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Field-based comparison of herbicides for control of parrotfeather (*Myriophyllum aquaticum*)

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ABSTRACT

Nonnative parrotfeather (*Myriophyllum aquaticum*) is an aquatic nuisance species of concern in many regions worldwide where it has become established, and a frequent target for management and control. In a field study, effectiveness of three chemical treatments (imazapyr, imazapyr + carfentrazone, and 2,4-D + carfentrazone) to control parrotfeather was tested in four locations along the Chehalis River (Washington State), and the range of natural conditions encountered was used to test differences in treatment efficacy across varying depths and plant densities. When evaluated 6 wk after treatment, parrotfeather cover was reduced by 67 to 69% in plots treated with imazapyr and imazapyr + carfentrazone compared with nontreated controls. Plots treated with 2,4-D + carfentrazone exhibited signs of substantial regrowth within the same time period, however, resulting in a net effectiveness of only 23% control when regrowth was accounted for. Evaluation of plot cover 1 yr after treatment corresponded with the observed trends in effectiveness at 6 wk posttreatment. There was no evidence for interactions of water depth or plant density with effectiveness of any treatment; however, accounting for variability within and between sites did help explain total variance in response. Field-based experiments such as these can help reduce uncertainty and facilitate development of realistic treatment plans for aquatic nuisance weeds.

Key words: carfentrazone, 2, 4-D, habitat variability, imazapyr, parrotfeather, random effects.

INTRODUCTION

Nonnative parrotfeather (*Myriophyllum aquaticum* (Vell. Verdc) is an invasive macrophyte of concern in many freshwater ecosystems worldwide (Hussner and Champion 2012). Documented ecological impacts of parrotfeather include alteration of physical habitat structure, reductions in dissolved oxygen, and modification of the composition of macroinvertebrate and macrophyte communities (Oborn and Hem 1962, Stiers et al. 2011, Kuehne et al. 2016); all of these have the potential to negatively influence valued goods and services provided by freshwater ecosystems (Schultz and Dibble 2012). Furthermore, parrotfeather is extremely difficult to manage once established, elevating the importance of identifying treatments with maximum effectiveness in field settings (Hussner and Champion 2012).

The effectiveness of numerous herbicides to control parrotfeather has been previously tested. Although application type (foliar vs. injection), treatment timing, and herbicide concentrations vary across these studies, overall trends indicate that the systemic herbicides imazapyr, 2,4-D, and triclopyr are among the most effective options for control of parrotfeather (Hussner and Champion 2012, Wersal et al. 2017); these herbicides have, however, been only rarely compared against each other (see Patten 2007, Wersal and Madsen 2010). The systemic herbicide glyphosate and contact herbicide diquat—both relatively well tested—are typically not recommended because of potential for rapid regrowth (Westerdahl and Getsinger 1988, Moreira et al. 1999). The contact herbicide carfentrazone-ethyl (hereafter "carfentrazone") is also not recommended for stand-alone use on parrotfeather because of comparatively low rates of control (Glomski et al. 2006, Richardson et al. 2008, Wersal and Madsen 2010), but has shown potential for use as an additive in combination with 2,4-D (Gray et al. 2007). Differences between the effectiveness of foliar and injection treatments for parrotfeather appear to be minimal, although foliar treatments are likely to be simpler and less expensive to implement (Patten 2007, Wersal and Madsen 2010).

Previous research investigating herbicide efficacy for parrotfeather has mainly been conducted as small-scale mesocosm or greenhouse trials (but see Moreira et al. 1999, Hofstra et al. 2006). Although controlled conditions clearly simplify comparisons across treatments, it can be difficult to translate these results to a field setting. In natural conditions, recolonization from untreated areas and plant recovery within plots can factor into the apparent effectiveness of herbicide treatments (Hofstra et al. 2006). Recolonization is difficult to mimic in greenhouse conditions, whereas trials based on short timescales (i.e., 2 to 4 wk) do not allow assessment of plant recovery (Wersal and Madsen 2007). Field-based studies also offer important opportunities to understand the range and potential influence of site-level habitat variability (e.g., water exchange, submersed foliage, overall plant abundance) on herbicide efficacy (Getsinger et al. 1997, Hofstra et al. 2006).

