BioOne RESEARCH EVOLVED

Off-Target Impacts of Graminoid-Specific Herbicide on Common Camas (*Camassia quamash*) Growth, Abundance, Reproduction, and Palatability to Herbivores

Author(s): Alexandra E. Lincoln, Rachel K. Brooks and Sarah T. Hamman
Source: Northwest Science, 92(3):166-180.
Published By: Northwest Scientific Association
https://doi.org/10.3955/046.092.0304
URL: http://www.bioone.org/doi/full/10.3955/046.092.0304

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Alexandra E. Lincoln, Center for Natural Lands Management, 120 Union Street SE, #215, Olympia, Washington 98501

Rachel K. Brooks, U.S. Fish and Wildlife Service, Washington Fish and Wildlife Office, 510 Desmond Drive SE, Lacey, Washington 98503

and

Sarah T. Hamman[1], Center for Natural Lands Management, 120 Union Street SE, #215, Olympia, Washington 98501

Off-Target Impacts of Graminoid-Specific Herbicide on Common Camas (*Camassia quamash*) Growth, Abundance, Reproduction, and Palatability to Herbivores

Abstract

Invasive grass removal with herbicide is an important component of the restoration process in many prairie and grassland ecosystems. Management of invasive grasses in areas with high concentrations of native plants necessitates investigation of off-target herbicide effects on sensitive native species. Two graminoid-specific herbicides, Fusilade (active ingredient fluazifop-P-butyl) and Envoy Plus (active ingredient clethodim), are frequently used to control invading broadleaf pasture grasses in Pacific Northwest prairies with little knowledge of how these chemicals impact native plants. One such native plant, common camas (*Camassia quamash*), is a characteristic forb of these prairies that often grows in areas treated with these herbicides. Because camas is a critical resource for native pollinators and holds ethnoecological significance to native peoples, it is important that management methods do not negatively impact this plant. The objective of this study was to understand if and how various seasonal applications of clethodim and fluazifop may impact camas. We implemented a factorial design testing the effects of herbicide type (fluazifop, clethodim, control) and application season-frequency (combinations of mid-spring, late-spring, fall) on camas growth, foliar cover, reproduction, and palatability to herbivores. Our results show that herbicide treatments may reduce leaf length and increase flower and seed production, but do not influence seed viability or palatability to herbivores. The observed effects are not likely to be ecologically detrimental, suggesting that repeat applications of either fluazifop or clethodim can be safely used in areas with high concentrations of this iconic prairie species.

Keywords: prairie, restoration, fluazifop-P-butyl, clethodim

Introduction

Invasive plant removal is often a primary step in the restoration process for disturbed areas worldwide (Solecki 1997, Ansley and Castellano 2006, Rowe 2010, Wilcox and Whillans 1999). While many techniques have been used to remove non-native plants, targeted herbicide application remains one of the most effective and efficient methods for removing one or more non-native, invasive species within a restoration landscape (Hamill et al. 2004). Different species can be targeted by varying the active ingredient, seasonal timing or application methods (Dennehy et al. 2011). The expanding use of both the variety and the volume of herbicides in restoration, and the increasing need to strategically remove invasive plants within a native landscape, has amplified concern about off-target impacts on native species.

While extensive environmental testing occurs for each new herbicide prior to release for widespread purchase and use, this testing has been considered insufficient (Boutin et al. 2012) and does not typically include effects of herbicides or surfactants on native species (Olszyk et al. 2013). For example, current standard U.S. Environmental Protection Agency (EPA) phytotoxicity tests for herbicide registration frequently do not include native terrestrial plant species, but instead focus on crop species, aquatic invertebrates, birds, fish, algae, and aquatic plants (U.S. Environmental

[1]Author to whom correspondence should be addressed: Email: shamman@cnlm.org

Protection Agency 2012a, 2012b; Wendel and Orrick 2014).

Many perennial grassland and prairie ecosystems have been subject to invasion by non-native forage grasses, often due to either historic introduction for livestock or by invasion from nearby agricultural lands (Mack 1981, Rogler and Lorenz 1983). Removing non-native grasses within the context of a native ecosystem over large acreages typically requires selective herbicide. This practice is common in the Pacific Northwest, where grass-specific herbicides are often successfully applied to target non-native grasses invading prairie habitats (Stanley et al. 2011b, Wold et al. 2011).

Two post-emergent grass-specific herbicides, fluazifop-P-butyl (Butyl (R)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate; CAS#79241-46-6), hereafter "fluazifop", and clethodim (2-{(E)-1-[(E)-3-Chloroallyloxyimino] propyl}-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; CAS#99129-21-2), are often used in grassland restoration (Harker and O'Sullivan 1991, Dennehy et al. 2011). These two chemicals are especially useful in Pacific Northwest prairie restoration, since they target invasive grasses while leaving Roemer's fescue (*Festuca roemeri* (Pavlick) Alexeev), a common native bunch grass, without injury at tested rates (Olszyk et al. 2013). The active ingredients in clethodim and fluazifop are acetyl-CoA carboxylase (ACCase) inhibitors, preventing the conversion of acetyl CoA to malonyl-CoA in the first step of fatty acid synthesis within plastids (Walker et al. 1988, Cronan and Waldrop 2002, Sasaki and Nagano 2004). Inhibition of fatty acid synthesis via herbicide application results in the destruction of the grass meristem and eventually plant death (Barnes 2004, Walker et al. 1988). However, fluazifop and clethodim only target and inhibit one of the two structurally different forms of ACCase, making them graminoid-specific (Rendina and Felts 1988). Grasses contain the sensitive form of ACCase (a homomeric form) while forbs contain both the sensitive version in the cell cytosol and a tolerant version (a heteromeric form) in the cell plastids (Sasaki et al. 1995, Konishi et al. 1996, Délye 2005). While forbs are generally considered tolerant to these herbicides, herbicide application may disrupt membrane lipids in some broadleaf species (Luo et al. 2004), resulting in off-target effects and phytotoxicity. Fluazifop treatment has been shown to induce wilting and necrosis of bristly starbur (Luo and Matsumoto 2002), and to increase production of ethylene (Luo et al. 2004), a plant hormone that regulates growth, development and senescence of leaves, flowers, and fruit (Reid 1995). Thus, altered levels of ethylene in broadleaf plants induced by herbicide treatment may result in decreased plant health, manifesting in altered foliar growth, foliar cover, reproduction (flower production, seed production, or seed viability), and/or a change in palatability to herbivores.

The timing of herbicide application as well as the frequency of application may also impact whether off-target effects are observed (Crone et al. 2009). Both target and off-target effects of herbicide vary with season of treatment due to changing plant physiology (Lanini and Radosevich 1982, Ruffner and Barnes 2010). For example, translocation of foliage-applied herbicides may be reduced in times of heat and water stress surrounding summer months and increased in cooler seasons (Lanini and Radosevich 1982, Reynolds et al. 1988), which may impact herbicide effectiveness. Additionally, application during periods when target species are actively growing and non-target species remain dormant could minimize off-target effects (Ruffner and Barnes 2010), and thus we may expect off-target effects to be more apparent in the spring when native plants are actively growing and temperatures are cooler.

