

# Control of *Lepidium latifolium* (perennial pepperweed) and recovery of native plants in tidal marshes of the San Francisco Estuary

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**Abstract** Several management techniques are effective in controlling *Lepidium latifolium* (perennial pepperweed) in rangelands and hay meadows; however, this invader's rapid spread into sensitive aquatic habitats throughout the western US calls for alternative control strategies. To evaluate control methods for use in tidal marshes of San Francisco Estuary, we tested chemical, mechanical, and biological methods in field and greenhouse experiments. In a field experiment in three brackish marshes spanning the estuary, application of the herbicide glyphosate to re-growth of *L. latifolium* following hand-removal reduced *L. latifolium* cover by an average of 80% after 2 years and led to a 60% increase in native vegetation cover. Glyphosate alone was less effective at reducing *L. latifolium* cover (20% decrease) and increasing native cover (34% increase). Preliminary tests of a potential biological control, a native parasitic plant, were not successful, thus plots intended for field trials were instead used to test the newly approved herbicide imazapyr, which showed promise in controlling *L. latifolium*. An additional greenhouse experiment found large reductions in stem lengths with either glyphosate following

clipping or imazapyr with or without clipping, all significantly more so than glyphosate alone. We conclude that an integrated management approach of applying glyphosate following mechanical removal can be effective at reducing *L. latifolium* cover and allowing recovery of native tidal marsh plants, providing a useful solution for controlling smaller, accessible infestations of the invader. Our preliminary tests of imazapyr suggest that it may be very effective at controlling *L. latifolium* in tidal marshes, although further assessment of non-target effects and native plant recovery are needed to evaluate its relative merit.

**Keywords** *Cuscuta* · Dodder · Glyphosate · Imazapyr · Integrated pest management · Invasive species · Native plants · Pickleweed · *Sarcocornia* · Wetlands

## Introduction

Invasive species have become the largest threat, next to habitat loss, to native communities around the world (Manchester and Bullock 2000; Ailstock et al. 2001). Wetland habitats are susceptible to non-native species introductions due to their downstream positions on the landscape, which can increase the probability of foreign propagule arrival as well as

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disturbances such as excess nutrient and sediment delivery that can enhance invasion success (Zedler and Kercher 2004; Tyler et al. 2007). The close proximity of many coastal wetlands to human populations and activities (e.g., shipping, aquaculture, farming, gardening) can further increase opportunity for invader transport and establishment (Grosholz 2002; Holland et al. 2004; Byers 2009). In tidal marshes of California (USA) alone, a growing number of successful plant invaders are displacing native plants and habitats. These include *Spartina alterniflora* (smooth cordgrass), which has hybridized with the native *Spartina foliosa* (Ayres et al. 2004), several subspecies of the sea lavender *Limonium ramosissimum* (M. Page, pers. comm.; G. Archbald, unpubl. data), and *Tamarix* sp. (salt cedar) (Whitcraft et al. 2008), among many others. The consequences of allowing stands of non-native species to proliferate can include reductions in biodiversity, loss of habitat, changes in trophic interactions, and alteration of important ecosystem processes (Chornesky and Randall 2003; Levin et al. 2006; Whitcraft et al. 2008).

*Lepidium latifolium*, perennial pepperweed, has only recently become recognized as a threat to tidal wetlands in California (Andrew and Ustin 2009; Leininger and Foin 2009; Reynolds and Boyer 2010). *L. latifolium* is a broad-leafed perennial forb in the mustard family (*Brassicaceae*), native to Central Asia and southeastern Europe (Young et al. 1997). Its distribution around the world is growing with documented occurrences in the United Kingdom (Burton 1997), Austria (Melzer and Barta 1994) and Spain (Romero and Amigo 1992). In the US, its introduction can be traced to sugar beet seed shipments during the 1930s (Robbins et al. 1951). It is now found along the eastern seaboard and all western states, except for Arizona (Miller et al. 1986; Young et al. 1995) and is included on the Noxious Weed List for 42 states. *L. latifolium* has prolific seed production and spreads vigorously through creeping rootstock, making it a threat in agricultural lands and freshwater wetlands in the western US (Blank and Young 1997; Young et al. 1997). While this species has higher seed production and viability in fresher, drier marshes (Leininger and Foin 2009), it is capable of establishing monotypic stands >20 m in diameter in more saline tidally inundated marshes of San Francisco Estuary, competing with native marsh species such as *Sarcocornia pacifica* (pickleweed) (Reynolds and Boyer 2010).

*L. latifolium* also potentially threatens rare tidal marsh fauna including the California Clapper Rail (*Rallus longirostris obsoletus*) and the Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*) (Shellhammer 1982; Alberston and Evans 2000) as well as rare plants such as Soft Bird's Beak (*Cordylanthus mollis* ssp. *mollis*) and Suisun Thistle (*Cirsium hydrophilum* var. *hydrophilum*) (Grewell et al. 2003; Fiedler et al. 2007). Stands of *L. latifolium* in multiple tidal marshes in the Estuary have soils with lower moisture, salinity, organic matter, carbon to nitrogen ratios, and higher pH than adjacent areas of native marsh plants with similar elevation and distance from channels, as well as differences in canopy characteristics that may influence insect and bird communities (Reynolds and Boyer 2010). In view of both the existing invasion and the potential for future spread and alteration of tidal marsh structure and function, effective control methods are needed.

Herbicides are the primary tool used to control invasive plants (Geyer et al. 2002), but their overuse elicits concern for non-target species. Integrated management techniques are gaining popularity with land managers and by definition require tactics to lower economic and environmental impacts while focusing on containment rather than complete eradication of the targeted species (Hobbs and Humphries 1995; Geyer et al. 2002; Major et al. 2003). Integrated techniques, such as mowing, burning, and clipping in conjunction with herbicide application, have been successful in controlling invasive plants in many areas (Kay 1995; Ailstock et al. 2001; Major et al. 2003). Similar efforts to integrate chemical and mechanical techniques have increased effectiveness of *L. latifolium* control in rangelands and hay meadows of eastern California (Young et al. 1998; Wilson et al. 2008). However, the most effective herbicide used in these efforts, chlorsulfuron, is not approved for use in aquatic habitats, and grazing, tilling, and mowing are less feasible for regularly inundated marshes.