In the Chehalis River, Washington, parrotfeather is typically found in backwater sloughs and riparian wetlands;

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Table 1. Characteristics of the four sites (and plots within sites) used in field-based comparison. Plot characteristics of depth, plant density, and maximum stem height are summarized as the mean ± SD of quadrat measurements in pretreatment surveys.

Site	Latitude	Longitude	No. of Blocks	Total Area Treated (m^2)	Mean Plot Size (m^2)	Mean Plot Depth (cm)	Mean Plant Density (% Cover)	Mean Max. Stem Height (cm)
1	46°50′24.468″N	123°14′58.92″W	5	1,361	68 ± 14	15 ± 12	74 ± 19	26 ± 7
2	46°55′0.6594″N	123°18′15.4794″W	4	962	60 ± 14	18 ± 15	18 ± 11	11 ± 5
3	46°55′53.4714″N	123°18′31.32″W	4	637	40 ± 9	14 ± 9	29 ± 21	19 ± 8
4	46°56′53.5914″N	123°20′33.36″W	5	1,161	58 ± 13	3 ± 4	85 ± 13	29 ± 7

these areas share general features of slow-moving water and dense vegetation, but can also vary greatly in characteristics such as water depth, amount of light, and severity of the parrotfeather infestation. Parrotfeather is distributed along approximately 70 km of river that is at times unnavigable because of spring/fall flooding and summer low flows, restricting opportunities for site visits and retreatment. Multiple agencies (two state, three county, and one tribal) are responsible for and have conducted monitoring and control efforts for parrotfeather along parts of the river; however, the work is opportunistic and depends entirely on agency time and resources. Control methods and timing also vary, and rarely include formal evaluation of treatment success. These types of challenges are not uncommon in restoration and management scenarios, particularly for rivers (Bernhardt et al. 2007); systematic, field-based investigations of treatment options that account for natural variability can thus help managers optimize and better evaluate their treatment plans (Getsinger et al. 1997, Hussner et al. 2017).

There are 12 herbicides currently permitted for aquatic use in Washington State (Hamel 2012); not all are appropriate in flowing water, and use of some is restricted on salmon-bearing waters. Given that the number of treatments would be relatively small to allow sufficient replication, we investigated the effectiveness of three chemical treatments to suppress parrotfeather in field locations along the Chehalis River. The three treatments—imazapyr[1], 2,4-D[2] + carfentrazone[3], and imazapyr + carfentrazone—were contrasted with nontreated reference areas.

Imazapyr is a systemic herbicide that binds to the acetohydroxyacid synthase enzyme, thereby inhibiting synthesis of three critical branch-chain amino acids (Shaner and Mallipudi 1991), and has shown effectiveness in reducing parrotfeather biomass in greenhouse and field trials (Patten 2007, Wersal and Madsen 2007). 2,4-D is a systemic herbicide that works by mimicking the hormone indole-3-acetic acid (an auxin); the molecular mechanisms whereby 2,4-D (at high doses) causes uncontrolled growth leading to plant death are not precisely known, but may involve mediation by multiple hormones (Song 2014). The effectiveness of 2,4-D for managing parrotfeather is variable: some research indicates high efficacy (Moreira et al. 1999, Wersal and Madsen 2010), whereas other reports indicate potential for rapid plant recovery (Westerdahl and Getsinger 1988, Patten 2007), variability in response to foliar versus injection treatments, and sensitivity to exposure time (for injection treatments) (Patten 2007, Wersal and Madsen 2010). Carfentrazone is a contact herbicide that disrupts photosynthesis by inhibiting the enzyme protoporphyrinogen oxidase in plant chloroplasts (WSSA 2002); it has been registered for aquatic use in Washington State for just a few years (Hamel 2012). Prior research has demonstrated that it has relatively low effectiveness as a stand-alone treatment for managing parrotfeather (Glomski et al. 2006); however, when used in combination with a systemic herbicide, efficacy may be increased (Gray et al. 2007). The objectives of this research were to contrast the effectiveness of 2,4-D + carfentrazone with imazapyr (alone, and in combination with carfentrazone to further evaluate and contrast its effectiveness as an additive) to suppress nonnative parrotfeather, and investigate the role of environmental variability on treatment success.