Despite the potential for off-target effects of grass-specific herbicides on native plants, there is very little published literature on the topic. In one study, *Sanguisorba occidentalis* Nutt., a grassland forb native to the western U.S., was found to be moderately sensitive to fluazifop, showing decreased weight, height, and relative growth rate with treatment (Olszyk et al. 2013). Work in an Australian shrubland indicated that fluazifop impacted numerous non-target species (including four understory monocots) throughout their development (Rokich et al. 2009). However, another study evaluating the phytotoxicity of

graminicides on wildflowers in the U.K. showed effects to be temporary and unlikely to reduce the competitive ability of these species over time (Blake et al. 2011). Finally, Colwell (1984) found that fluazifop-butyl was not phytotoxic to the common red onion (*Allium cepa* L.), a forb in the *Liliaceae* family.

There are over 160 vascular plant species native to the prairies and oak woodlands of the south Puget Sound, Washington, including over 110 species of forbs (Dunwiddie et al. 2006). Common camas (*Camassia quamash* (Pursh) Greene), hereafter "camas", is an iconic native monocot with a blue-purple flower, found throughout the historic range of prairies in this region. Prairie and oak savanna ecosystems in the Pacific Northwest are among the most critically endangered ecosystems in the United States, with only 3% of original prairie lands remaining (Noss et al. 1995, Crawford and Hall 1997, Floberg et al. 2004, Dunwiddie and Bakker 2011). These ecosystems, once managed carefully by Native Americans (Boyd 1986, Walsh et al. 2010), have been dramatically impacted as Euro-American settlers excluded fire (Weisberg and Swanson 2003) and introduced non-native species, including several perennial grasses, to the area over the past 200 years (Dunn and Ewing 1997). In many prairies of western Washington State, camas is a dominant species and makes up a sub-community that is of high conservation priority due to low regeneration potential and high use by wildlife (Erickson 1978). Camas is one of the most common native species in prairies in Thurston County, WA, composing over 10% of plant cover in many areas (Blazina 2017). Camas interacts with multiple families of insects (Parachnowitsch and Elle 2005) and has been recognized as an important pollen and nectar source for native pollinators (Schultz 2001, Adamson et al. 2015), including the endangered Taylor's checkerspot butterfly (U.S. Fish and Wildlife Service 2010), as well as a significant food source for deer and elk (Miller et al. 1981). Additionally, camas has been highlighted as a culturally important or even a cultural keystone species due to its use by many Native American tribes as a primary food source (Beckwith 2004, Garibaldi and Turner 2004, Higgs 2005, Tomimatsu et al. 2009). Thus, camas holds a unique position as an ecologically and culturally important species for Pacific Northwest prairies (Garibaldi and Turner 2004), and conservation managers should be conscious of how restoration efforts impact this key player in the landscape.

To add to our understanding of the unintended impacts of herbicide use, and to investigate how management practices may influence the conservation of a culturally and ecologically important species, we examined the effects of fluazifop and clethodim application on camas. Our specific aims were to determine if various seasonal timing and frequency of herbicide treatments affected camas growth, abundance, reproductive output and viability, and palatability to herbivores. We hypothesized that herbicide application across all season-frequency treatments would not have any lasting negative effects on growth, reproduction, foliar cover, or palatability to herbivores, though temporary effects may be observed with treatments during the period of most active camas growth (April–early May).

Methods

Study Area

We conducted this study at two sites in Thurston County, WA: the 1020-acre Glacial Heritage Preserve (46.865537, –123.050834), owned by Thurston County and managed by the Center for Natural Lands Management, and the 965-acre Scatter Creek Wildlife Area (46.827652, –123.016656), owned and managed by the Washington State Department of Fish and Wildlife. Both of these sites contain upland prairie with a diverse mix of native species and non-native grasses, forbs, and shrubs. The two dominant non-native grass species in the study sites were the invasive broadleaf tall oatgrass (*Arrhenatherum elatius* (L.) P. Beauv. ex J. & C. Presl) and colonial bentgrass (*Agrostis capillaris* L.), both commonly targeted by clethodim and fluazifop applications. Additionally, native camas was fairly evenly distributed throughout the study areas, with higher densities found at Glacial Heritage than at Scatter Creek. Neither of the areas we chose for this study had received any previous grass-specific herbicide treatments, but some locations at Scatter Creek

had been periodically brush cut in the winter to remove the invading shrub Scotch broom (*Cytisus scoparius* (L.) Link).

Experimental Design

Since effects of herbicide applications can vary by season (Lanini and Radosevich 1982, Ruffner and Barnes 2010), we employed a factorial design testing effects of three herbicide treatments crossed with six application season-frequency treatments. We applied these treatments evenly to three replicate experimental arrays containing 18 plots arranged in a stratified random block design in areas containing the target non-native grasses and camas at each prairie site. The herbicide treatments included Envoy Plus (active ingredient clethodim) plus Nufilm® (a surfactant), and Fusilade DX (active ingredient fluazifop-P-butyl) plus Nufilm®, which were compared to a control treatment (water application). Season-frequency treatments included: 1) a mid-spring application (MS); 2) a late spring application (LS); 3) a mid-spring and a late spring application (MS-LS); 4) a mid-spring and a fall application (MS-F); 5) a late spring and a fall application (LS-F), and 6) a mid-spring, a late spring, and a fall application (MS-LS-F). We applied mid-spring applications in late April or early May, late spring applications in late May, and fall applications in late October of each year from 2012–2014. The exact dates of application were based on the height of the tall oatgrass to reflect the timeframes when land managers would apply herbicide: 20–30 cm (mid-spring), over 30 cm (late spring), and 10–20 cm (fall). Fusilade treatments were 0.75% Fusilade and 0.25% Nufilm, while Envoy treatments were 0.5% Envoy and 0.25% Nufilm. We mixed herbicides with water and applied at a rate of approximately 200 ml m^{-2} (which is equivalent to 85.53 quarts ac^{-1} or 2.56 lbs ha^{-1} of Envoy and 3.84 lbs ha^{-1} of Fusilade) evenly across each 2 m x 2 m plot with a backpack sprayer. We applied the herbicides at the prescribed times for three years (2012–2014) and collected various metrics described below for up to four years (2012–2015) to determine short- and long-term impacts. Data in 2012 were collected prior to treatment in that year to establish baseline values.

Field Measurements

Due to site, weather and resource constraints not all metrics were recorded in every year at each site (Table 1). To evaluate camas abundance over time, each year we estimated percent cover of camas to the nearest percentage in each plot using a 1 m x 1 m quadrat placed at the plot center. In addition, we harvested one randomly selected plant per plot at Glacial Heritage in May 2013 and 2014 when plants were at peak growth to measure dry biomass. While this results in a small sample size of three plants per chemical-season treatment per site, we chose this methodology to avoid significant soil disturbance and reductions in camas density inside plots. We also conducted counts of the total number of flowers m^{-2} in 2013, 2014 and 2015.

In May 2014 and 2015, when camas was in full bloom, we selected the five plants closest to the center point of each plot at Glacial Heritage and measured a suite of plant traits: the number of leaves produced by the plant regardless of size or health ("leaves/plant"), the length of the tallest leaf measured from its base ("leaf length"), the width of the tallest leaf at its widest point ("leaf width"), presence of a flowering stem ("stem presence"), the height of the flowering stem (if present) from the ground to the top of the stem excluding pedicels ("stem height"), the number of flowers regardless of health or phenophase ("flowers/plant"), and evidence of grazing by deer or other herbivores ("grazing").

To determine reproductive output via seed and seedpod production, we covered five randomly chosen camas stems containing seedpods in each plot at Glacial Heritage with a fine mesh bag to capture all seed produced by the plant in 2015. These plants were not necessarily the same plants observed for the traits listed above. We collected the bags in June 2015 and counted seeds and seedpods by hand. We determined seedpods to be successful if they contained at least one seed. Additionally, we collected seeds by hand from five camas plants per plot at Glacial Heritage in 2014 for viability testing. After collection, we stored seeds in envelopes at 3 °C for 5–6 months prior to germinating (Drake and Ewing 1997).

