J. Aquat. Plant Manage. 41: 1- 6

Persistence and Non-target Impact of Imazapyr Associated with Smooth Cordgrass Control in an Estuary

KIM PATTEN[1]

ABSTRACT

The herbicide (±-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid (imazapyr) has shown potential to control smooth cordgrass (*Spartina alterniflora* Loisel), a noxious weed in many estuaries throughout the world. Research was conducted under tidal estuary conditions in Willapa Bay, Washington, to determine imazapyr's persistence and aquatic risk and impact to non-target estuary species. Persistence of imazapyr in water and sediment followed an exponential decay. The maximum levels of imazapyr found in water and sediment samples after application were 3.4 µg/ml and 5.4 µg/g, respectively, which are 5 to 6 orders of magnitude lower than levels needed to affect aquatic invertebrates and fish. Imazapyr in water and sediment samples approached the zero asymptote at 40 and 400 hours respectively, with half-lives of <0.5 and 1.6 days, respectively. Water collected 6 or 60 m from outside the spray zone with the first incoming tide was 99% lower than the maximum water concentration collected at the edge of the spray zone. In comparison to imazapyr applied to bare mud, an application to a smooth cordgrass canopy resulted in 75% less herbicide in the underlying sediment. The amount of imazapyr found in the interstitial water within the sediment was slightly less than that found in sediment. Japanese eelgrass (*Zostera japonica* Aschers and Graebn) was killed by imazapyr when it was applied to a dry canopy, but sites were reinfested within 1.0 year of treatment. Applications of imazapyr to native eelgrass (*Zostera marina* L.) and Japanese eelgrass covered by a thin film of tidal water had no effect. Smooth cordgrass treated with imazapyr was colonized by Japanese eelgrass and *Salicornia* (*Salicornia virginica* L.) within 12 to 20 months MAT. The osmoregulatory capacity of Chinook salmon (*Oncorhynchus tshawytscha* (WALBAUM)) smolts based on plasma sodium level and gill AT-Pase was not affected by imazapyr at concentrations up to 1600 µg/l. Based on these studies, imazapyr had a short half-life in estuary conditions and there was a very large margin of safety between the maximum concentration of imazapyr that occurs following application and the concentrations that could result in toxicity to invertebrates and fish. There was no direct or indirect evidence of long-term persistence or impact following its use in an estuary. It is unlikely that imazapyr used to control smooth cordgrass will pose a risk to non-target estuarine organisms.

Key words: aquatic risk assessment, Chinook salmon, eelgrass, estuary, glyphosate, seawater challenge tests, *Salicornia virginica* L, *Spartina alterniflora, S. anglica, S. X townsendii, Zostera japonica, Zostera marina* L.

INTRODUCTION

Estuaries serve as productive rearing grounds for numerous species of fish, and important breeding, migration and wintering grounds for a variety of migrating birds and other wildlife. They form an economic base for many communities involved with commercial fishing, mariculture, tourism and shipping. These waters, however, are among the most heavily-invaded by nuisance species of all ecosystems in the world (Grosholz 2002). Currently, the biological viability of many of these estuaries on the West Coast, USA, and New Zealand is being compromised due to invasion by a tall, deep-rooted perennial saltmarsh grass known as smooth cordgrass, *S. alterniflora.* Several other species are problematic: *S. anglica* (CE Hubbard) in the West Coast, USA, Europe, Australia and New Zealand; and *S. X townsendii* (H and J Groves) in Western Europe and Australia (Daehler and Strong 1996, Gray et al. 1997, Kriwoken and Hedge 2000, Shaw 1997). Long-term ecological impacts of invasive *Spartina* include drastic decline in shorebird populations, eelgrass beds, and waterfowl (Gray et al. 1997).