MATERIALS AND METHODS

Four sites along the Chehalis River were selected for field-based comparisons of chemical treatments (Table 1). All sites occurred within a 20-km stretch of the river (minimum distance between sites was 2 km), and were in backwaters or sloughs partially or fully created by beaver dams. The dams create shallow, silty areas of very low flow that seem to facilitate parrotfeather establishment and growth in the 0- to 1-m depth zone along the shoreline. We used a randomized complete block design to partition variance due to unknown or unmeasured environmental variation within sites (e.g., aspect, soil condition) that could potentially affect treatment effectiveness. Depending on the total extent of parrotfeather present in a site, four or five adjacent blocks were established parallel to the shore (Table 1, Figure 1a). Each block ($n = 18$) contained one replicate of the four treatments: nontreated reference, imazapyr, imazapyr + carfentrazone, and 2,4-D + carfentrazone. Plot size was kept consistent within blocks (minimum plot dimensions = 4 by 7 m, maximum = 10 by 10 m), but might vary across blocks on the basis of the total amount of parrotfeather and configuration of shoreline patches (Table 1, Figure 1a).

Line-intercept sampling was used pre- and posttreatment to measure plant presence or absence at 1-m intervals along each transect (Figure 1b) (Madsen 1999); presence or absence was evaluated within a 15-cm^2 area underneath the intercept point. Water depth, maximum stem height, and visually estimated percent cover were measured for each plot within a 0.5-m^2 quadrat placed at eight points; quadrats were placed 1 m inside the plot perimeter to avoid edge effects while also avoiding disturbance of plants (Figure 1b).

Chemical treatments were applied on 13 and 14 July 2015 using backpack sprayers and walking or wading around

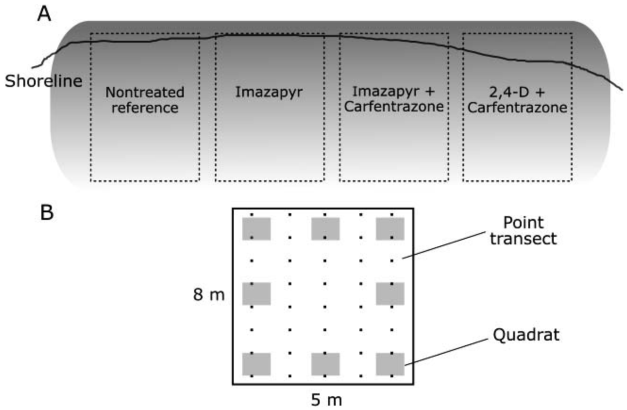

Figure 1. Schematic diagram of (a) a block of treatments (dotted lines) established along an area of shoreline with parrotfeather (shaded) and (b) sampling methods used in each treatment replicate (i.e., plot). Points indicate 1-m intervals of the point transect where presence or absence of plants was recorded, and shaded boxes show placement of quadrats for measurements of depth and plant cover within a plot.