TABLE 1. Data collection from Glacial Heritage (GH) and Scatter Creek (SC) by year.

	2012[a]	2013	2014	2015
Percent cover	GH, SC	GH, SC	GH, SC	GH, SC
Biomass	-	GH	GH	-
Flowering Stem presence and height	-	-	GH, SC	GH, SC
Number of leaves per plant	-	-	GH, SC	GH, SC
Length & width of tallest leaf	-	-	GH, SC	GH, SC
Leaf grazing	-	-	GH, SC	GH, SC
Number of flowers m^{-2}	-	GH, SC	GH, SC	GH, SC
Number of flowers per plant	-	-	GH, SC	GH, SC
Seedpod success	-	-	-	GH
Seeds per seedpod	-	-	-	GH
Germination success	-	-	GH	GH

[a] Data were collected prior to herbicide treatment.

Germination and Seed Vigor

To initiate germination of collected seeds, we followed the protocols developed by Guerrant and Raven (1995), subjecting seeds to cold-moist stratification to mimic natural conditions in the field. We first imbibed seeds in a 1:10 dilution of 3% hydrogen peroxide solution for 24 hours. We then divided seeds from each plot into three replicate groups, which we placed into petri dishes lined with moistened Whatman #1 filter paper. We placed the petri dishes into a dark germination chamber set to 3 °C for 60 days (Guerrant and Raven 1995), checking dishes weekly during the cold-moist stratification period to keep filter paper moist. At the end of the 60-day stratification period, we altered the germination chamber settings to a 12 hr light:12 hr dark cycle at 15 °C and 7 °C respectively, to initiate germination. We monitored seeds for germination, defined as having a 2 mm radical, every 3–4 days for 24 days. We randomized the location of petri dishes within the germination chamber after each monitoring period. To calculate the time to 50% germination (T50), a measure of seed vigor, we followed the equation used by Coolbear et al. (1984):

$$\mathrm{T_i} + \frac{(N/2 - n_i)(\mathrm{T_j} - \mathrm{T_i})}{n_j - n_i}$$

where $\mathrm{T_i}$ = day prior to 50% germination, $\mathrm{T_j}$ = day following 50% germination, N = final number of germinants, n_i = number of seeds germinated by day $\mathrm{T_i}$, and n_j = number of seeds germinated by day $\mathrm{T_j}$.

Statistical Analysis

We utilized generalized linear mixed effects models to determine the effect of chemical type and season of treatment on our various measurements of abundance, growth, reproduction, and grazing. We considered chemical-season treatments and year as unordered fixed effects (for variables with multiple years of data) and both experimental array and prairie site as random effects, where arrays (which contained all stratified randomized treatments) were considered blocks. We included the interaction between chemical and season of treatment in all models to consider how effects of herbicide may vary with season. We ran all analyses in R version 3.4.0 (R Core Team 2017), and used the "lme4" R package to build the models (Bates et al. 2014). We used a log link function to analyze raw count data (numbers of flowering plants, flowers/plant, leaves/plant, and seedpods/plant), a logit link function for binomial outcome and proportion data (grazing and stem presence, seedpod success, germination success), and an identity link function for continuous variables (plant biomass, stem height, leaf length, leaf width, percent cover, T50, and average number of seeds produced per seedpod). We log-transformed continuous data, when necessary, to meet assumptions of homogeneity of variance. Significance of fixed effects was determined with a likelihood ratio

test, and pairwise comparisons between chemical and season of treatment groups were tested with *Z*-tests (or *t*-tests for those with an identity link) using the summary function. Alpha of 0.05 was used to determine significance.

Results

Growth

There was no evidence that plant biomass was significantly impacted by either the chemical or season of treatment (χ^2 =1.21, P = 0.55 and χ^2 = 1.30, P = 0.73 respectively), but biomass varied between the two years measured (χ^2 = 3.82, P = 0.05; Table 2), with greater biomass observed in 2013 than 2014. Similarly, the number of leaves produced per plant was different between years sampled (χ^2 = 7.75, P = 0.005), but was not affected by either chemical (χ^2 = 5.65, P = 0.06) or season of treatment (χ^2 = 4.83, P = 0.44) (Table 2). However, leaf length differed significantly between treatment chemicals (χ^2 = 10.85, P = 0.004), and years (χ^2 = 22.51, P < 0.001), with the effect of chemical varying by season of treatment (χ^2 =20.33, P = 0.03) (Tables 2 & 3). Leaves were generally shorter in plants from fluazifop-treated plots compared to the control (t = –3.149, P = 0.04), particularly in LS, LS-F, and MS-LS-F plots at Scatter Creek. In contrast, leaf width varied by year (χ^2 = 8.89, P = 0.003), but there was no evidence that chemical or season of treatment influenced the width of leaves (χ^2 = 0.84, P = 0.66; χ^2 = 10.51, P = 0.06) (Table 2).

Chemical treatment significantly predicted the presence of a flowering stem in sampled plants (χ^2 = 9.29, P = 0.01) (Table 2); camas treated with either fluazifop (Z = 2.80, P = 0.005) or clethodim (Z = 2.46, P = 0.014) were more likely to have a flowering stem than camas in the control group (Table 4). Stem height did not differ across chemical treatment (χ^2 = 0.31, P = 0.86) or season of treatment (χ^2 = 5.23, P = 0.39), but it did differ between years (χ^2 = 4.63, P = 0.031) (Table 2). The difference in stem length between years was not substantial at Glacial Heritage, but on average, longer stems were observed in 2014 at Scatter Creek than in 2015.

Abundance

The best predictor of camas foliar cover was year (χ^2 = 29.11, P < 0.001). Many plots experienced an increase in percent cover of camas in 2013, one year after the first treatment, but percent cover returned to the baseline values observed in 2012 in subsequent years (Figure 1). The effect of season of treatment was also significant (χ^2 = 11.46, P = 0.04) (Table 2), with lower cover in LS-F than MS season treatment plots (t = 2.49, P = 0.03) or MS-F plots (t = 2.52, P = 0.03), though no patterns of change in foliar cover with frequency of treatment were observed. There was no evidence that treatment with either chemical changed camas abundance significantly compared to the control (χ^2 = 2.34, P = 0.31) (Table 2).

Reproduction

Flower production differed by year (χ^2 = 57.63, P < 0.001) and chemical treatment (χ^2 = 1651.4, P < 0.001) (Table 2), with more flowers m^{-2} in plots treated with either clethodim (Z = 39.23, P < 0.001) or fluazifop (Z = 26.40, P < 0.001) than in control plots. The difference in flower production between clethodim-treated plots and control plots increased over time; while the average number of flowers in control groups decreased from 2013 to 2015, the average number of flowers in clethodim-treated groups increased over the same time period. Season of treatment also significantly impacted flowers m^{-2} (χ^2 = 980.57, P < 0.001), but the effect varied with chemical treatment (χ^2 = 803.58, P < 0.001) (Tables 2 & 3). Generally, more flowers were observed in plots that included a mid-spring application, and plots treated in all three seasonal periods showed the highest flower production compared to the control (Table 3). When flower production was examined on a per plant level, the treatment effects were less pronounced. Chemical was not a significant predictor of flowers per plant (χ^2 = 5.59, P = 0.06) (Table 2), but year was important, with more flowers per plant observed in 2014 than 2015 (t = –2.37, P = 0.02). Flower production per plant also varied with season of treatment (χ^2 = 14.05, P = 0.02), with the most flowers produced in the MS-F treatment (Tables 2 & 3).