Eradication or management of *Spartina* is limited by lack of effective chemical, mechanical or biological controls. *N*-(phosphonomethyl) glycene (glyphosate) is the only herbicide registered for aquatic use in an estuary in the USA. The label rate of 4.2 kg ae/ha for aerial applications has been ineffective. While the higher label rate for hand application (5% v/v applied at 934 l/ha) is more effective, it lacks consistency and may cost more than $2000/ha. Only 2 ha per airboat per day can be effectively treated, and application requires exacting weather conditions and expensive equipment for transporting water, equipment, crew and chemicals (Patten and Stenvall 2002). Alternatives to glyphosate in Australia and New Zealand include the herbicides fluazifop-P butyl ester (Fusilade) and haloxyfop methyl ester (Gallant), respectively (Pritchards 1992, Shaw 1997). In the United States, excellent smooth cordgrass control has been achieved with imazapyr (Patten 2002). U.S. EPA registration efforts for the use of imazapyr in freshwater systems are ongoing. Use of imazapyr within estuaries, however, requires additional risk

[1]Washington State University Long Beach Research and Extension Unit, 2907 Pioneer Road, Long Beach, Washington 98631, pattenk@cahe.wsu.edu. Received for publication June 24, 2002 and in revised form September 16, 2002.

imazapyr and glyphosate in a series of experiments between 1999 and 2000. On August 16, 1999, 1 m^2 plots were treated with 0.84 or 1.68 kg ae/ha imazapyr, or 3.63 or 14.4 kg ae/ha of glyphosate, 480 g ae/l Isopropylamine salt. Hasten and R11 were used for surfactants (1%v/v) with imazapyr and glyphosate, respectively. Treatments were applied with a CO_2 back-pack sprayer, using a 1.5 m boom equipped with Teejet 11001 nozzles. Spray volume was 94 l/ha, except for 14.4 kg ae/ha glyphosate, which was delivered at 940 l/ha. There were four replications per plot. Trials were located at six sites for Japanese eelgrass and two sites for native eelgrass. Two Japanese eelgrass sites were high enough in the tidal zone to allow for herbicide application to a dry canopy (4 to 6 hours dry time). Four Japanese eelgrass and all native eelgrass sites were low enough in the tidal zone so that their canopies were always covered with a thin layer of water during herbicide applications. Plots were evaluated based on a visual rating of percent ground covered by eelgrass canopy compared to the control 9 to 14 months after treatment (MAT). In 2000, a two factorial experiment with herbicides applied at 1.68 kg ae/ha of imazapyr or 7.5 kg ae/ha of glyphosate and application dates July 15, 2001 or September 15, 2001, was applied to four replicated 10 m^2 stands of Japanese eelgrass. The experiment was conducted on dry and wet Japanese eelgrass sites as previously described. Percent canopy cover and canopy dry density were evaluated on four 0.25m^2 per plot and compared to an untreated control. Data were arcsine transformed to stabilize variances and subjected to ANOVA. Means were separated by Duncan's New Multiple Range Test (α =0.05). Data are presented as nontransformed values.

Post-Treatment Colonization by Native Tidal Marsh Plants. The potential for imazapyr to persist in sediment following smooth cordgrass control and to affect the subsequent colonization by two estuary plants, Japanese eelgrass and *Salicornia,* was measured. The site used to evaluate eelgrass colonization was near the Palix River and was treated with imazapyr at 1.68 kg ae/ha and glyphosate at 18.0 kg ae/ha in August 1998 (Patten 2002). Japanese eelgrass colonization was measured by visually estimating percentage of clonal coverage 20 MAT on plots that had greater than 85% control 12 MAT. Data were collected on eight plots per treatment. Plot size was 9 m^2. The site used to evaluate *Salicornia* colonization was near Oysterville and was treated in July through October with imazapyr at 1.68 kg ae/ha and glyphosate at 8.4 kg ae/ha in 1999 (Patten 2002). Smooth cordgrass control achieved on the imazapyr and glyphosate plots was 40 to 97% and 0%, respectively. *Salicornia* colonization was based on number of plants per plot. Data were collected on 23 plots per herbicide. Plot size was 9 m^2.