In this study, we evaluated several treatment methods at multiple field sites and in pots to assess the potential for *L. latifolium* control in tidal marsh habitats along a salinity gradient in San Francisco Estuary. These included application of two herbicides, glyphosate and imazapyr; either herbicide in combination with mechanical control methods; as well as a potential biological control, a native parasitic plant,

*Cuscuta salina* (dodder). Glyphosate is commonly used on weeds and is considered safe for aquatic systems (Leson and Associates 2005), but had not been tested on *L. latifolium* in tidal marsh conditions. Imazapyr gained approval for aquatic use in California during the first year of our study, thus prompting us to add limited tests of its effectiveness in controlling *L. latifolium* in the second year. This herbicide had been successful in the treatment of invasive *Spartina* (cordgrass) in Washington State, and appears to have lower toxicity to aquatic organisms compared to glyphosate (Kay 1995; Patten 2004; Leson and Associates 2005). In seasonal wetlands, early season mowing followed by glyphosate application has been shown to be more effective at controlling *L. latifolium* than glyphosate alone (Renz and DiTomaso 1998). As mowing is difficult in tidal marsh habitat and can have large impacts on native species, hand-removal was deemed a promising low-impact alternative. Hand-removal of *L. latifolium* is commonly used by volunteer groups around San Francisco Estuary, but alone is not effective for sustained control (Burdick, pers. obs.; Save the Bay, unpubl. data); hence, we were motivated to develop integrated methods that could increase efficacy of grassroots efforts. Finally, in the northern San Francisco Estuary (San Pablo and Suisun Bays), *Cuscuta subinclusa*, a native holoparasitic vine, frequently and perhaps preferentially parasitizes *Lepidium latifolium* (Benner 2005; Burdick and Boyer, pers. obs.). Benner (2005) experimentally infected *L. latifolium* with *C. subinclusa*, which resulted in reduced seed production, viability, and root biomass of the invader. We set out to test whether a native congener, *C. salina*, which is more widespread throughout the estuary and the only *Cuscuta* species found at all of our study sites, affects *L. latifolium* in a similar manner. We predicted that this parasite alone would not effectively control *L. latifolium*, but that plants formerly treated using other control methods would be more susceptible to parasitism; if so, a natural biological control might add to the tools that could be integrated into a treatment strategy.

Overall, we hypothesized that a combination of treatments would produce the most effective control strategy. We focused our study both on evaluating methods of *L. latifolium* control and recovery of native plant species, as the latter is a critical component in the restoration of natural structure and function to these marshes after removal of the invader.

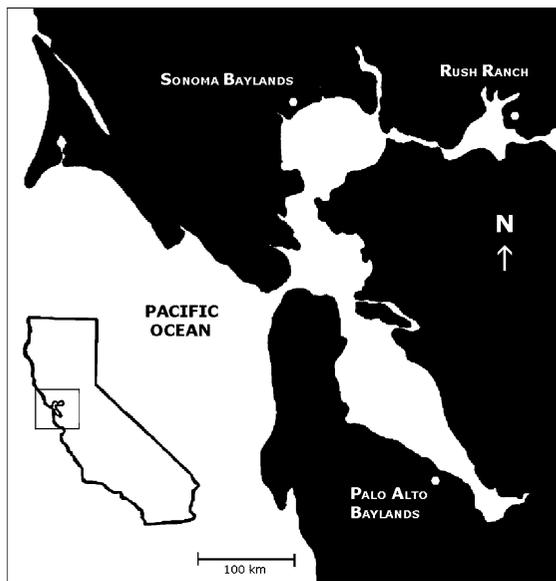
## Methods

### Field experiment

In February 2005, we set up a field experiment across multiple San Francisco Estuary tidal marshes to test the effects of glyphosate (Rodeo<sup>®</sup>, Monsanto, St. Louis, MO; active ingredient: isopropylamine salt of glyphosate, *N*-[phosphonomethyl] glycine) in comparison to glyphosate applied to re-growth following hand removal. The design included six treatments: a non-manipulated control, glyphosate addition, glyphosate following hand removal, and each of these treatments crossed with presence/absence of dodder (*Cuscuta salina*) as a biological control treatment to be applied in the second year. As the dodder treatment was not successful (see below), the experiment necessarily became focused on the glyphosate and glyphosate following hand removal treatments. However, in the second year we capitalized on the availability of the extra set of control plots to test the newly approved herbicide, imazapyr (Habitat<sup>®</sup>, BASF, Florham Park, NJ; active ingredient: isopropylamine salt of imazapyr).

We conducted the field experiment in three different regions of San Francisco Estuary (Fig. 1) to represent a range of salinities and plant community composition. Rush Ranch (N 38°12'226'', W 122°01'528'') is an 838-hectare brackish tidal marsh on Suisun Bay. This site had the most extensive *L. latifolium* infestation of the three study sites, with dense stands and thick layers (up to 10 cm) of *L. latifolium* thatch. Research plots prior to manipulation had an average of 100% cover of *L. latifolium* and 15% cover of native vegetation. The second study site was a brackish/salt marsh located adjacent to the 174 hectare Sonoma Baylands (N 38°06'847'', W 122°29'015'') in the San Pablo Bay National Wildlife Refuge, along the Petaluma River. Prior to manipulation, plots had an average of 95% *L. latifolium* cover and 35% native vegetation cover. The final study site was a salt marsh located in the Palo Alto Baylands Nature Preserve (N 37°27'413'', W 122°07'365''), a 785-hectare preserve in the South San Francisco Bay. The plots had an average of 97% *L. latifolium* cover and 28% native vegetation cover.