TABLE 2. Statistical significance of fixed factors from mixed effects models. Bold text identifies *P*-values < 0.05.

Variable	χ^2 Statistic	*P* value
Plant Biomass		
Year	3.82	0.05
Chemical	1.21	0.55
Seasonality	1.30	0.73
Chemical:Seasonality	5.61	0.47
Leaves per Plant		
Year	7.75	**0.005**
Chemical	5.65	0.059
Seasonality	4.83	0.44
Chemical:Seasonality	9.09	0.52
Leaf Length		
Year	22.51	**< 0.001**
Chemical	10.85	**0.004**
Seasonality	13.36	**0.020**
Chemical:Seasonality	20.33	**0.026**
Leaf Width		
Year	8.89	**0.003**
Chemical	0.84	0.66
Seasonality	10.51	0.062
Chemical:Seasonality	7.60	0.67
Flowering Stem Presence		
Year	1.26	0.262
Chemical	9.29	**0.01**
Seasonality	3.54	0.617
Chemical:Seasonality	7.61	0.667
Flowering Stem Height		
Year	4.63	**0.031**
Chemical	0.31	0.86
Seasonality	5.23	0.39
Chemical:Seasonality	12.49	0.25
Percent Cover		
Year	29.11	**< 0.001**
Chemical	2.34	0.31
Seasonality	11.46	**0.04**
Chemical:Seasonality	4.85	0.90
Flowers m^{-2}		
Year	57.63	**< 0.001**
Chemical	1651.4	**< 0.001**
Seasonality	980.57	**< 0.001**
Chemical:Seasonality	803.58	**< 0.001**
Flowers per Plant		
Year	5.65	**0.017**
Chemical	5.59	0.061
Seasonality	14.05	**0.015**
Chemical:Seasonality	9.78	0.46
Seedpods per Plant		
Chemical	23.42	**< 0.001**
Seasonality	5.31	0.38
Chemical:Seasonality	9.77	0.46
Seedpod Success		
Chemical	0.94	0.63
Seasonality	3.15	0.68
Chemical:Seasonality	6.23	0.80
Seeds per Seedpod		
Chemical	1.06	0.59
Seasonality	4.78	0.44
Chemical:Seasonality	6.27	0.79
T50		
Chemical	16.89	**< 0.001**
Seasonality	8.26	0.14
Chemical:Seasonality	29.39	**0.001**
Germination Success		
Chemical	1.12	0.57
Seasonality	7.89	0.16
Chemical:Seasonality	6.14	0.80
Proportion Grazed		
Year	0.76	0.38
Chemical	1.00	0.61
Seasonality	34.61	**< 0.001**
Chemical:Seasonality	17.01	0.07

Seed production, as measured by the proportion of seedpods successfully producing seed ("seedpod success") and the number of seeds per successful seedpod, was not significantly altered by either chemical treatment or the season of treatment (Table 2). However, the number of seedpods per plant was impacted by chemical treatment ($\chi^2 = 23.42$, $P < 0.001$) (Table 2), with plants in clethodim-treated plots producing more seedpods than plants in both fluazifop-treated plots and control plots (Table 4). The vigor of the seeds, measured by the time to 50% germination (T50), was affected by chemical treatment ($\chi^2 = 16.89$, $P < 0.001$) (Table 2). On average, it took 1.14 days longer for 50% of the seeds from plots treated with clethodim to germinate compared to the control, while seeds from fluazifop-treated plots were comparable to the control. There was also a significant interaction between chemical and season of treatment ($\chi^2 = 29.39$, $P = 0.001$) (Table 2), with higher T50 observed in clethodim-treated plots in MS and LS season treatments (Table 3).

TABLE 3. Effects of chemical and season of treatment on camas growth, reproduction, and herbivory variables. Values are presented as mean ± 1 SD. Different lowercase letters indicate significant differences between seasons within each chemical treatment and different uppercase letters represent significant differences between chemical treatments ($\alpha = 0.05$). MS = mid-spring, LS = late spring, and F = fall.

		Season of Treatment					
	Chemical	MS	LS	MS-LS	MS-F	LS-F	MS-LS-F
Leaf length	Clethodim^{AB}	22.31 ± 6.93^{a}	19.35 ± 4.92^{b}	20.83 ± 7.85^{ab}	20.86 ± 8.96^{b}	21.10 ± 6.89^{ab}	19.69 ± 6.28^{b}
	Fluazifop^{B}	19.93 ± 7.43^{ab}	21.19 ± 8.63^{a}	17.71 ± 6.02^{b}	21.39 ± 7.39^{a}	21.06 ± 5.79^{a}	21.00 ± 7.59^{ab}
	Control^{B}	21.78 ± 5.73^{ab}	23.06 ± 6.37^{a}	21.45 ± 5.51^{ab}	22.17 ± 4.86^{ab}	22.43 ± 6.32^{a}	20.08 ± 6.33^{b}
Flowers m^{-2}	Clethodim^{A}	106.56 ± 164.22^{a}	73.94 ± 85.29^{b}	94.00 ± 115.24^{c}	127.00 ± 206.06^{d}	63.67 ± 79.95^{e}	143.22 ± 158.29^{f}
	Fluazifop^{B}	55.94 ± 100.07^{a}	57.22 ± 84.56^{a}	87.22 ± 117.22^{b}	111.17 ± 197.55^{c}	81.83 ± 122.38^{b}	108.22 ± 122.31^{c}
	Control^{C}	55.94 ± 90.10^{a}	61.89 ± 76.54^{b}	55.83 ± 79.15^{a}	72.44 ± 95.27^{c}	38.22 ± 60.13^{d}	37.28 ± 66.59^{d}
T50	Clethodim^{A}	65.74 ± 2.94^{a}	64.74 ± 2.66^{ab}	63.90 ± 1.40^{bc}	63.09 ± 0.73^{c}	63.79 ± 1.87^{bc}	64.31 ± 2.04^{bc}
	Fluazifop^{AB}	62.84 ± 0.57^{a}	63.59 ± 1.25^{ab}	64.67 ± 2.20^{b}	62.71 ± 1.55^{a}	64.00 ± 1.29^{ab}	63.88 ± 0.73^{ab}
	Control^{B}	62.88 ± 0.55	62.64 ± 0.57	63.17 ± 0.76	63.29 ± 0.65	62.87 ± 0.92	63.86 ± 1.29
Proportion grazed	Clethodim	0.19 ± 0.40^{a}	0.19 ± 0.39^{a}	0.27 ± 0.45^{a}	0.16 ± 0.37^{b}	0.26 ± 0.44^{a}	0.45 ± 0.50^{c}
	Fluazifop	0.31 ± 0.47^{a}	0.19 ± 0.40^{a}	0.14 ± 0.35^{a}	0.14 ± 0.35^{b}	0.20 ± 0.40^{a}	0.34 ± 0.48^{c}
	Control	0.35 ± 0.48^{a}	0.29 ± 0.46^{a}	0.21 ± 0.41^{a}	0.02 ± 0.15^{b}	0.25 ± 0.44^{a}	0.37 ± 0.49^{c}

TABLE 4. Effects of chemical on flowering stem presence and seedpods per plant. Values are presented as mean ± 1 SD. Different letters represent significant treatment differences ($\alpha = 0.05$).