plots; maximum stem heights were 22 ± 14 cm (mean ± SD). Chemical treatments were mixed using the maximum recommended concentration for emergent vegetation (imazapyr: 1,680 g ae ha^{-1}; 2,4-D: 2,100 g ae ha^{-1}; carfentrazone: 224 g ai ha^{-1}); a nonionic surfactant[4] (0.5% v/v) and marker dye[5] were added to all tank mixes. On the basis of prior experience, 467 L ha^{-1} (50 gal ac^{-1}) using backpack sprayers was the target spray rate; however, during preliminary testing in the field this was adjusted downward to 340 L ha^{-1} to avoid weighting down of plants and dripping of excess chemicals into the water. Weather conditions were warm (26 to 32 C) for the week after application, with no precipitation to interfere with absorption of chemicals; wind was also minimal (0 to 3 mph) on application days, and drift of herbicide treatments between plots was not apparent.

Plots were resurveyed using the same method 6 wk after treatment (WAT). An estimate of percent cover in the whole plot was visually assessed 1 yr after treatment (YAT). Visual assessments are less precise than point-transect sampling but useful for evaluating trends in treatment efficacy.

Data and statistical analysis

The proportion (P) of grid cells with parrotfeather was summed for the pre- (1) and posttreatment (2) survey 6 WAT. To account for differences between plots before treatment, effectiveness of the chemical treatment was calculated as ($[P1 - P2]/P1$); this metric is the percent change in plot cover (hereafter "percent control"). The mean water depth, mean cover, and mean maximum stem height were calculated for each plot using quadrat data to examine interactions of these covariates with treatments; however, a strong correlation between plant cover and maximum stem height (Pearson's correlation, $R = 0.89$) caused us to drop stem height from further analysis. The range of values represented by the final covariates was 0 to 43 cm for mean plot depth and 4 to 100% for mean plot cover.

Linear mixed-effects models (LME) were used to analyze and compare treatment effectiveness while accounting for the variability in field settings. LME accounts for the nested structure of plots within blocks and sites, and allows analysis of both random (unplanned) effects and fixed (planned or measured) effects on total variance. Chemical treatment was the fixed effect, and block and site were included as random effects (block nested within site). Response variables of percent control 6 WAT and percent cover 1 YAT met criteria for heteroscedasticity. Percent cover 1 YAT was arcsine transformed before analyses to improve normality; residual and diagnostic plots for models were also examined for evidence of departures from model assumptions.

Model fit and the variance explained by the random effects were evaluated by calculating the marginal R^2 (associated with fixed effects) and conditional R^2 (fixed plus random effects) for each model (Nakagawa and Schielzeth 2013). The significance of each random effect (i.e., block or site) was tested using ANOVA to compare models without the effect to the full model, with P-values obtained using likelihood ratio tests (Pinheiro and Bates 2000). Intraclass correlation coefficients (ICCs) for the random effects were also calculated for each model; ICC measures the correlation within group observations to reflect the relative importance of random effects in explaining variance (Merlo et al. 2005).

To test the effect of water depth and plant cover on the effectiveness of different treatments, additional LMEs were conducted that included interactions of treatment with mean depth and mean plant cover for each plot. The significance of all fixed effects (including interaction terms) was tested using ANOVA (Pinheiro and Bates 2000), with *post hoc* contrasts (Tukey's honestly significant difference) for significant treatment differences; P-values were obtained using the Satterthwaite approximation for degrees of freedom (Luke 2016). LME analyses and *post hoc* tests were conducted using the lmerTest and multcomp packages (Hothorn et al. 2008, Kuznetsova et al. 2016) and model R^2 was calculated using the MuMIn package (Barton 2016) in the R statistical computing environment.[6]