At each of the three sites, the field experiment consisted of 5 randomized blocks with six 2 m × 2 m plots each (with 2 m spaces between plots). Plots were



**Fig. 1** Field experiment sites, San Francisco Estuary, California, USA

surveyed with a Topcon Laser Level to achieve similar elevation within and among blocks ( $\pm 3$  cm). During March 2005, *L. latifolium* in two plots within each block was mechanically removed using volunteers pulling plants and digging out roots to  $\sim 20$  cm depth with shovels. We estimate that removal of  $20 \text{ m}^2$  of dense *L. latifolium* by hand required 30 h of volunteer time. In April 2005, the two glyphosate plots were treated with a 1.25% glyphosate solution (1.6 oz per gallon water), the minimum dosage recommended using the chemical label guidelines, using a backpack sprayer until leaves were coated. In July 2005, the resprouting *L. latifolium* in the two plots with prior hand-removal were sprayed with a glyphosate solution at the same dosage.

In April 2006, the second plot in each block originally designated as a control (intended for later *Cuscuta salina* addition) was treated with a 0.64% imazapyr solution and 0.32% surfactant solution (Competitor<sup>®</sup>, Wilbur-Ellis; active ingredients: ethyl oleate, sorbitan alkyl polyethoxylate ester, dialkyl polyoxy-ethylene glycol), also at the minimum dosage recommended, using a backpack sprayer. Although we had not added a surfactant previously to glyphosate in an effort to limit the solution's impact on non-target organisms, it was recommended that we add a surfactant as a necessary component of imazapyr application (E. Grijalva, Invasive *Spartina*

Project, pers. comm.). To be conservative, imazapyr was not tested at Rush Ranch due to proximity of an endangered plant, *Cordylanthus mollis* ssp. *mollis* (Soft Bird's Beak).

We assessed the vegetation cover before (April 2005) and after treatments (quarterly through April 2007) using a gridded  $1 \text{ m}^2$  quadrat placed in the center of each plot; presence of each plant species was noted for every second space in the grid (50 spaces possible). Due to layering of plant species, total percent cover could exceed 100%. Measures from duplicate plots for glyphosate and glyphosate following hand removal (intended for *C. salina* addition) were averaged for each block. We ran repeated measures ANOVA (site, block, treatment, and interactions) on arcsine square root transformed plant cover data, followed by within-subjects contrasts using SPSS 15.0.

On each sampling date, a 2.5 cm corer was used to collect soil from each plot to a 5 cm depth for relative measures of pore-water salinity through saturated soil pastes. As no differences were found by treatment, we averaged values for each block to permit comparisons of mean salinity by site. We further compared edaphic conditions among the 3 sites through a one-time (March 2007) assessment of sediment organic content (as loss on ignition in a  $500^\circ\text{C}$  muffle furnace for 3 h) and bulk density (as g dry mass per volume of cored sediment) from the control-plot cores.

#### Herbicide comparison in pots

To directly test the effectiveness of glyphosate versus imazapyr, with and without mechanical control treatments, 60 individual *L. latifolium* were grown (from seed) in 5-gallon pots for 4 months at San Francisco State University's Romberg Tiburon Center, Tiburon, California, USA. The treatments included clipping the plants to the sediment surface, clipping followed by application of either herbicide to re-growth, herbicide application alone at the same time as clipping, and an un-manipulated control ( $n = 10$ ). The concentration of herbicide in the solution was the same as in the field experiment; however, unlike in the field, the surfactant Competitor<sup>®</sup> was added to the glyphosate solution as well as the imazapyr solution (0.8 and 0.4 oz, respectively, in proportion to the quantity of herbicide as per label guidelines). Adding surfactant to both

herbicides allowed for a more direct comparison of the two herbicides than was conducted in the field. Clipping was chosen as the mechanical treatment for this pot experiment because hand-pulling would have completely removed the young plants, unlike in the field where well-established root systems permit emergence of new shoots following hand-pulling efforts. Six months after treatments, plant heights were measured and data were analyzed with 2-way ANOVA (herbicide, clipping) using SPSS 15.0. One-way ANOVA was followed by a Tukey test to permit pair-wise comparisons of all treatment combinations.

### Biological control

Having established a duplicate set of plots in each block at each field site, our objective was to compare the ability of *Cuscuta salina* to parasitize *Lepidium latifolium* in plots where no other control methods had been applied in the previous year (the duplicate control plots) relative to plots that had previously received either glyphosate alone or glyphosate following hand removal. During the second year of the field study, we attempted to infect *L. latifolium* in the second replicate plot of each treatment with *C. salina*. In early-March 2006, we added *C. salina* seed to half of the plots at each field site, collected from the same marsh the previous fall, at a density of 40 g of *C. salina* seed and chaff (~200 seeds) per m<sup>2</sup>. After 3 weeks, we returned to assess the number of seedlings coiled around and attached to *L. latifolium*.

In addition, we tested *C. salina* infestation potential on *L. latifolium* in the greenhouse at the Romberg Tiburon Center. We grew individual *L. latifolium* in 5-gallon pots from seed collected from the three study sites. After 6 months of growth, in February 2006, we added an average of 20 *C. salina* seeds from the appropriate site to 10 pots per site, leaving 10 pots as controls. We recorded *C. salina* germination and

number of parasitic coils around each *L. latifolium* shoot (Benner 2005) for 5 weeks.

## Results

### Patterns among field experiment sites

The three study sites were chosen to represent a gradient of soil salinities from north to south and, indeed, soil salinities were found to be consistently lowest at Rush Ranch, intermediate at Sonoma Baylands, and highest at Palo Alto Baylands (Table 1). In addition, Rush Ranch soils were approximately 2 × higher in organic matter and 2 × lower in bulk density compared to the other two sites.