	Clethodim	Fluazifop	Control
Stem presence (proportion)	0.31 ± 0.46^{a}	0.32 ± 0.47^{a}	0.22 ± 0.41^{b}
Seedpods per plant	6.90 ± 2.52^{a}	5.13 ± 2.14^{b}	5.25 ± 1.76^{b}

The overall proportion of seeds successfully germinating ("germination success") was not affected by chemical ($\chi^2 = 1.12$, $P = 0.57$) or season of treatment ($\chi^2 = 7.89$, $P = 0.16$) (Table 2).

Palatability to Herbivores

The proportion of plants grazed by herbivores differed significantly by season of treatment ($\chi^2 = 34.61$, $P < 0.001$), but not chemical ($\chi^2 = 1.00$, $P = 0.61$) (Table 2). Plants treated in mid-spring and fall experienced less grazing than plants in other treatment groups, and those in MS-LS-F season treatment groups experienced more grazing (Table 3).

Discussion

The spread of non-native annual and perennial grasses in native grasslands and arid lands throughout the United States has led to loss of biodiversity (Rosentreter 1994) and endangered species (Dangremond et al. 2010), and dramatically altered disturbance regimes (Brooks et al. 2004), plant-soil interactions (Jordan et al. 2008), and plant-insect interactions (Wilcove et al. 1998). Because of this, a range of control efforts involving herbicide (Dennehy et al. 2011), mowing (Wilson and Clark 2001), and prescribed burning (Stanley et al. 2011a) have been used to try to remove invasive grasses with varying levels of success, depending

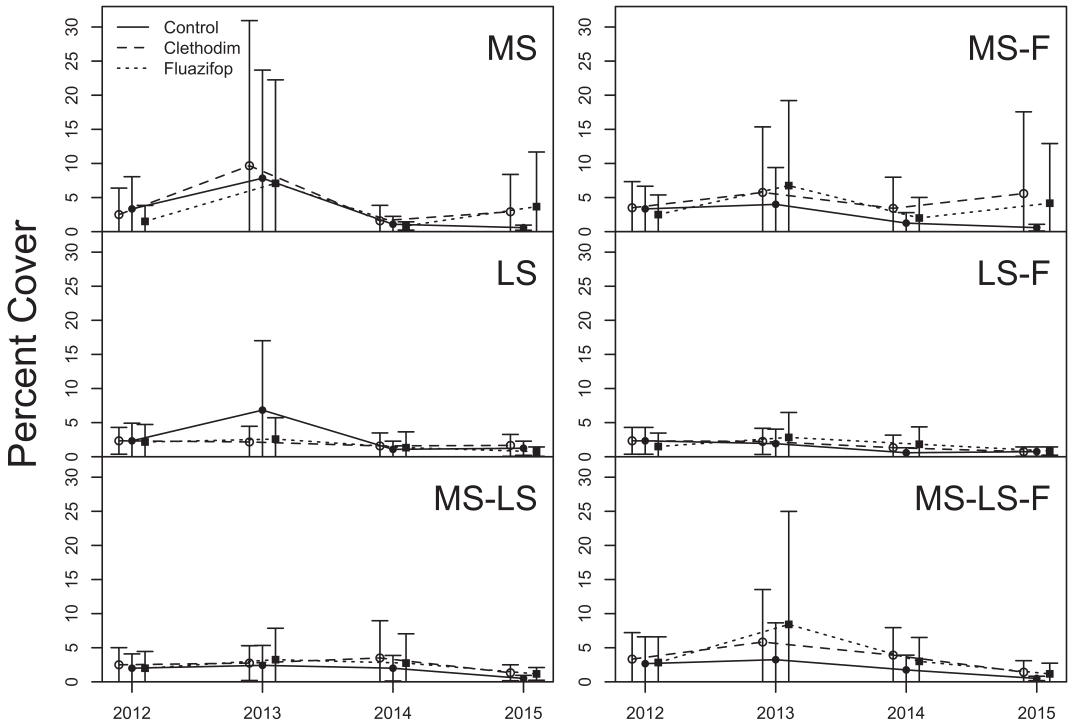

Figure 1. Camas abundance over time, chemical treatment, and season of treatment. "LS" = late spring, "MS" = mid-spring, and "F" = fall. Values are presented as mean ± 1 SD. Significant differences were observed by year ($\chi^2 = 29.11$, $P < 0.001$) and season of treatment ($\chi^2 = 11.46$, $P = 0.04$).

on the species and the landscape context. Selective herbicide is increasingly used as an invasive plant treatment, due to the ability to strategically target individual species or functional groups, where fire and mowing cannot (Hamill et al. 2004). Though initially and successfully developed for use in agriculture (Aktar et al. 2009), selective herbicide use is also increasingly used as a tool in restoration. However, the direct and indirect effects of these selective herbicides on native species in these restoration settings are rarely assessed in peer-reviewed literature (Zavaleta et al. 2001, Crone et al. 2009; however, see Hitchmough et al. 1994). This study evaluated off-target impacts of two graminoid-specific herbicides used extensively throughout Pacific Northwest grasslands on the growth and reproductive capacity of a culturally and ecologically important species, *Camassia quamash*. Overall, we found that neither fluazifop nor clethodim application had a sustained negative influence on the growth, abundance, reproduction, or herbivory of camas.

Chemical Treatment

Herbicide application reduced camas leaf length and increased the frequency of flowering stems. Observations of reduced leaf length could simply be a product of increased grazing, however we did not observe a corresponding increase in the proportion of grazed plants in chemical-treated groups compared to the control, suggesting that these shorter leaves are instead a function of physiology. This decrease in leaf length could be indicative of plant stress and reduced health, potentially from decreased flavonoid production resulting from clethodim and fluazifop treatment (Luo et al. 2004). However, the overall reduction in mean leaf length compared to control groups

was minimal (1–5 mm), and biomass remained unchanged between chemical treatment groups, thus these small changes in growth may not have substantial ecological ramifications.

The increase in flowering stem production and flowers m^{-2} in herbicide-treated groups, as well as the increase in seedpod production in clethodim-treated groups compared to fluazifop-treated and control groups, may also support the hypothesis of decreased plant health if plants are under stress and this reflects a stress-induced last-ditch attempt at reproduction (Southwick and Davenport 1986). Yet anecdotally, camas plants in herbicide-treated plots appeared no less robust in coloration or any other visual characteristics than plants in control plots. More likely, these changes may indicate a release from competition with non-native grass species. Results from this same field experiment show that the abundance of invasive pasture grasses significantly decreased in treated plots (66% decrease in tall oatgrass at Glacial Heritage and 47–68% decrease at Scatter Creek; Freed et al. 2015), allowing camas plants greater access to sunlight and other resources. Increased camas visibility to pollinators, as a result of decreased grass cover, may also have contributed to the increased seedpod production observed. Additionally, the reduction in leaf length in chemically treated plots could have been caused by the reduced need for the camas to compete with the taller invasive grasses to gain access to sunlight. A hypothesized release from competition follows prior work showing an increase in wildflower cover following herbicide application (Blake et al. 2013), though here we did not observe a change in foliar cover over the time period of the study.

The time to 50% germination (T50) slightly but significantly increased with herbicide treatment, suggesting that plants treated with herbicide may have a somewhat reduced competitive ability in seedling establishment. However, this average delay of one day may not be substantial enough to have ecological consequences.