Chinook Salmon Smolts. Seawater challenge tests (Clarke and Blackburn 1977) were conducted by Parametrix Environmental Toxicology Laboratory (58008 Lake Washington Blvd. N.E., Suite 200, Kirkland WA 98033) using their Protocol #1023 to assess the potential effect of acute exposure of imazapyr on the osmoregulatory capabilities of Chinook salmon smolts. Smolts weighing 5.7 to 15.1 grams live-weight were obtained from Grovers Creek Salmon Hatchery, Poulsbo, WA, and acclimated for 3 weeks in spring water with a salinity of 0 g/l and a hardness of 74 to 90 mg/l. Five salmon smolts were placed in each replicated test chamber with 95 L of water at 13 C, containing imazapyr at 0, 50, 100, 200, 400, 800, or 1,600 µg/l. Water was obtained from Gold Creek Trout Farm in Woodinville, WA. There were two chambers per concentration. Salmon were not fed 48 hours prior to or during the test. Dissolved oxygen, pH, and temperature were recorded daily. Mortality was recorded daily and any dead fish were removed immediately. The seawater challenge was initiated following the 96-hour herbicide exposure. At 96 hours, test chambers were drained to 10 to 15% of initial volume by siphon and refilled with fresh seawater. Final salinity was measured in all test chambers with a hand-held refractometer. After 24 hours, live fish were netted, killed by a blow to the head, measured for length, and then weighed. Blood was collected by cutting off the tail and collecting a sample into an ammonia heparinized microhematocrit capillary tube, which was then placed into a cooler packed with dry ice until centrifugation. Immediately following blood collection, gill arches were removed and gill filaments were placed in a cold sucrose/EDTA/imidizole buffer and stored over dry ice for shipping. Hematocrit tubes were centrifuged in an IEC clinical centrifuge for 15 minutes to separate plasma from the cells. Plasma samples were analyzed for sodium levels and gill tissue analyzed for gill ATPase activity. Plasma sodium concentrations were determined with a Microelectrodes, Inc., combination micro-sodium probe calibrated with seven standards of NaCl, ranging in concentration from 100 to 200 mM and recalibrated every 20 to 30 samples. Gill ATPase assays were performed using the Zaugg (1982) method except that color was developed by stannous chloride, not UV absorption. Standards for protein and phosphate were included with each group of 20 to 30 samples. Data were analyzed using linear and nonlinear regression analysis and are presented as means and standard errors using all fish.

RESULTS AND DISCUSSION

Imazapyr Persistence

Persistence of imazapyr in water and sediment followed exponential decay upon application to estuary mud (Figures 1 and 2). The maximum level found in water and sediment after application was 3.4 µg/ml and 5.4 µg/g, respectively. Water and sediment concentrations approached the zero asymptote at 40 and 400 hours, respectively. Imazapyr concentration in water decreased rapidly within a short distance away from the spray zone. Water collected just 6 or 60 m from outside the spray zone at the 1st incoming tide had imazapyr concentration equivalent to water collected at the 7th tide at the immediate edge of the spray zone. In comparison to imazapyr applied to bare mud, an application to a smooth cordgrass canopy resulted in a five-fold (5.4 to 1.4 µg/g) reduction in herbicide in the underlying sediment. The amount of imazapyr found in the interstitial water within the sediment was slightly less than that found in sediment at the 1st and 28th tidal sequence.

The maximum concentration of 5.4 µg/g fw in sediment following a direct herbicide application converts to 2.7 µg/g dw, which is within the range of 0.116 to 20.8 µg/g dw reported for glyphosate (Kilbride 2001, Paveglio et al. 1996). In contrast to glyphosate, however, the rate of imazapyr dissi-

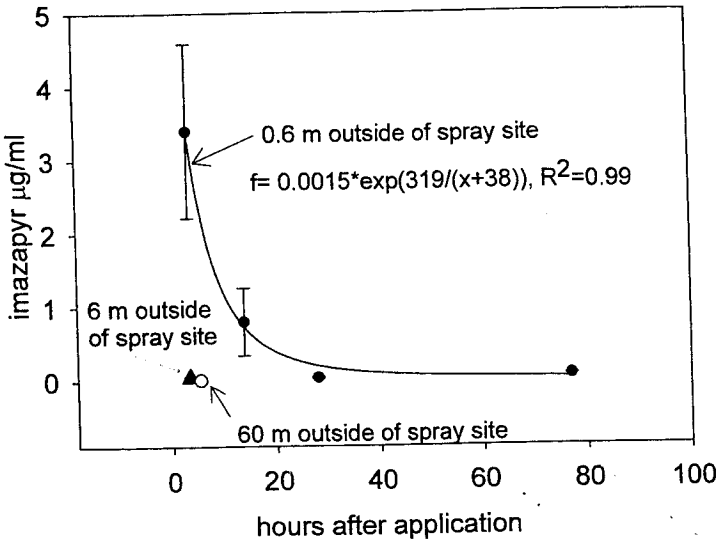

Figure 1. Persistence of imazapyr in estuarine water in Willapa Bay, WA, following the direct application of herbicide to an unvegetated tide flat. Data represent mean values ± SE.

pation in sediment was much more rapid. Imazapyr sediment residues were reduced to approximately 1% of early post treatment levels 29 DAT; however, Paveglio et al. (1996) found glyphosate was only reduced to about 30% at 51 DAT. Based on these data, the first order half-life of imazapyr in estuary sediment was 1.6 days, whereas for glyphosate, half-life in estuary sediment exceeded 119 days at some sites (Paveglio et al. 1996).