Patterns in vegetation communities also differed among sites (Table 2). Rush Ranch had the highest species richness (species with at least 1% cover averaged across all dates and treatments), with 13 species compared to 7 or 9 at Sonoma Baylands and Palo Alto Baylands, respectively. Rush Ranch also had a greater number of native species in plots (8) compared to 5 for the other two sites. The most common native species at Rush Ranch was *Schoenoplectus americanus* (chairmaker's bulrush), followed by (in similar abundance) *Distichlis spicata* (salt grass), *Juncus kelloggii* (Kellogg's dwarf rush), *Hypericum anagalloides* (tinker's penny), and *Symphotrichum divaricatum* (southern annual saltmarsh aster). *Sarcocornia pacifica* was most common at the other two sites. In addition, *Jaumea carnosa* (fleshy jaumea) was abundant at Sonoma Baylands, while *Grindelia stricta* var. *angustifolia* (marsh gumplant) and *Frankenia salina* (alkali heath) were also present in low abundances. At Palo Alto Baylands, *J. carnosa* was generally less abundant and *F. salina* more abundant than at Sonoma Baylands, with *G. stricta* and *D. spicata* also present.

**Table 1** Mean (±1 SE) soil salinity (PSU)<sup>a</sup>, organic matter (%), and bulk density (g cm<sup>-3</sup>) in control plots at each field experiment site

	Salinity (grand mean)	Salinity (high)	Salinity (low)	Organic matter	Bulk density
Rush Ranch	10.7 (0.9)	13.1 (0.6)	8.5 (0.5)	19.3 (2.4)	0.56 (0.12)
Sonoma Baylands	15.9 (1.6)	20.0 (1.4)	12.8 (1.3)	8.1 (0.7)	1.00 (0.02)
Palo Alto Baylands	29.4 (0.9)	33.5 (2.7)	22.2 (0.9)	7.9 (0.5)	1.20 (0.05)

<sup>a</sup> Grand means of soil salinities (from saturated soil pastes) were calculated across all seasons; "high" and "low" are the averages for July and March, respectively

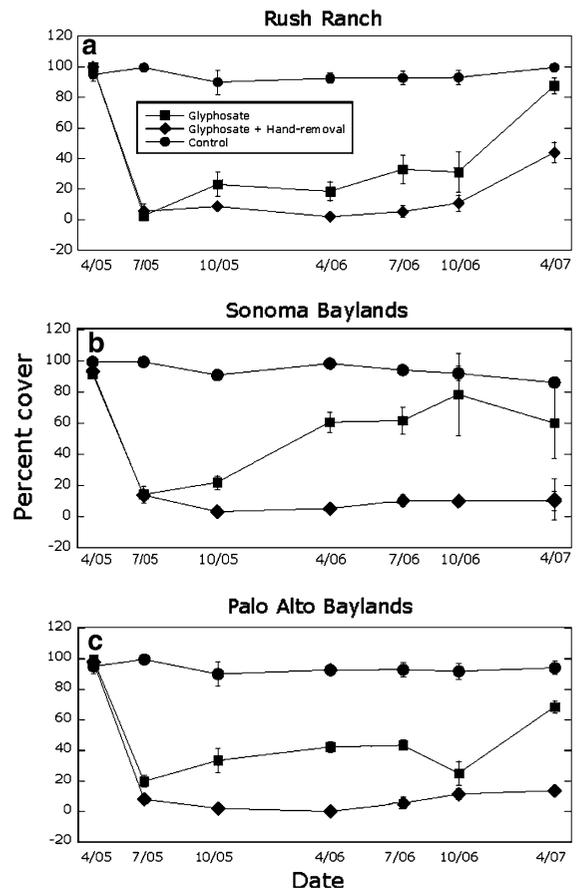
**Table 2** Percent cover of all plants with >1% cover (averaged across all treatments and seasons) in at least one of the three study sites

	Rush Ranch (%)	Sonoma Baylands (%)	Palo Alto Baylands (%)
Native species			
<i>Cuscuta salina</i>	2	1	<1
<i>Distichlis spicata</i>	9	0	3
<i>Frankenia salina</i>	0	4	12
<i>Fragaria vesca</i>	1	0	0
<i>Grindelia stricta</i>	0	3	5
<i>Hypericum angalloides</i>	8	0	0
<i>Jaumea carnosa</i>	0	22	4
<i>Juncus kelloggii</i>	6	0	0
<i>Sarcocornia pacifica</i>	1	48	32
<i>Schoenoplectus americanus</i>	32	0	0
<i>Symphyotrichum divaricatum</i>	8	0	0
Non-native species			
<i>Apium graveolens</i>	3	0	0
<i>Atriplex prostrata</i>	6	4	2
<i>Lepidium latifolium</i>	53	38	56
<i>Lotus sp.</i>	1	0	0
<i>Picris echioides</i>	10	0	0
<i>Raphanus sativus</i>	0	0	5
<i>Salsola soda</i>	0	0	3

*L. latifolium* was by far the dominant non-native species at all sites, but the annual *Atriplex prostrata* (fat hen) was also present at low abundances (Table 2). In addition, the non-natives *Apium graveolens* (wild celery), *Lotus sp.* and *Picris echioides* (Bristly ox-tongue) were present at Rush Ranch, and *Raphanus sativus* (wild radish) and *Salsola soda* (oppositeleaf Russian thistle) were present at Palo Alto Baylands.