RESULTS AND DISCUSSION

Parrotfeather cover was significantly reduced by all three chemical treatments compared with reference plots, although short- and long-term effectiveness differed (Table 2). Six weeks after treatment, imazapyr and imazapyr + carfentrazone reduced parrotfeather cover by 67 to 69% compared with nontreated reference plots. The 2,4-D + carfentrazone treatment was significantly less effective; rapid and uniform resprouting observed in these plots resulted in percent control of only 23% compared with nontreated plots. One year after treatment, the percent cover as visually assessed in plots corresponded inversely with the trends in percent control 6 wk after treatment, with the lowest percent cover in the imazapyr-only plots, followed by imazapyr + carfentrazone; the percent cover in 2,4-D + carfentrazone plots was somewhat reduced but did not statistically differ from nontreated reference areas (Table 2). There was no evidence for a significant

Table 2. Percent control 6 wk after treatment (WAT) and percent cover observed 1 yr after treatment (YAT) for suppression of nonnative parrotfeather in field sites. Letter groups within a column designate statistically different treatments as determined using Tukey's honestly significant difference for *post hoc* contrasts (Satterthwaite's approximation for degrees of freedom, $P = 0.05$).

Treatment	Herbicide Rate ha^{-1}	Percent Control 6 WAT[1]	Percent Cover 1 YAT[2]
Non-treated reference[3]	—	0.0 a	72 a
2,4-D + Carfentrazone	2,100 g ae + 224 g ai	23.4 b	61 ab
Imazapyr + Carfentrazone	1,680 g ae + 224 g ai	67.4 c	49 b
Imazapyr	1,680 g ae	68.9 c	25 c
Model fit and random effects			
Conditional R^2		0.73	0.61
Proportion of variance due to random effects		0.26	0.36
ICC_{Block}		0.35	0.14
ICC_{Site}		0.08	0.40

[1]Mean percent change in extent of plot cover based on point-transect measurements before and then 6 wk after treatment.
[2]Mean percentage of plot covered as visually assessed 1 yr after treatment (YAT).
[3]Parrotfeather extent declined by 5.9% in reference plots 6 wk after treatment (WAT); this decline was set to zero to compare net effectiveness (i.e., percent control) of herbicide treatments.

interaction of any chemical treatment with depth (ANOVA, $F_{3,49} = 0.82$, $P = 0.49$) or plant cover (ANOVA, $F_{3,49} = 1.34$, $P = 0.27$).

LME models explained a high amount of variance for both response variables (conditional $R^2 > 0.60$, Table 2), and the combined random effects explained substantial variance in models for both percent control (26% variance) and percent cover (36% variance) (Table 2). In comparisons of random-effects models using ANOVA, for percent control 6 WAT the effect of block was significant ($\chi^2[1] = 13.26$, $P < 0.01$), whereas site was not ($\chi^2[1] = 0.38$, $P = 0.54$); this relationship was inverted for percent cover 1 YAT, with a significant effect of site ($\chi^2[1] = 7.94$, $P < 0.01$) but not block ($\chi^2[1] = 1.87$, $P = 0.17$). The ICCs for block and site effects support the ANOVA results, with a higher ICC associated with the block effect for percent control, and with site for percent cover (Table 2).

Of the three chemical treatments tested, imazapyr alone provided the most effective long-term control option on the basis of a single treatment in field settings. The addition of carfentrazone to imazapyr offered no short- or long-term advantages in control. In short-term trials, Gray et al. (2007) reported suppression of parrotfeather using 2,4-D with an addition of carfentrazone. In this study, observations 1 yr after treatment suggest that long-term effectiveness of imazapyr may have been slightly reduced by the addition of carfentrazone, with higher percent cover observed in the imazapyr + carfentrazone versus imazapyr-only plots. The highest percent cover 1 yr after treatment was observed in the plots treated with 2,4-D + carfentrazone, which was consistent with evidence of rapid plant recovery (mean maximum stem height ± SD = 9 ± 8 cm) when measured 6 wk after treatment. This potential pattern of resprouting from intact stolons within 4 to 5 wk has been noted in previous studies using 2,4-D (Westerdahl and Getsinger 1988, Wersal and Madsen 2010). Overall, our results underscore the vital importance of conducting tests over extended time periods (i.e., > 5 to 6 WAT when regrowth is apparent) and in ways that account for rates and extent of plant regrowth (Hofstra et al. 2006, Wersal and Madsen 2007, Emerine et al. 2010).