The initial maximum imazapyr concentration of 3.4 µg/ml found in seawater just 0.6 m outside the treated zone occurred after the incoming tidal water passed over the treated area was greater than the 0.26 µg/ml reported for glyphosate (Paveglio et al. 1996). Dilution of imazapyr concentration by the tidal prism occurred very rapidly. Concentrations of samples taken only minutes later, just an additional 5.4 m outside the spray zone, were reduced by 99%. Imazapyr concentration in interstitial water was not appreciably different from sediment concentration at those same time periods. First order half-life of imazapyr in estuary water was < 0.5 day.

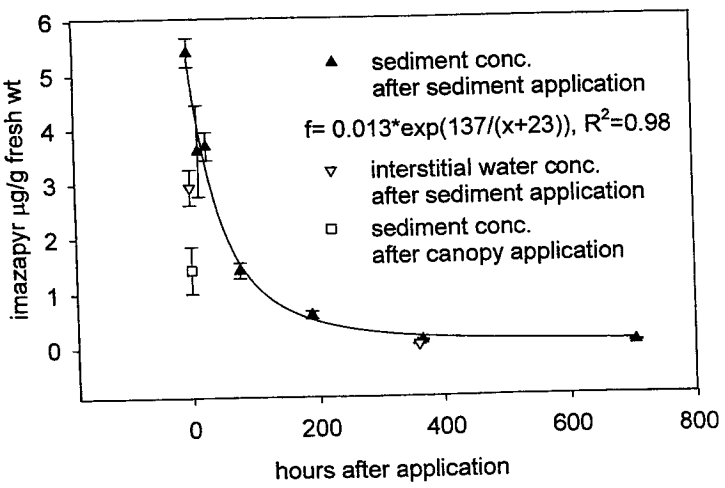

Figure 2. Persistence of imazapyr in estuarine sediment in Willapa Bay, WA, following the direct application of herbicide to an unvegetated tide flat. Data represent mean values ± SE.

Determining the relative risk of imazapyr to aquatic invertebrates and fish species requires an estimate of typical exposure concentration. It is realistic to use a geometric mean of imazapyr concentrations over 76 hours and use values obtained within the spray zone for sediment and 0.6 to 60 m outside the spray zone for water. These values for water and sediment were 0.1 µg.ml and 3.2 µg/g fw, respectively. The LC50 of imazapyr for aquatic invertebrate and fish is >100 mg/l, and the No Effect Level (NOEL) for invertebrates and fish is 97 and 43 to 92, respectively (Rubin 1999). The margin of safety of imazapyr is > 10,000 for fish and aquatic invertebrates in water and > 40 fold for benthic invertebrates. These large margins of safety are similar to the values reported for the maximum glyphosate exposure (Pavgelio et al. 1996).

Additional margins of safety should be realized owing to the attenuation of active ingredients by the dense smooth cordgrass canopy during an actual spray event. Based on these data (Figure 2), the smooth cordgrass canopy intercepts at least 75% of the imazapyr following a 100 l/ha spray volume application.

Non-target Effects on Plants

Japanese eelgrass was killed by a direct application of herbicides, if the eelgrass canopy was dry at application time (Tables 1 and 2). Under the same conditions, damage from imazapyr was greater than that from glyphosate. This response was completely nullified if applications were made to Japanese eelgrass with a film of water over the canopy. Under these conditions, imazapyr had no effect on native eelgrass. All sites where Japanese eelgrass was killed by imazapyr completely recovered within 12 MAT. In smooth cordgrass meadows treated with herbicides there was no evidence that residual herbicide affected the colonization of *Salicornia* or Japanese eelgrass (Table 3). Colonization by these species occurred more in imazapyr-treated plots than glyphosate-treated plots.

