ENVIRON 2012). For consumption of treated eelgrass, the hazard quotient for acute-ingestion exposure, subchronic ingestion dose, and chronic subacute-ingestion dose for three indicator species: mallard (*Anas platyrhynchos* L.), rainbow trout (*Oncorhynchus mykiss* Walbaum), and Dungeness crab (*Metacarcinus magister* Dana), ranged from 0.0001 to 0.003 (ENVIRON 2012). These are several orders of magnitude below what would be considered a hazard. Nevertheless, hunters have expressed concern that certain waterfowl species, like wigeon (*Anas americana* Gmelin), which forage on Japanese eelgrass, could be affected by consuming eelgrass treated with imazamox. The approximately 1 mg ai $L^{-1}$ of imazamox found in Japanese eelgrass 24 h after treatment is three orders of magnitude less than the 1,950 mg ai $L^{-1}$ avian $LD_{50}$ (ENVIRON 2012). In addition, the imazamox residue would be very short-lived. The shoots rapidly degrade posttreatment and, like other aquatic plants, the desorption rate is rapid. Vassios (2010), for example, found that 46% of imazamox was rapidly desorbed in Sago pondweed [*Stuckenia pectinata* (L.) Börner] in the first 12 h after treated plants were transferred to water with no herbicides.

A review of the potential risks of imazamox suggests that nontarget aquatic macrophytes could be at risk if imazamox concentrations were to build up in aquatic sediments (New York State Dept. of Environ. Conserv. 2003). These results suggest that concerns about high sediment concentration are not warranted. Because of the high solubility of imazamox in water (> 4000 mg ai $L^{-1}$), rapid tidal flushing and low binding affinity for sediment ($K_{oc}$ [binding coefficient] = 5.3), the level of sediment imazamox found (5 μg $kg^{-1}$) is likely to drop below the detection limits (0.5 μg $kg^{-1}$) within a short period. Overall, the levels of imazamox found in water, sediment, and vegetation in this study were several orders of magnitude lower than the $LC_{50}$ toxicity of imazamox for the most sensitive aquatic organisms (> 100 mg ai $L^{-1}$) (EPA 2009, ENVIRON 2012, WA Dept. Ecology 2014). Based on these results, the short exposure to the imazamox concentration found in this study is unlikely to present a risk to the aquatic ecosystem.

### Conclusions for integrated control

These trials indicate imazamox is an efficacious treatment for invasive eelgrass and, when applied under the right conditions, it is not likely to result in nontarget impacts to estuarine species of concern. The lowest effective doses of imazamox to manage established plants and seedlings are 0.14 and 0.04 kg ai $ha^{-1}$, respectively. Application too early in the spring would miss controlling newly germinating Japanese eelgrass seedlings, which peak in mid March and tail off into early June (Ruesink et al. 2010). Application later in the season is problematic because of the rapidly growing Japanese eelgrass canopy slowing or preventing tidal dewatering during low tides and ultimately leaving the canopy with a protective water layer. Dense mats of Japanese eelgrass reduce water flow by up to 40% compared with nonvegetated mudflats (Tsai et al. 2010). In addition, application of imazamox to a site that doesn't fully dewater increases the potential of nontarget impact to native eelgrass. In these situations, imazamox more easily drains off-site, thus concentrating in the swales containing native eelgrass. The ideal spray window would be from late April to early June, after most seed germination occurs, but while the tidal flats are still dewatered during low tides. Since these sites are mostly dry during a low tide, applications of imazamox to control Japanese eelgrass during this period would help minimize the risk for nontarget impacts to native eelgrass. Risk to native eelgrass can also be minimized by avoiding spraying near or over pools or near drainage swales containing native eelgrass.

## SOURCES OF MATERIALS

[1] TeeJet technologies, P.O. Box 7900, Wheaton, IL 60187-7901.

[2] Imazamox (Clearcast), SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

[3] Competitor, Wilbur-Ellis Co., P.O. Box 16458, Fresno, CA 93755.

[4] Amber HDPE bottles, Nalge Nunc International Corporation, 75 Panorama Creek, Dr., Rochester, MY 14625.

[5] SePRO Lab Services, 16013 Watson Seed Farm Rd., Whitakers, NC 27891-9114.

[6] LC-20 HPLC systems, Shimadzu Scientific Instruments, 7102 Riverwood Dr., Columbia, MD 21046.

[7] ISO 17025 Standard: General requirements for the competence of testing and calibration laboratories, International Organization for Standardization, ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland.

[8] Pacific Agricultural Laboratory, 12505 NW Cornell Rd., #4, Portland, OR 97229.

[9] SYSTAT Software, Inc., 1735 Technology Dr., Suite 430, San Jose, CA 95110.

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