Season of Treatment

The effects of chemical treatment differed by season of treatment for one growth metric (leaf length) and two reproductive metrics (flowers m^{-2}, T50). The reduction in leaf length compared to the control was greatest after a late spring treatment with clethodim. It is possible smaller leaves could result in less photosynthetically-fixed carbon and, hence, lower growth rates. However, the lack of change in plant biomass across all treatments suggests that the plants with smaller leaves were able to compensate. Average flowers m^{-2} increased the most in plots treated with herbicide over multiple seasons (i.e., higher frequency of treatment), suggesting that each application may have an additive positive effect on camas reproduction, or, more likely, an additive negative effect on the competing non-native grasses. Beyond this one frequency-dependent result, no other metrics were consistently related to frequency of herbicide treatment, and, contrary to our expectation, there was no obvious pattern of increased magnitude of effects during mid-spring when camas is actively growing.

While the effect of chemical on T50 varied by season of treatment, the proportion of germinated seeds was overall very high across chemical-season treatments (all over 80%, most over 90%). Thus, differences in seed viability may not be ecologically significant, although the increase in flower production in chemical-treated groups may benefit camas reproduction. However, we note that the findings here reflect seed germination when seeds were grown in an herbicide-free environment. Seeds germinating in the field could be exposed to chemicals, particularly those with soil persistence. Clethodim photodegrades rapidly when exposed to light (half-life of 6–10 minutes) (Sandin-Espana et al. 2016) and there is evidence of fairly rapid (< 24 hours) microbial degradation of fluazifop to less- or non-toxic by-products in both the laboratory and the field (Smith 1987, Badawi et al. 2015). For both chemicals, the degradation products are more persistent and labile and effects of these products on native seed are unknown, especially in the context of varying seasonal conditions (light exposure, moisture). Thus, we cannot completely rule out some degree of inhibited germination as observed by Rokich et al. (2009) and Wagner and Nelson (2014).

Management Implications and Future Questions

The variables explored in this study revealed that herbicide application has minimal short-term impacts on the growth and reproduction of camas. It is possible that any substantial negative impacts caused by herbicide application were mitigated by positive impacts associated with release from competition with invasive broadleaf grasses (Wilson and Clark 2001, Andreu and Vilà 2011, Cox and Allen 2011). While camas reproduction increased under grass-specific herbicide treatment, this did not translate into increased abundance during the timeframe of this study.

While this study attempted to address impacts of herbicide to camas plants and populations over multiple years, the effects of herbicide on camas may extend over timeframes longer than three years. Applying herbicide for more than three consecutive years is not uncommon, and may have negative indirect impacts on native plants through two mechanisms. First, repeat herbicide applications often create large amounts of litter, which can inhibit native germination or plant growth through light limitation (Eliason and Allen 1997). Second, repeat applications of herbicide with the same mechanism of action (i.e., targeting the same enzyme) can select for resistance to the chemical (Tranel and Wright 2002, Wang et al. 2017), and the resulting herbicide-resistant non-native grasses may continue to compete with native plants. Some species develop resistance to herbicide in as few as three years of intensive agricultural use (*Conyza canadensis* L. resistance to glyphosate; VanGessel 2001), while others have taken 8-10 years with three treatments per year (*Lolium multiflorum* Lam. resistance to glyphosate; Perez and Kogan 2003).

Off-target herbicide impacts may also extend beyond camas and have implications in plant-insect interactions (Kearns et al. 1998, Russell and Schultz 2010, Brittain and Potts 2011) and plant-microbe symbioses (Darine et al. 2015). Recent work evaluating both of these grass-specific herbicides on three *Euphydras* butterfly species found that herbicide treatment altered iridoid glycoside (defense compounds typically obtained from host plants) profiles in larvae, potentially changing their palatability to predators (Schultz et al. 2016). Glaeser and Schultz (2014) found that when applied in the early spring, fluazifop did not have any negative impacts on the behavior or demographic responses of the silvery blue butterfly (*Glucopsyche lygdamus*) and that it actually enhanced vegetative structure preferred by the butterfly. Thus, to fully understand how targeted herbicides influence potentially sensitive native species it is important to consider the timing of the application relative to the life history of each sensitive plant and animal species. This type of evaluation ensures that the widespread use of any new chemical will not be detrimental to the conservation and restoration of native communities.

The impacts of these two chemicals on human health and other ecosystems should be considered when making decisions about herbicide use. A recent review suggests that fluazifop poses higher risks to human and aquatic health than clethodim (Thurston County Health Department 2015). Additionally, the potential for bioaccumulation of herbicide residuals in camas bulbs is a major consideration for those interested in harvesting this culturally important species for consumption. More information on bioaccumulation potential for these compounds is needed.

Strategic application of graminoid-specific herbicides has become a valuable and widespread tool for the removal of invasive grasses in Pacific Northwest prairie habitats (Stanley et al. 2011b), with little knowledge of off-target impacts on native plants. Results from the present study suggest that clethodim has minimal impacts on camas, with a projected increase in reproductive effort and a small decrease in foliar growth and delay in seed germination. Therefore, we recommend that land managers use clethodim for invasive grass management to minimize environmental and human health impacts.

Acknowledgments

The authors would like to thank Tim Leque, Adam Martin, and Alex Bell for assistance in the field and Deborah Rogers and three anonymous reviewers for reviews of earlier drafts of the manuscript.

Funding and support for this project were provided by the Department of Defense Army Compatible Use Buffer Program, the Washington Service Corps AmeriCorps Program sponsored by the Washington Fish and Wildlife Conservation Office (USFWS) and the Center for Natural Lands Management.

Literature Cited

Adamson, N. L., B. Borders, J. K. Cruz, S. F. Jordan, K. Gill, J. Hopwood, E. Lee-Mäder, A. Minnerath, and M. Vaughan. 2015. Pollinator Plants: Maritime Northwest. Xerces Society for Invertebrate Conservation, Portland, OR.

Aktar, W., D. Sengupta, and A. Chowdhury. 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology 2:1-12.

Andreu, J., and M. Vilà. 2011. Native plant community response to alien plant invasion and removal. Management of Biological Invasions 2:81-94.

Ansley, R. J., and M. J. Castellano. 2006. Strategies for savanna restoration in the southern Great Plains: effects of fire and herbicides. Restoration Ecology 14:420-428.

Badawi, N., A. E. Rosenbom, P. Olsen, and S. R. Sørensen. 2015. Environmental fate of the herbicide fluazifop-P-butyl and its degradation products in two loamy agricultural soils: A combined laboratory and field study. Environmental Science & Technology 49:8995-9003.

Barnes, T. G. 2004. Strategies to convert exotic grass pastures to tall grass prairie communities. Weed Technology 18:1364-1370.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Beckwith, B. R. 2004. The queen root of this clime: ethnoecological investigations of blue camas (*Camassia leichtlinii* (Baker) Wats., *C. quamash* (Pursh) Greene; Liliaceae) and its landscapes on southern Vancouver Island, British Columbia. Ph.D. Dissertation, University of Victoria, Victoria, British Columbia.

Blake, R. J., D. B. Westbury, B. A. Woodcock, P. Sutton, and S. G. Potts. 2011. Investigating the phytotoxicity of the graminicide fluazifop-P-butyl against native UK wildflower species. Pest Management Science 68:412-421.

Blake, R. J., B. A. Woodcock, D. B. Westbury, P. Sutton, and S. G. Potts. 2013. Novel management to enhance spider biodiversity in existing buffer strips. Agricultural and Forest Entomology 15:77-85.

Blazina, A. J. 2017. Historical disturbance and recent management factors driving *Quercus garryana* vegetation communities in the Puget Sound Lowlands. M.S. Thesis, University of Washington, Seattle.