The use of imazapyr in an estuary does have the potential to temporarily affect eelgrass populations. Similar to glyphosate (Bulthuis and Shaw 1993), this is most likely to occur when applications are made directly to eelgrass when there is no protective film of water over the plants (upper intertidal). The risk could be minimized by judicious spray applications. Should a deleterious overspray occur, long term consequences at > 1 year are minor, as Japanese eelgrass appears to quickly re-establish itself in treated plots. Furthermore, the confinement of native eelgrass to subtidal and lower intertidal areas severely limits the occasions that foliage would be dry enough to be susceptible to imazapyr.

Under terrestrial conditions, imazapyr can be persistent in some soils (Vizantinopoulus and Lolos, 1994), causing problems with replanting. Under estuarine conditions, there were no indications that persistence would be an issue. Sediment residue data indicated rapid exponential decay. Plant data indicated that species like Japanese eelgrass or *Salicornia* could rapidly establish in eelgrass beds or smooth cordgrass

TABLE 1. NON-TARGET EFFECTS OF IMAZAPYR AND GLYPHOSATE ON JAPANESE AND NATIVE EELGRASS AT SEVERAL LOCATIONS IN WILLAPA BAY, WA, IN 1999 TO 2000[a].

		% Canopy cover											
		Site 1 dry Japanese eelgrass		Site 2 dry Japanese eelgrass		Site 3 dry Japanese eelgrass		Site 4 dry Japanese eelgrass		Site 5 dry Japanese eelgrass		Site 6 and 7 dry Japanese eelgrass	
Herbicide	Rate kg ae/ha	3 MAT[b]	11 MAT	7 MAT	11 MAT	7 MAT	11 MAT	7 MAT	11 MAT	7 MAT	11 MAT	7 MAT	11 MAT
Imazapyr	0.84	2 a	100	20 a	100	—	—	100	100	—	—	100	100
Imazapyr	1.68	9 a	100	30 ab	100	90	100	100	100	23	100	100	100
Glyphosate	3.63	40 b	100	73 b	100	65	100	100	100	28	100	100	100
Glyphosate	14.40	34 a	100	53 ab	100	—	—	100	100	—	—	100	100

[a]Mean separation within columns by Duncan's New Multiple Range Test (P = 0.05).
[b]MAT – months after treatment.

TABLE 2. NON-TARGET EFFECTS OF IMAZAPYR AND GLYPHOSATE ON JAPANESE EELGRASS AT TWO LOCATIONS IN NAHCOTTA, WA, IN 2000, 2001[a,b].

		Canopy cover (%)						Shoot dry wt (g/m²)			
		October 5, 2000		June 5, 2001		August 31, 2001		September 2, 2000		September 9, 2001	
Treatment	Rate kg ae/ha	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Imazapyr	1.68	0 a	100	88 a	100	93	100	4 b	90	51	92
Glyphosate	7.5	100 b	100	99 b	100	97	100	22 a	93	43	85
Control		100 b	100	100 b	100	98	100	28 a	88	50	83

[a]Data for application dates were pooled, since time of application was not significant.
[b]Mean separation within columns by Duncan's New Multiple Range Test (P = 0.05).

meadows that were killed by imazapyr. In contrast, uncontrolled smooth cordgrass meadows remained monocultures, completely uncolonized by native plant species.

Non-target Effect on Chinook Salmon Smolts

The osmoregulatory capacity of Chinook smolts based on plasma sodium level and gill ATPase was not affected by imazapyr at concentrations up to 1600 µg/l (Figure 3). Imazapyr, even at levels 470-fold above the maximum water concentrations found in this study (worst-case scenario), did not cause a decrease in the osmoregulatory capacity of Chinook salmon smolts. Although the gill ATPase activities of 12 to 65 µmolP_i per mg protein/hour found in this study were lower than what would be expected for fully smolted salmon (Zaugg 1982), the mean control plasma sodium concentration of 150.2 ± 11.2 meq/l (SE) was under the acceptable osmoregulatory levels of ≤170 meq/l achieved by fully developed Chinook salmon smolt after 24 hours of seawater exposure (Clarke and Blackburn 1977). This ability of control organisms to reach acceptable plasma sodium levels in

TABLE 3. RESIDUAL HERBICIDE EFFECTS ON THE INFESTATION OF MUDFLATS WITH JAPANESE EELGRASS AND SALICORNIA FOLLOWING SMOOTH CORDGRASS CONTROL WITH HERBICIDES IN WILLAPA BAY, WA IN 1998.