#### Field experiment: glyphosate versus glyphosate following hand removal

By July 2005, 3 months after treatments, glyphosate (G) and glyphosate following hand removal (G + H) had both reduced percent cover of *L. latifolium* 80–100% from initial levels at all three study sites (Fig. 2). Overall, the three sites showed similar patterns in *L. latifolium* cover, and treatment effects



**Fig. 2** Percent cover of *Lepidium latifolium* before (April 2005) and after treatments of glyphosate or glyphosate following hand-removal, relative to un-manipulated controls at **a** Rush Ranch, **b** Sonoma Baylands, and **c** Palo Alto Baylands. Data are means (five blocks) of means (duplicate plots within blocks), except for control plots in **b** and **c**, in which one replicate within each block was used to test imazapyr beginning in 2006 (see Fig. 5). Bars are  $\pm 1$  SE

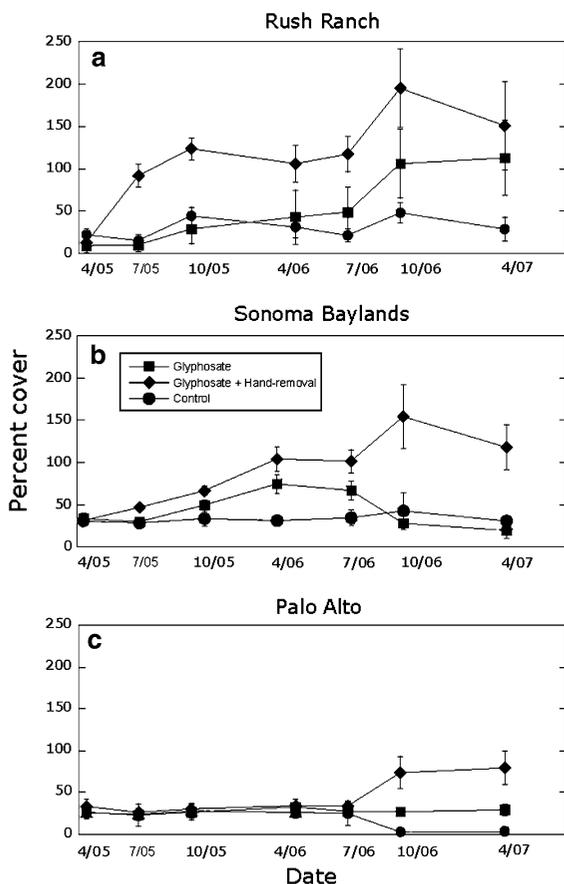
were consistent across sites (Table 3). There was a significant effect of time and a significant interaction between treatment and time; while control plots at all sites consistently had nearly 100% cover of *L. latifolium*, the G and G + H treatments resulted in substantially lower *L. latifolium* cover overall, although cover increased somewhat toward the end of the 2 years (Fig. 2). The G + H treatment was significantly more effective than G alone over the course of the experiment (within subjects contrast, Table 3). Examining patterns in greater detail, 1 year following treatment (spring 2006), G + H had

reduced *L. latifolium* cover by 95, 90, and 97% at Rush Ranch, Sonoma Baylands, and Palo Alto Baylands, respectively, compared to reductions of 80, 40 and 60% with G alone. 2 years after treatment, G + H was still the most effective treatment, with 58, 80, and 90% reductions in cover, compared to 16, 35, and 20% with G alone at the three sites, respectively.

Native plant recovery varied by treatment, and with study site and time after treatment (Fig. 3; Table 3). There was a significant effect of treatment on native plant recovery; G + H led to overall greater cover of native plants than G alone (within subjects contrast, Table 3), while control plots with no *L. latifolium*

removal began and remained comparatively low in native plant cover (Fig. 3). There was a significant interaction of treatment and time, as native plant cover generally increased over the 2-year period, primarily due to the G + H treatment (Fig. 3). There was also a significant difference in native plant cover by site, with much lower cover at Palo Alto Baylands (Fig. 3; Table 3). There was an interaction between treatment and site, with treatment effects generally greatest at Rush Ranch, followed by Sonoma Baylands and then Palo Alto Baylands (Table 3).

Rush Ranch native plant cover in G + H plots increased from 13 to 124% after 6 months, and after 2 years finished at 151% cover, while in the G plots native plant cover increased from 9 to 29% after 6 months, and after 2 years finished at 113%



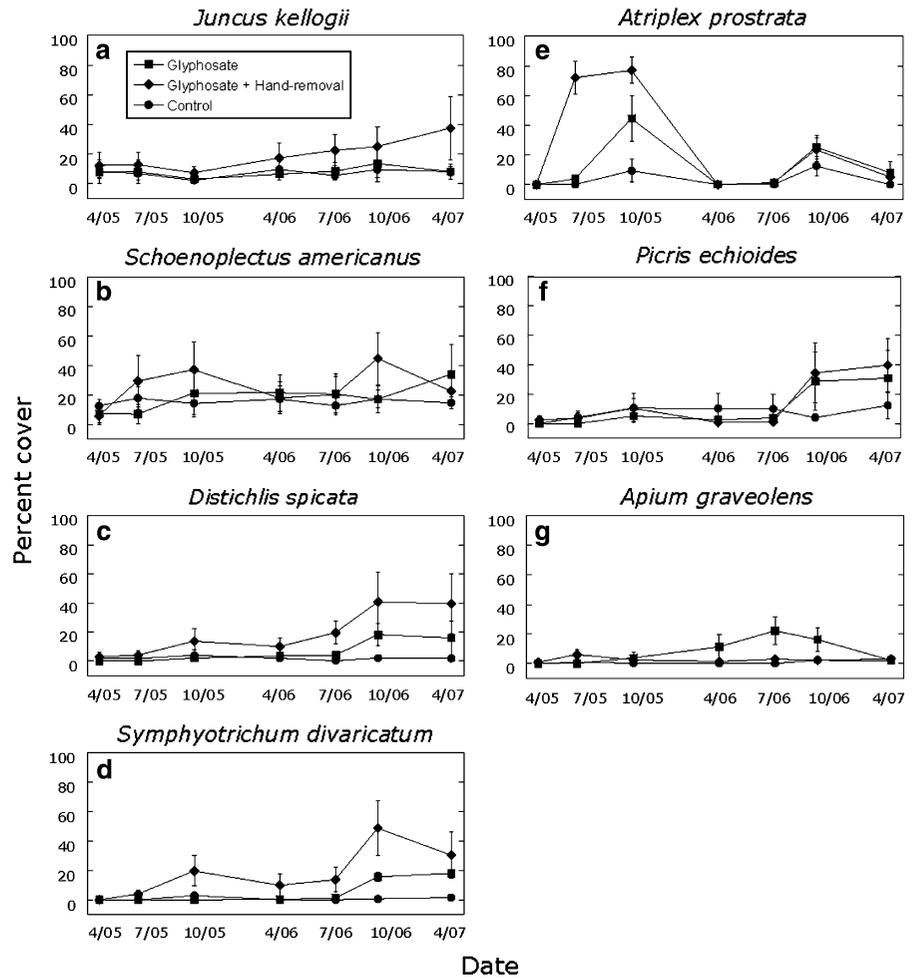
**Fig. 3** Percent cover of all native plants, before (April 2005) and after treatments of glyphosate or glyphosate following hand-removal, relative to un-manipulated controls at **a** Rush Ranch, **b** Sonoma Baylands, and **c** Palo Alto Baylands. Data are means (five blocks) of means (duplicate plots within blocks), except for control plots in **b** and **c**, in which one replicate within each block was used to test imazapyr beginning in 2006 (see Fig. 5). Bars are  $\pm 1$  SE