Boutin, C., K. Aya, D. Carpenter, P. Thomas, and O. Rowland. 2012. Phytotoxicity testing for herbicide regulation: shortcomings in relation to biodiversity and ecosystem services in agrarian systems. Science of the Total Environment 415:79-92.

Boyd, R. T. 1986. Strategies of Indian burning in the Willamette Valley. Canadian Journal of Anthropology 5:65-86.

Brittain, C., and S. G. Potts. 2011. The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. Basic and Applied Ecology 12:321-331.

Brooks, M. L., C. M. D'Antonio, D. M. Richardson, J. B. Grace, J. E. Keeley, J. M. DiTomaso, R. J. Hobbs, M. Pellant, and D. Pyke. 2004. Effects of invasive alien plants on fire regimes. BioScience 54:677-688.

Colwell, S. G. 1984. Response of seedling onions (*Allium cepa*) to fluazifop-butyl and bromoxynil. M.S. Thesis, The University of Arizona, Tucson.

Coolbear, P., A. Francis, and D. Grierson. 1984. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. Journal of Experimental Botany 35:1609-1617.

Cox, R. D., and E. B. Allen. 2011. The roles of exotic grasses and forbs when restoring native species to highly invaded southern California annual grassland. Plant Ecology 212:1699-1707.

Crawford, R. C., and H. Hall. 1997. Changes in the South Puget Prairie landscape. *In* P. V. Dunn and K. Ewing (editors), Ecology and Conservation of the South Puget Sound Prairie Landscape. The Nature Conservancy of Washington, Seattle, WA. Pp. 11-15.

Cronan, J. E., and G. L. Waldrop. 2002. Multi-subunit acetyl-CoA carboxylases. Progress in Lipid Research 41:407-435.

Crone, E. E., M. Marler, and D. E. Pearson. 2009. Non-target effects of broadleaf herbicide on a native perennial forb: a demographic framework for assessing and minimizing impacts. Journal of Applied Ecology 46:673-682.

Dangremond, E. M., E. A. Pardini, and T.M. Knight. 2010. Apparent competition with an invasive plant hastens the extinction of an endangered lupine. Ecology 91:2261-2271.

Darine, T., C. Alaeddine, B. Fethi, and M. Ridha. 2015. Fluazifop-P-butyl (herbicide) affects richness and structure of soil bacterial communities. Soil Biology and Biochemistry 81:89-97.

Délye, C. 2005. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Science 53:728-746.

Dennehy, C., E. R. Alverson, H. E. Anderson, D. R. Clements, R. Gilbert, and T. N. Kaye. 2011. Management strategies for invasive plants in Pacific Northwest prairies, savannas, and oak woodlands. Northwest Science 85:329-351.

Drake, D., and K. Ewing. 1997. Germination requirements of 32 native Washington prairie species. *In* P. V. Dunn and K. Ewing (editors). Ecology and Conservation of the South Puget Sound Prairie Landscape. The Nature Conservancy of Washington, Seattle. Pp. 181-187.

Dunn, P. V., and K. Ewing. 1997. Ecology and Conservation of the South Puget Sound Prairie Landscape. The Nature Conservancy of Washington, Seattle, WA.

Dunwiddie, P. W., and J. D. Bakker. 2011. The future of restoration and management of prairie-oak ecosystems in the Pacific Northwest. Northwest Science 85:83-92.

Dunwiddie, P. W., E. Alverson, A. Stanley, R. Gilbert, S. Pearson, D. Hays, J. Arnett, E. Delvin, D. Grosboll, and C. Marschner. 2006. The vascular plant flora of the south Puget Sound prairies, Washington, USA. Davidsonia 17:51-69.

Eliason, S. A., and E. B. Allen. 1997. Exotic grass competition in suppressing native shrubland re-establishment. Restoration Ecology 5:245-255.

Erickson, W. R. 1978. Classification and interpretation of Garry oak (*Quercus garryana*) plant communities and ecosystems in southwestern British Columbia. M.S. Thesis, University of Victoria, Victoria, British Columbia.

Floberg, J., M. Goering, G. Wilhere, C. MacDonald, C. Chappell, C. Rumsey, Z. Ferdana, A. Holt, P. Skidmore, T. Horsman, E. Alverson, C. Tanner, M. Bryer, P. Iachetti, A. Harcombe, B. McDonald, T. Cook, M. Summers, and D. Rolph. 2004. Willamette Valley-Puget Trough-Georgia Basin Ecoregional Assessment, Volume One: Report. The Nature Conservancy of Washington, Seattle.

Freed, S., D. W. Hays, D. Wilderman, and S. T. Hamman. 2015. Invasive Plant Control Across Army Compatible Use Buffer Lands, Annual Progress Report. Center for Natural Lands Management, Olympia, WA.

Garibaldi, A., and N. Turner. 2004. Cultural keystone species: implications for ecological conservation and restoration. Ecology and Society 9:1-18.

Glaeser, R. M., and C. B. Schultz. 2014. Characterizing a contentious management tool: the effects of a grass-specific herbicide on the silvery blue butterfly. Journal of Insect Conservation 18:1047-1058.

Guerrant, E. O., Jr., and A. Raven. 1995. Seed germination and storability studies of 69 plant taxa native to the Willamette Valley wet prairie. The Berry Botanic Garden, Portland, OR.

Hamill, A. S., J. S. Holt, and C. A. Mallory-Smith. 2004. Contributions of weed science to weed control and management. Weed Technology 18:1563-1565.

Harker, K. N., and P. A. O'Sullivan. 1991. Synergistic mixtures of sethoxydim and fluazifop on annual grass weeds. Weed Technology 5:310-316.

Higgs, E. 2005. The two-culture problem: ecological restoration and the integration of knowledge. Restoration Ecology 13:159-164.

Hitchmough, J., R. A. Kilgore, J. W. Morgan, and I. G. Shears. 1994. Efficacy of some grass-specific herbicides in controlling exotic grass seedlings in native grassy vegetation. Plant Protection Quarterly 9:28-34.

Jordan, N. R., D. L. Larson, and S. C. Huerd. 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. Biological Invasions 10:177-190.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29:83-112.

Konishi, T., K. Shinohara, K. Yamada, and Y. Sasaki. 1996. Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. Plant and Cell Physiology 37:117-122.

Lanini, W. T., and S. R. Radosevich. 1982. Herbicide effectiveness in response to season of application and shrub physiology. Weed Science 30:467-475.

Luo, X. Y., and H. Matsumoto. 2002. Susceptibility of a broad-leaved weed, *Acanthospermum hispidum*, to the grass herbicide fluaxifop-butyl. Weed Biology and Management 2:98-103.

Luo, X. Y., Y. Sunohara, and H. Matsumoto. 2004. Fluazifop-butyl causes membrane peroxidation in the herbicide-susceptible broad leaf weed bristly starbur (*Acanthospermum hispidum*). Pesticide Biochemistry and Physiology 78:93-102.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. Agro-Ecosystems 7:145-165.

Miller, R. F., W. C. Krueger, and M. Vavra. 1981. Deer and elk use on foothill rangelands in northeastern Oregon. Journal of Range Management 34:201-204.

Noss, R. F., E. T. LaRoe, and J. M. Scott. 1995. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. U.S. Department of the Interior, National Biological Service, Washington, DC.

Olszyk, D., M. Blakeley-Smith, T. Pfleeger, E. H. Lee, and M. Plocher. 2013. Effects of low levels of herbicides on prairie species of the Willamette Valley, Oregon. Environmental Toxicology and Chemistry 32:2542-2551.