		Japanese eelgrass	Salicornia
Herbicide	Rate kg ae/ha	20 MAT (% Coverage ± Std. Err.)	12 MAT (plant/m² ± Std. Err.)
Imazapyr	1.68	34 ± 16	1.06 ± 0.2
Glyphosate	18.00	11 ± 9	—
Glyphosate	8.40	—	0
Control		0	0

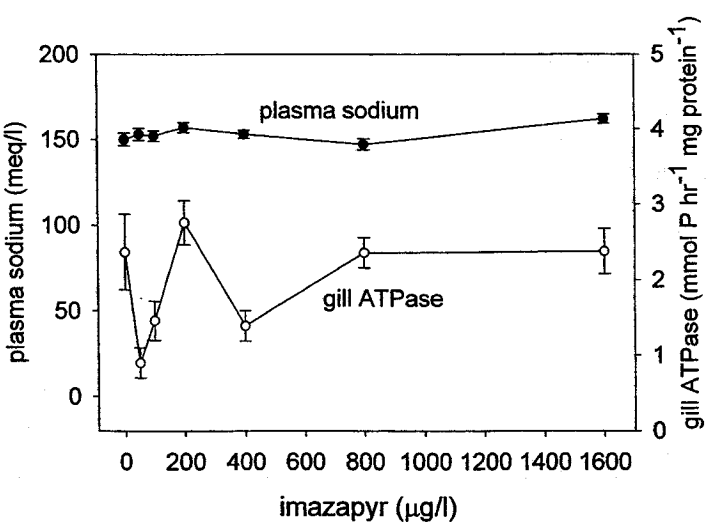

Figure 3. Plasma sodium concentrations and gill ATPase of Chinook salmon smolts as a function of imazapyr concentration during a saltwater challenge test. Data represent mean values ± SE. Regression analysis was not significant.

assessments. Fate and persistence of imazapyr under tidal conditions may vary considerably from terrestrial or freshwater conditions. In the Pacific Northwest there are special regulatory concerns on any activities that may pose a risk to salmon. Non-target impacts of imazapyr on the osmoregulatory capabilities of salmon smolts, or critical estuary habitat species, like eelgrass, are critical to know for fully assessing risk. The objectives of this study were to evaluate imazapyr's use in an estuary in terms of: 1) persistence; 2) short-term aquatic risk assessment; 3) risk to non-target species; and 4) effect on salmonid smoltification processes.

MATERIAL AND METHOD

Study Area

Research was conducted under tidal estuary conditions in Willapa Bay, Washington, between 1999 and 2001. 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.

Imazapyr Persistence

Mudflat Applications. Imazapyr, 480 g ae/l isopropylamine salt, was applied at 1.68 kg ae/ha to an area 30 by 33 m of bare mudflat on the upper intertidal zone of Willapa Bay near the outlet of the Bear River (46° N, 124° W). The treatment area was aligned parallel with the tidal wetting front to enhance the uniformity of water sample collection. The transient time for the tide to cover the 0.28-m elevation gain from the low end to the high end of the plot averaged 13 minutes. Application was made with a CO_2 back-pack sprayer on August 27, 2001 between 6:45 and 7:20 a.m., approximately 1.5 hours after the tide receded off the site, using a 3-m boom equipped with six Teejet 11001 nozzles pressurized to 345 kPa, applying 97 l/ha. Mudders® (Forestry Suppliers, Inc., POB 8397 Jackson, MS 39284), expandable outershoes, were worn by the applicator to allow walking across the soft mud at a constant pace. Agridex at 1% (v/v) was used as a surfactant. Water and sediment temperatures at application were 13 C and 19 C, respectively. The soft sediment was 49% moisture and 51% dry weight, pH 7.9, organic matter 5.4%, and 18.3, 65.5, 16.2% sand, silt, clay, respectively.

Water samples were collected by burying a 1.0-l wide-mouth sample jar so that only 1.0 cm of jar lip showed above the mud. The jars were placed 0.3, 6 and 60 m beyond the upper tidal end of the plot. There were three samples collected per location, each spaced 10 m apart along a linear plane of the tidal front. Jars were capped by hand immediately after they filled with the incoming tide water. An initial control sample was collected 76 m in front of the lower tidal end of the plot in a similar fashion as the lst tide advanced, 3.1 hours after treatment (HAT). For the location 0.3 cm past the higher tidal end of plot, water samples were collected following the 1st, 2nd, 3rd and 7th tides to cover the plots after application. This corresponded to 3.5, 14, 28 and 77 HAT. For the 6 and 60 m locations past the end of the plot, water samples were only collected following the 1st tidal flush after application. Samples were frozen 0.5 hours after collection. Since there was only minor wave action with the incoming tide, the water samples were normally fairly free of sediment.