An important feature of our study design and analysis is assessing the influence of natural variability within and between sites on treatment effectiveness. Several greenhouse and field-based studies have suggested that herbicide efficacy may be influenced by factors such as depth, plant cover, and growth stage of parrotfeather (Moreira et al. 1999, Emerine et al. 2010). However, without systematic testing of these factors, practitioners are largely left to guesswork to determine optimal settings at which to apply treatments. In this field test, chemical control was reasonably consistent across and within four distinct sites that differed with respect to area, depth, and overall abundance of parrotfeather. In measurements that accounted for starting condition of plots (percent control 6 WAT), the combined random effects were moderately important (Table 2), with a significant block effect that explained a larger proportion of variance than site. However, an examination of the random-effects intercepts suggested that this was largely driven by a highly anomalous trend (toward increased cover) in only 1 of 18 total blocks, underscoring the general consistency and relatively low influence of site and patch variability on the fixed-treatment effects. Random effects were more important when the response variable did not account for starting condition of plots (percent cover 1 YAT), with a significant site effect that explained most of the random-effects variance; specifically, this site effect is accounting for the two shallower sites having consistently higher percent cover compared with the deeper sites (Table 1). That the (relatively minor) block effect at 6 WAT was not present 1 YAT is not surprising given plant recovery within sites over that duration. Overall, this analysis should give confidence to managers by demonstrating consistency in herbicide efficacy between and within sites. However, our results also support the desirability of accounting for site and patch variability in the design and analysis of field-based comparisons (e.g., sufficient replication, blocking, mixed-effect models) to improve interpretation of treatment outcomes.

In addition to consistency of fixed treatment effects across and within sites, there was no evidence of treatment interactions specifically with water depth or plant cover. For parrotfeather, these factors tend to correspond, where density of emergent biomass (and stem height) generally decreases with increasing water depth. By intentionally selecting sites that differed with respect to depth (i.e., two shallow and two deep) we could test for these interactions across a comprehensive range of plot depths (0 to 0.4 m) and plant cover (4 to 100%), further emphasizing that managers can expect to obtain reasonably consistent results across different environmental conditions.

One potential factor of importance that we did not test is whether there is optimal timing of herbicide applications for parrotfeather control. Two studies suggest that 2,4-D treatments are most effective when applied to young, actively growing parrotfeather plants (Westerdahl and Getsinger 1988, Moreira et al. 1999); overall, however, there are few systematic tests of herbicide application timing for parrotfeather as have been conducted for some other species (e.g., Spencer et al. 2011, Hofstra et al. 2013). Comparisons of long-term control with single versus multiple treatments within a growing season would also be of interest to managers; however, given the logistical and resource challenges of invasive plant control, knowing when to apply a single treatment may be the more critical research need to support management efforts. Ideally, new research will evaluate these factors in field-based trials to continue to develop our understanding of how environmental contexts impede or facilitate management of invasive macrophytes (Hussner et al. 2017).

SOURCES OF MATERIALS

[1]Habitat, SePro Corporation, 11550 North Meridian Street, Carmel, IN 46032.

[2]2,4-D Amine, Southern Agricultural Insecticides, Inc., P.O. Box 218, Palmetto, FL 34220.

[3]Stingray, SePro Corporation, 11550 North Meridian Street, Carmel, IN 46032.

[4]Competitor, Willbur-Ellis, 3300 S Parker Road, Aurora, CO 80014.

[5]Blazon, Milliken Chemical, 1440 Campton Road, Inman, SC 29349.

[6]R: A language and environment for statistical computing. R Foundation for Statistical Computing, Wirtschaftsuniversität Wien, Welthandelsplatz 1, 1020 Vienna, Austria.

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