**Table 3** Results of repeated-measures ANOVA over the six sampling dates after treatments began in the field experiment, followed by within-subjects contrasts of effects of glyphosate versus glyphosate following hand removal

	df <sup>a</sup>	F	P
<i>Lepidium latifolium</i>			
Site	2.00	0.44	0.6590
Treatment	1.48 (2)	212.77	0.0000
Time	1.72 (5)	11.58	0.0020
Site $\times$ treatment	2.96 (4)	0.46	0.7130
Site $\times$ time	3.43 (10)	2.48	0.1050
Treatment $\times$ time	4.18 (10)	6.51	0.0010
Site $\times$ treatment $\times$ time	8.36 (20)	2.64	0.0250
Glyphosate versus glyphosate + hand-removal (G versus G + H)	1.00	48.22	0.0001
Native plant recovery			
Site	2.00	2.91	0.0214
Treatment	1.48 (2)	34.82	0.0000
Time	1.91 (5)	9.11	0.0050
Site $\times$ treatment	2.59 (4)	7.41	0.0040
Site $\times$ time	3.12 (10)	3.14	0.1130
Treatment $\times$ time	5.17 (10)	12.43	1.0001
Site $\times$ treatment $\times$ time	7.91 (20)	2.78	0.3100
Glyphosate versus glyphosate + hand-removal (G versus G + H)	1.00	53.69	0.0001

<sup>a</sup> Degrees of freedom for within-subjects effects (time, treatment, and interactions) are Greenhouse-Geisser corrected to meet the assumption of sphericity. Degrees of freedom if sphericity had been assumed are included in parentheses to aid reader understanding of design

**Fig. 4** Percent cover of the most common native (a–d) and non-native (e–g) species in plots at Rush Ranch (besides *Lepidium latifolium*). Treatments included glyphosate, glyphosate following hand-removal, and un-manipulated controls. Data are means (five blocks) of means (duplicate plots within blocks); bars are  $\pm 1$  SE



(Fig. 3a). Recovering native assemblages at Rush Ranch were dominated by *Juncus kellogii*, *Schoenoplectus americanus*, *Distichlis spicata*, and *Symphytotrichum divaricatum*, all of which tended to increase cover most following the G + H treatment and mostly in the second year after treatment (Fig. 4). The most common non-native plants after *L. latifolium* removal were *Atriplex prostrata*, *Picris echioides*, and *Apium graveolens*. The annual *A. prostrata* produced high cover quickly, then declined in the second year as both native and other non-native species became more abundant in the plots (Fig. 4).

It took longer to discern an effect of *L. latifolium* removal on native plant recovery at both Sonoma and Palo Alto Baylands (Fig. 3b, c). At both of the sites, recovery was primarily due to a single species, *Sarcocornia pacifica*, thus individual species responses are not shown. The site adjacent to Sonoma

Baylands showed no difference among treatments in October 2005, 6 months after treatment (Fig. 3b); however, by April 2006, native plant cover increased in the G + H plots in comparison with the G only and control plots. Over the 2-year period, percent cover increased from 25 to 111% in the G + H treatment (Fig. 3b). While G alone also increased native plant cover by 1 year after treatment, its effects were short-lived; native cover in October 2006 (25%) was very similar to initial levels (22%).

An increase in native plant cover following treatment took longest and was least pronounced at Palo Alto Baylands (Fig. 3c). It was the end of the second growing season after treatment before we observed substantial increases in native plant cover in the G + H plots (from 27 to 89%), which remained through the end of the experiment the following spring. In the G only plots, there was virtually no

change in native plant cover ( $\sim 28\%$ ) over the course of the experiment (Fig. 3c).

#### Field experiment: imazapyr

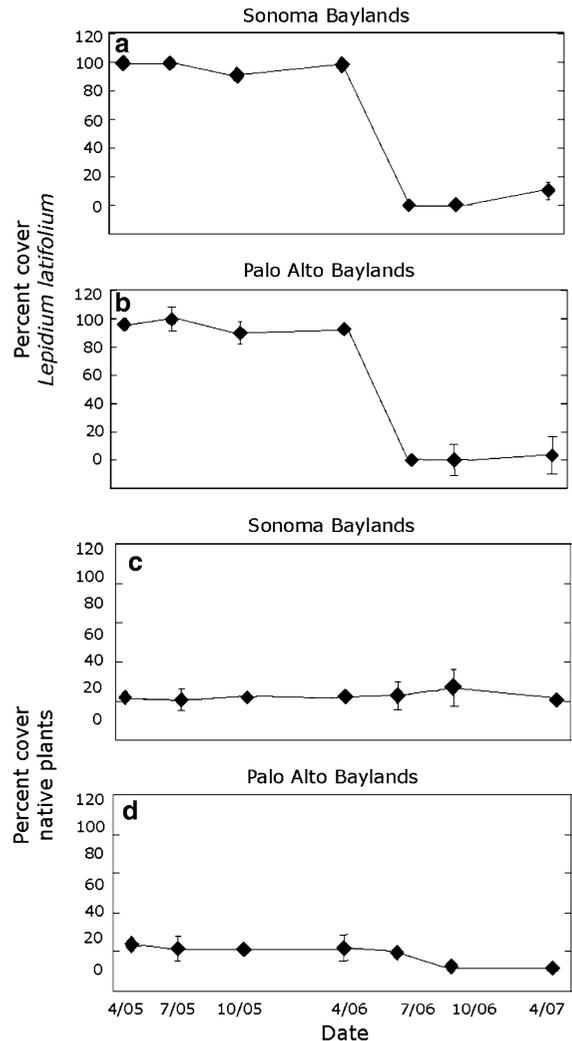
Application of imazapyr in April 2006 at Sonoma Baylands and Palo Alto Baylands led to complete removal of aboveground cover of *L. latifolium* by the July sampling date (Fig. 5a, b). One year after treatment, mean percent cover remained reduced by 91% at Sonoma Baylands and 94% at Palo Alto Baylands. There was no evidence of native plant recovery in the first year following imazapyr application at either site (Fig. 5c, d). While we can only compare imazapyr and glyphosate effects at two sites and in two different years, poor native plant recovery at Sonoma Baylands following imazapyr application contrasts with glyphosate results, as the latter led to greatly increased native plant cover within the first year following application (Fig. 3b).