Sediment samples were collected in the treatment area along three transects parallel to the incoming tide, approximately 10 m apart. Transects were in the middle of the boom pattern 1.5 m away from the walking line. Intact sediment cores were collected by pressing a new plastic square container with dimensions of 8 cm in depth by 3 cm in width into the sediment, lifting out the intact core and removing any sediment extending beyond the container lip. There were three subsamples collected (10 m apart) and pooled for each transect. New nitrile gloves were used for each transect line for handling sediments to avoid cross-contaminations. Untreated samples were collected from the same tidal elevation, but 100 m south of the application site. Treatment samples were collected after application and after the 1st, 2nd, 6th, 14th, 28th and 56th tidal sequences following application (1, 14, 27, 77, 184, 366, and 703 HAT). Samples were frozen 0.5 hour after collection.

Canopy Applications. An additional population of smooth cordgrass which was 1.7 m tall and approximately 2 m above MLLW was selected for a study to determine how much imazapyr penetrates through a dense plant canopy and reaches the mudflat. These plants were sprayed with 1.68 kg ae/ha of imazapyr at 97 l/ha spray volume. Three replicated plots of 3 by 4 m in surface area were treated. Three sediment subsamples were collected 0.5 HAT from under the canopy and pooled for each replication in the manner previously described.

Analysis of Residue Samples. Water and sediment samples were shipped and received frozen and stored at <-4 C until analysis. For analysis, samples were allowed to reach room temperature and made homogenous through mechanical agitation before sample aliquots were taken. The interstitial water from selected sediment samples was obtained utilizing 0.45 µm inert filter centrifuge tubes in conjunction with high-speed centrifugation. All water samples were filtered and serial dilutions were brought to a known volume in 70/30 ACN/aqueous ammonium formate buffer in preparation for LC MS/MS analysis. For sediment samples, imazapyr was extracted from the matrix utilizing a three-step extraction. This consisted of three shaking/centrifugation procedures utilizing 0.1 N NaOH for the first procedure and 70/30 ACN/0.1 N NaOH for the subsequent procedures. The decants were combined in a volumetric flask, brought to a known volume, and serial diluted utilizing 70/30 ACN/aqueous ammonium formate buffer in preparation for LC MS/MS analysis. Samples were analyzed using a Perkin Elmer series 200 PE HPLC with a Sciex API 3000 Biomolecular Mass Analyzer. The column was Columbus C-18 100 × 2 mm with a Mobile Phase/Gradient: A: 100% (H_2O/4 mM ammonium formate/0.1% formic Acid); B: 100% (methanol/4 mM ammonium formate/0.1% formic acid); flow rate was 300 µl/min. The composition flow was 90% A, 10% B; 10% A, 90% B; 10% A, 90% B; 90% A, 10% B, and 90% A and 10% B at 0, 0.5, 2.5, 2.6 and 5 minutes respectively. The limit of quantification was 0.01 mg/kg.

Non-target Effects

Direct application to Eelgrass. Established stands of native eelgrass and Japanese eelgrass were directly over-sprayed with

spite of their gill ATPase activities indicates that the population used in this study osmoregulated well.

ACKNOWLEDGMENT

The author would like to acknowledge the Washington State Commission on Pesticide Registration for funding this project.

LITERATURE CITED

Bulthuis, D. A. and T. Shaw. 1993. Effects of application of glyphosate on the eelgrasses *Zostera marina* and *Zostera japonica* in Padilla Bay, WA. Padilla Bay National Estuarine Res. Tech. Rept. No. 8. 40 pp.

Daehler, C. C. and D. R. Strong. 1996. Status, prediction, and prevention of introduced cordgrass (*Spartina* spp.) invasions in Pacific estuaries, USA. Biol. Conserv. 8:51-58.

Clarke, W. C. and J. Blackburn. 1977. A seawater challenge test to measure smolting of juvenile salmon. Environ. Canada Fisheries and Marine Service. Tech. Rpt. 705. 11pp.

Gray, A. J., A. F. Raybould and S. L. Brown. 1997. The environmental impact of *Spartina Anglica*: past, present and predicted, p. 29-45. *In:* K. Patten (ed.). Proc. 2nd Inter. *Spartina* Conf.