Parachnowitsch, A., and E. Elle. 2005. Insect visitation to wildflowers in the endangered Garry Oak, *Quercus garryana*, ecosystem of British Columbia. The Canadian Field-Naturalist 119:245-253.

Perez, A., and M. Kogan. 2003. Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. Weed Research 43:12-19.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Reid, M. S. 1995. Ethylene in plant growth, development, and senescence. *In* P. J. Davies (editor). Plant Hormones and Their Role in Plant Growth and Development. Martinus Nijhoff Publishers, Dordrecht, Netherlands. Pp. 486-508.

Rendina, A. R., and J. M. Felts. 1988. Cyclohexanedione herbicides are selective and potent inhibitors of acetyl-CoA carboxylase from grasses. Plant Physiology 86:983-986.

Reynolds, D. B., T. G. Wheless, E. Basler, and D. S. Murray. 1988. Moisture stress effects on absorption and translocation of four foliar-applied herbicides. Weed Technology 2:437-441.

Rogler, G. A., and R. J. Lorenz. 1983. Crested wheatgrass: early history in the United States. Journal of Range Management 36:91-93.

Rokich, D. P., J. Harma, S. R. Turner, R. J. Sadler, and B. H. Tan. 2009. Fluazifop-p-butyl herbicide: implications for germination, emergence and growth of Australian plant species. Biological Conservation 142:850-869.

Rosentreter, R. 1994. Displacement of rare plants by exotic grasses. *In* Proceedings of Ecology and Management of Annual Rangelands. USDA Forest Service General Technical Report INT-GTR-313. Intermountain Research Station, Ogden, UT. Pp. 170-175.

Rowe, H. I. 2010. Tricks of the trade: techniques and opinions from experts in tallgrass prairie restoration. Restoration Ecology 18:253-262.

Ruffner, M. E., and T. G. Barnes. 2010. Natural grassland response to herbicides and application timing for selective control of tall fescue, an invasive cool-season grass. Invasive Plant Science and Management 3:219-228.

Russell, C., and C. B. Schultz. 2010. Effects of grass-specific herbicides on butterflies: an experimental investigation to advance conservation efforts. Journal of Insect Conservation 14:53-63.

Sandín-España, P., B. Sevilla-Morán, C.López-Goti, M. M. Mateo-Miranda, and J. L. Alonso-Prados. 2016. Rapid photodegradation of clethodim and sethoxydim in soil and plant surface model systems. Arabian Journal of Chemistry 9:694-703.

Sasaki, Y., T. Konishi, and Y. Nagano. 1995. The compartmentation of acetyl-coenzyme A carboxylase in plants. Plant Physiology 108:445-449.

Sasaki, Y., and Y. Nagano. 2004. Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. Bioscience, Biotechnology, and Biochemistry 68:1175-1184.

Schultz, C. B. 2001. Restoring resources for an endangered butterfly. Journal of Applied Ecology 38:1007-1019.

Schultz, C. B., J. L. Zemaitis, C. C. Thomas, M. D. Bowers, and E. E. Crone. 2016. Non-target effects of grass-specific herbicides differ among species, chemicals and host plants in *Euphydryas* butterflies. Journal of Insect Conservation 20:867-877.

Smith, A. E. 1987. Persistence studies with herbicide fluazifop-butyl in Saskatchewan soils under laboratory and field conditions. Bulletin of Environmental Contamination and Toxicology 39:150-155.

Southwick, S. M., and T. L. Davenport. 1986. Characterization of water stress and low temperature effects on flower induction in citrus. Plant Physiology 81:26-29.

Solecki, M. K. 1997. Controlling invasive plants. *In* S. Packard and C. F. Mutel (editors), The Tallgrass Restoration Handbook: for Prairies, Savannas, and Woodlands. Island Press, Washington DC. Pp. 251-278.

Stanley, A. G., T. N. Kaye, and P. Dunwiddie. 2011a. Multiple treatment combinations and seed addition increase abundance and diversity of native plants in Pacific Northwest prairies. Ecological Restoration 29:35-44.

Stanley, A. G., P. W. Dunwiddie, and T. N. Kaye. 2011b. Restoring invaded Pacific Northwest praries: management recommendations from a region-wide experiment. Northwest Science 85: 233-246.

Thurston County Health Department. 2015. Terrestrial herbicide reviews. Public Health and Social Services, Olympia, WA. Available online at http://www.co.thurston.wa.us/health/ehipm/terrestrialreview.html (accessed 05 May 2018).

Tomimatsu, H., S. R. Kephart, and M. Vellend. 2009. Phylogeography of *Camassia quamash* in western North America: postglacial colonization and transport by indigenous peoples. Molecular Ecology 18:3918-3928.

Tranel, P. J., and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Science 50:700-712.

U.S. Environmental Protection Agency. 2012a. Ecological effects test guidelines, OCSPP 850.4150: vegetative vigor. EPA 712-C-011. Office of Chemical Safety and Pollution Prevention. Washington, DC.

U.S. Environmental Protection Agency. 2012b. Ecological effects test guidelines, OCSPP 850.4300: terrestrial plants field study. EPA 712-C-009. Office of Chemical Safety and Pollution Prevention, Washington, DC.

U.S. Fish and Wildlife Service. 2010. Recovery Plan for the Prairie Species of Western Oregon and Southwestern Washington. U.S. Fish and Wildlife Service, Portland, OR.

VanGessel, M. J. 2001. Glyphosate-resistant horseweed from Delaware. Weed Science 49:703-705.

Wagner, V., and C. R. Nelson. 2014. Herbicides can negatively affect seed performance in native plants. Restoration Ecology 22:288-291.

Walker, K. A., S. M. Ridley, T. Lewis, and J. L. Harwood. 1988. Fluazifop, a grass-selective herbicide which inhibits acetyl-CoA carboxylase in sensitive plant species. Biochemical Journal 254:307-310.

Walsh, M. K., C. Whitlock, and P. J. Bartlein. 2010. 1200 years of fire and vegetation history in the Willamette Valley, Oregon and Washington, reconstructed using high-resolution macroscopic charcoal and pollen analysis. Palaeogeography, Palaeoclimatology, Palaeoecology 297:273-289.

Wang, C-S., W-T. Lin, Y-J. Chiang, and C-Y. Wang. 2017. Metabolism of fluazifop-P-butyl in resistant Goosegrass (*Eleusine indica*) in Taiwan. Weed Science 65:228-238.

Weisberg, P. J., and F. J. Swanson. 2003. Regional synchroneity in fire regimes of western Oregon and Washington, USA. Forest Ecology and Management 172:17-28.

Wendel, C., and G. Orrick. 2014. Ecological risk assessment for the registration review of clethodim. U.S. Environmental Protection Agency Office of Pesticide Programs, Washington, DC.

Received 13 February 2018
Accepted 04 June 2018

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. Bioscience 48:607-615.

Wilcox, D. A., and T. H. Whillans. 1999. Techniques for restoration of disturbed coastal wetlands of the Great Lakes. Wetlands 19:835-857.

Wilson, M. C., and D. L. Clark. 2001. Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegetation Science 4:129-138.

Wold, E. N., J. E. Jancaitis, T. H. Taylor, and D. M. Steeck. 2011. Restoration of agricultural fields to diverse wet prairie plant communities in the Willamette Valley, Oregon. Northwest Science 85:269-287.

Zavaleta, E. S., R. J. Hobbs, and H. A. Mooney. 2001. Viewing invasive species removal in a whole-ecosystem concept. Trends in Ecology and Evolution 16:454-459.