#### Pot experiment: direct herbicide comparison with and without mechanical treatment

Both herbicides, with or without clipping, reduced *L. latifolium* heights by 2/3 or more compared to controls, when measured 6 months after application (Fig. 6). There was a significant interaction between clipping and herbicide ( $P = 0.017$ ), as clipping had no effect on its own but led to reduced plant heights in combination with herbicide. In the no-clip treatments, imazapyr had significantly greater effects on *L. latifolium* heights than did glyphosate (Fig. 6, Tukey test). Clipping increased effectiveness of glyphosate such that effects were comparable to that of imazapyr treatments. Clipping had no influence on imazapyr effects (Fig. 6).

#### Biological control

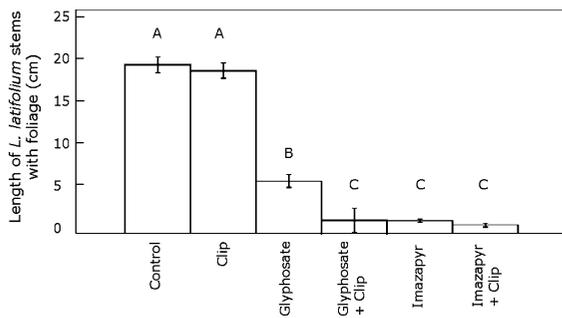
In the greenhouse, *Cuscuta salina* germination rates after 1 week averaged 35% (SE:  $\pm 3.55$ ) for Rush Ranch, 46% ( $\pm 3.43$ ) for Sonoma Baylands, and 41% ( $\pm 6.51$ ) for Palo Alto Baylands. Germination rates increased by the end of the third week, averaging 44% ( $\pm 3.52$ ) for Rush Ranch, 60% ( $\pm 4.68$ ) for Sonoma Baylands, and 56% ( $\pm 5.71$ ) for Palo Alto Baylands. Numbers of *C. salina* coils around *L. latifolium* also increased over time, reaching the



**Fig. 5** Percent cover of *Lepidium latifolium* (a, b) and native plants (c, d) before and after treatment with imazapyr in April 2006, at Sonoma Baylands and Palo Alto Baylands.  $N = 5$ ; bars are  $\pm 1$  SE

highest number of coils at 3 weeks in early March, averaging 7.2 ( $\pm 1.80$ ) for Rush Ranch, 3.3 ( $\pm 1.23$ ) for Sonoma Baylands, and 6.2 ( $\pm 1.33$ ) for Palo Alto Baylands. However, over the next 2 weeks, *C. salina* began to lose vigor (coiling ceased and plants appeared flaccid) and all *C. salina* were dead by the 5-week point (March 13, 2006). The loss of *C. salina* after 3 weeks of successful establishment was unexpected as weather conditions were mild and host plants were thriving.

As the greenhouse experiment was revealing strong parasitism of *C. salina* on *L. latifolium* at the time we added seed to the field plots, we expected to



**Fig. 6** Mean heights of *L. latifolium* in the pot experiment, 6 months after treatments of clipping, glyphosate alone, glyphosate following clipping, imazapyr, and imazapyr following clipping, compared to un-manipulated controls.  $N = 10$ ; error bars are  $\pm 1$  SE. Means labeled with different letters are significantly different according to a post hoc Tukey test

observe similar germination and coiling of seedlings around the invader in the field plots. However, upon returning to the field plots after 3 weeks, we found no evidence that *C. salina* seedlings had established.

## Discussion

With growing concern regarding the impacts of *Lepidium latifolium* in tidal marshes of the San Francisco Estuary and other sensitive aquatic habitats, resource managers are seeking solutions for control of the invader and prompt return of native communities (e.g., San Francisco Bay National Estuarine Research Reserve's *Lepidium* Science and Management Workshops, October 2008; [http://www.sfbaynerr.org/ctp/programs/program\\_detail.php?PROGID=BaQN7PL](http://www.sfbaynerr.org/ctp/programs/program_detail.php?PROGID=BaQN7PL)). In this study, we found that application of multiple control methods; i.e., mechanical removal followed by glyphosate application, enhanced the effectiveness of the herbicide in controlling dense infestations of *L. latifolium*. This finding was consistent in both a short-term pot experiment and a 2-year field experiment at multiple sites along a salinity gradient in the San Francisco Estuary. Our results concur with research on other aquatic invasive species, where combinations of mechanical and chemical treatment were employed (Kay 1995; Major et al. 2003) and are also consistent with previous experiments on *L. latifolium* in meadows and hay fields (Young et al. 1998; Wilson et al. 2008).