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. Trends Ecol. Evol.17:22-27.

Kilbride, K. M. and F. L. Paveglio. 2001. Long-term fate of glyphosate associated with repeated Rodeo applications to control smooth cordgrass (*Spartina alterniflora*) in Willapa Bay, Washington. Arch. Environ. Contam. Toxicol. 40:179-183.

Kriwoken, L. K. and P. T. Hedge. 2000. Exotic species and estuaries: managing *Spartina anglica* in Tasmania, Australia. Ocean Coast. Manage. 43:573-584.

Patten, K. 2002. *Spartina* control with Imazapyr. Weed Technol. 16:826-832.

Patten, K. and C. Stenvall. 2003. Control of smooth cordgrass (*Spartina alterniflora*): a comparison between various mechanical and chemical control methods for efficacy, cost and aquatic toxicity. Proc. 11th Int. Conf. on Aquatic Invasive Species. p. 306-316.

Paveglio, F. L, K. M. Kilbride, C. E. Grue, C. A. Simenstad and K.L. Fresh. 1996. Use of Rodeo and X-77 spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: 1. Environmental fate. Environ. Toxicol. Chem. 15:961-968.

Pritchards, G. 1992. Control of *Spartina* with herbicides, p. 23-29. *In:* G. Faser-Quick and A. Philips (eds.). Proc. of a conference on the problem of *Spartina* - The sea has weeds too. Inverloch, Victoria.

Rubin, L. 1999. Imazapyr (Arsenal, Chopper, and Stalker Formulations). Final Report to the USDA, Forest Service SERA TR 98-21-12-01b.

Shaw, W.B. 1997. *Spartina* Ecology, Control and eradication - recent New Zealand Experience, p. 32-38. *In:* K. Patten (ed.). Proc. 2nd Int. *Spartina* Conference.

Vizantinopoulos, S. and P. Lolos. 1994. Persistence and leaching of the herbicide imazapyr in soil. Bull. Environ. Contam. Toxicol. 52:404-410.

Zaugg, W. S. 1982. A simplified preparation for Adenosine Triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci. 39:215-217.

J. Aquat. Plant Manage. 41: 6- 12

Mechanical and Chemical Control of Smooth Cordgrass in Willapa Bay, Washington

WALTER W. MAJOR III[1], C. E. GRUE[1,3], J. M. GRASSLEY[1], AND L. L. CONQUEST[2]

ABSTRACT

We evaluated four methods to control smooth cordgrass (*Spartina alterniflora* Loisel), hereafter spartina, in Willapa Bay, Washington: mowing, mowing plus herbicide combination, herbicide only for clones, and aerial application of herbicide for meadows. We used a single-hand application of Rodeo® formulated at 480 g L^{-1} acid equivalence (ae) of the isopropylamine salt of glyphosate (Monsanto Agricultural Co., St. Louis, MO; currently Dow AgroSciences, Indianapolis, IN) with the non-ionic surfactant LI 700® (2.0% v/v) on clones, and a single aerial application with X-77 Spreader® (0.13% v/v) on large meadows. We compared efficacy using changes in stem density and stem height 1 yr post-treatment. Stem densities and heights within clones were reduced by all treatments. The mowing plus herbicide combination and single-hand spray were equally more efficacious than repeated mowing at two sites, whereas at a third site, the mowing plus herbicide combination was the most efficacious. Aerial application of the herbicide resulted in an average of 91% of intended deposition, but both treatment and control plots showed similar increases in stem density and decreases in stem height. A subsequent aerial application of glyphosate with the non-ionic surfactant, R-11® to the study area the following year resulted in no statistically significant change in stem density on our former treated plot, but stem height decreased. However, on our former control plot, stem density significantly decreased, whereas stem height increased. We conclude that the mowing plus herbicide combination consistently provided the best control of clones, but hand application of the herbicide was almost as efficacious. The aerial herbicide applications we monitored provided little or no control indicating the need to improve efficacy if aerial treatment is to be a viable control strategy.

Key words: estuary, glyphosate, herbicide, mowing, Rodeo®, *Spartina alterniflora*.

[1]Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, WA 98195.

[2]Center for Quantitative Studies and School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, WA 98195.

[3]Corresponding author: cgrue@u.washington.edu.

Received for publication 15 May 2000 and in revised form December 27 2002.