Glyphosate with prior hand-removal led to persistent large reductions in *L. latifolium* cover at Sonoma Baylands and Palo Alto Baylands during the 2 years of the experiment. However, the invader increased in cover somewhat during the second year at Rush Ranch. It is possible that higher soil salinity at Sonoma Baylands and Palo Alto Baylands presented more stressful conditions for new seedlings and vegetative re-growth of *L. latifolium* than at Rush Ranch. Spenst (2006) found that higher salinity corresponded with increased effectiveness of glyphosate applications on *L. latifolium* cover in both low and high density patches and that increased salinity levels depressed seed germination rates and increased the number of days until germination.

Within the first year after the initial treatment, glyphosate alone also performed relatively well. Our results are consistent with those of both Renz and DiTomaso (1998) and Spenst (2006) 1 year after treatment. Together, these studies suggest that application of glyphosate on a yearly basis could reduce cover and diminish the seed source. It is promising that we achieved these results without the addition of a surfactant, which can increase the toxicity of the solution to aquatic invertebrates (Leson and Associates 2005) and raise costs for land managers.

Because Rush Ranch initially had well-established patches of *L. latifolium*, with very little cover of native species in plots, we were expecting slower native plant recovery than at the other sites. However, Rush Ranch showed the first and most pronounced increase in native plant cover following *L. latifolium* removal, especially in plots with glyphosate following hand removal. In addition, successional patterns and overall species richness differed among sites during the recovery period. While primarily *Sarcocornia pacifica* colonized Palo Alto Baylands and the site adjacent to Sonoma Baylands, Rush Ranch plots were colonized by an average of six native species over the 2-year period. Lower salinities can lead to higher plant species richness in fresher tidal marshes of an estuary (e.g., Crain et al. 2004). However, *L. latifolium* may also return more quickly through higher germination rates in fresher marshes (Spenst 2006) and through vegetative growth, as seen in our data after 2 years at Rush Ranch. In addition, *L. latifolium* removal at Rush Ranch led to recruitment of other non-native species, most commonly *Picris echioides*, a Class-B rated invader (California

Invasive Plant Council), *Atriplex prostrata*, and *Apium graveolens*. Factors promoting native plant species diversity can similarly promote non-native plant species (Levine and D'Antonio 1999) after the initial invader is removed (e.g., Fig. 4). Land managers working in fresh and brackish marshes need to be aware of other potential invaders that may capitalize on *L. latifolium* removal, and might consider planting with native species to impede recruitment of other non-native species.

As imazapyr was applied to plots 1 year after the other treatments were performed and a surfactant was added to the solution, we cannot directly compare its effects to glyphosate in the field. Still, we showed that 1 year after initial treatment, imazapyr performed very well in controlling *L. latifolium* at the two sites where it was applied. In addition, when we directly compared glyphosate to imazapyr in the pot experiment, we found that imazapyr was more effective in reducing *L. latifolium* heights than was glyphosate, unless the latter was combined with clipping. However, the relatively low cover of native plants in imazapyr-treated field plots after 1 year suggests longer-term studies are needed. A control treatment that leads to delays in native canopy recovery after *L. latifolium* removal is not desirable, especially where rapid recovery of the natural structure and functions of tidal marshes is a primary goal. Frequent tidal flushing in lower marshes where non-native *Spartina alterniflora* and hybrids invade appears to minimize effects of imazapyr on native vegetation (I. Hogle, Invasive *Spartina* Project, pers. comm.), perhaps due to rapid photodegradation in aquatic solutions. However, less frequent inundation at higher intertidal elevations where *L. latifolium* occurs may lead to greater non-target effects of imazapyr (G. Block, San Pablo Bay National Wildlife Refuge, pers. comm.), as there is little or no photodegradation in soils (WSSA 1994). Further, reports are emerging of imazapyr leaking from roots of treated plants and damaging adjacent vegetation in terrestrial and riparian systems (J. DiTomaso, University of California, Davis, pers. comm.; Tu et al. 2004), suggesting that caution and further experimentation should guide use of this herbicide in any but the most frequently flooded environments.

Our attempts to enlist a native parasite, the marsh dodder *Cuscuta salina*, to enhance *L. latifolium*

control, did not succeed. While initial greenhouse results were promising, seedlings did not survive, nor did they recruit in field plots. However, managers of marshes where the congener *C. subinclusa* is common might still consider biological control as a possible integrated management technique in view of its observed affinity for *L. latifolium*, negative impacts on its growth, and apparently greater ease of manipulation onto host plants (Benner 2005).

In conclusion, this work represents an early step in evaluating a range of control methods for *Lepidium latifolium* in tidal marshes. The size and location of patches, proximity to rare species, funding, and the availability of volunteers are all considerations for anyone faced with managing this invader. Our data suggest that smaller, accessible stands of *L. latifolium* can be controlled effectively by a combination of glyphosate and hand removal, and at some sites (e.g., the more saline ones) the invader will remain absent for two or more years. This combination of treatments may be a good solution if managers wish to decrease herbicide use (by  $\sim 2/3$ ) and its potential toxicity to marsh organisms, while including local citizens in the conservation of natural resources. If a land manager does not wish to incorporate hand removal (or perhaps another mechanical surrogate), lesser and more temporary effects of glyphosate application alone suggest that yearly (at least) re-treatment will be necessary (Kay 1995; Spent 2006; this study). Further information is needed to evaluate the potential for imazapyr use on *L. latifolium* in tidal marshes, and particularly its effects on recovery of native plants over a longer period ( $>1$  year). In addition, little is known of the importance of removal of the thick layer of thatch that accumulates over time in *L. latifolium* stands. Suppression of germination through allelopathy has been seen in other members of the *Brassicaceae* (Kiemnec and McInnis 2002; Norsworthy 2003) and there is some preliminary evidence of negative effects of *L. latifolium* thatch on germination of native tidal marsh plants (Boyer, unpublished data). That both physical (light, space) and chemical constraints on natives may result from *L. latifolium* thatch build-up suggest further investigation is needed. Finally, in areas where repeated spraying of herbicide is required, the long-term effects of glyphosate and imazapyr application on native plants and fauna should be evaluated (Cornish and Burgin 2005).

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