

INVESTIGATING THE USE OF HERBICIDES TO CONTROL INVASIVE GRASSES IN
PRAIRIE HABITATS: EFFECTS ON NON-TARGET BUTTERFLIES

By

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Abstract

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Invasive grasses severely impact prairies in the northwestern United States, leading to the decline of several butterfly species. Controlling these invasive species is a high priority for land managers. The use of herbicides is a promising management technique, yet effects on nontarget butterflies are virtually unknown. We conducted a field investigation on the effects of a widely used grass-specific herbicide on butterfly habitat and demography using the Puget blue (*Icaricia icarioides blackmorei* Barnes and McDunnough) butterfly as a model species. Habitat use of adult silvery blue (*Glaucopsyche lygdamus* Doubleday), ochre ringlet (*Coenonympha tullia* Müller), and wood nymph (*Cercyonis pegala* Fabricius) butterflies was also quantified. The results indicate that the herbicide had very little to no impact on larval survival, flower species, or Puget blue oviposition, while adult butterflies spent significantly less time in sprayed plots than in controls. Given the necessity of invasive grass control in natural areas, we recommend several strategies to minimize herbicide effects on butterflies.

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INTRODUCTION

Invasive exotic plants are a significant problem in natural ecosystems worldwide (Andow et al. 1990, Cronk and Fuller 1995, Culliney 2005). They critically damage natural habitats (Cronk and Fuller 1995, Levine et al. 2003), leading to displacement or extirpation of native plants and animals (Culliney 2005). In the United States, invasive plants are linked to the decline of 33 butterfly species (New et al. 1995, Wilcove et al. 1998), and 15 of 18 recovery plans for threatened or endangered butterflies recommend invasive plant control (Schultz et al. 2008). Management of invasive plants, however, involves practices that may also be detrimental to these butterflies. This is challenging to managers who need to reduce invasive plants yet limit impacts on nontarget species.

Mechanical methods of invasive plant control, such as mowing and burning, have had limited success in butterfly habitats, therefore managers have turned to herbicides as an additional tool (Schultz et al. 2008). Herbicides inhibit cell division, photosynthesis, and various biochemical pathways, depending on the target plant and type of herbicide used, and cost an average of \$6.5 billion each year in the United States (Kiely et al. 2004). Evaluations of herbicide effects on butterflies and other insects are generally only for species of agricultural importance, such as pollinators, decomposers, and predators (e.g. Ahn et al. 2001, Haughton et al. 2001a, 2001b). These effects vary depending on site-specific (Haughton et al. 2001a and 2001b, Stark and Banks 2003) and species-specific variables (Kjaer and Heimbach 2001, Stark and Banks 2003), and on the herbicide used (Agnello et al. 1986a). Although some herbicides have no effect on certain insect species (Agnello et al. 1986b, Kjaer and Heimbach 2001), others may be toxic, leading to decreased survival, altered

development time, or reduced size of the insect species (Brown 1987, Ahn et al. 2001, Russell and Schultz 2009). They can also have an indirect effect on insect population abundance by creating an unsuitable habitat, such as the loss or avoidance of nectar and food plants (Feber et al. 1996, Pratt et al. 1997). Because results from agricultural studies are not always applicable to nontarget butterflies and other insects in a conservation context (Longley and Sotherton 1997), further investigation of toxicological and ecological effects on nontarget species is warranted (Pratt et al. 1997).

Herbicide use is a common method of invasive plant control in areas with or near populations of many endangered, threatened, and candidate butterfly species in the United States (Table 1). Although there is limited published information regarding herbicide effects on at-risk species, there are herbicide use guidelines for some of these species. Specific project treatment of a herbicide exists for a three-year project in Lassen County, California, in Carson wandering skipper (*Pseudocopaeodes eunus obscurus* Austin and Emmel) habitat. Project treatment allows for spot applications of a herbicide with backpack sprayers during the last two weeks in April and/or the first week in August, when the butterfly is in the pupa or egg stage, respectively (M. Haworth, pers. comm.). Similar guidelines exist for Karner blue (*Lycaeides melissa samuelis* Nabokov) habitat in Wisconsin, where spraying is allowed after September 1, when the butterfly is in the egg stage (C. Carnes, pers. comm.). In the San Francisco Bay area, spraying is not permitted in areas with known populations of five at-risk butterflies (Table 1), while herbicide effects on these butterflies are under evaluation (D. Kelly, pers. comm.). In pine woodland habitat of Colorado, where the Pawnee montane skipper (*Hesperia leonardus montana* Skinner) is found, weeds are controlled using

spot spraying techniques to minimize effects on non-target species (D. Bohon, pers. comm.). Where control of widespread invasive plants is necessary, herbicides can be applied using methods to minimize impacts on at-risk butterflies. For example, to control scotch broom (*Cytisus scoparius* L.) in Taylor's checkerspot (*Euphydryas editha taylori* Edwards) habitat in Oregon, spot spray methods are used to minimize overspray on food plants and larvae (A. Kitzman, pers. comm.).

Washington and Oregon prairies have experienced substantial degradation from human disturbance and invasive species, with an estimated 97% loss of native vegetation in Washington's Puget lowlands, and 99.9% loss of the Willamette Valley's prairie in Oregon (Crawford and Hall 1997, Noss et al. 1995). This has led to the decline of several prairie-dependent butterfly species in these areas. These species include Fender's blue (*Icaricia icarioides fenderi* Macy, federally endangered), Valley silverspot (*Speyeria zerene bremnerii* Edwards, federal species of concern), Taylor's checkerspot (federal candidate), and Mardon skipper (*Polites mardon* Edwards, federal candidate) (USFWS 2008, WDFW 2008). The latter three butterflies are present on the Fort Lewis Military Installation in Washington, which has one of the largest tracts of remnant native prairie in Western Washington. Two major invasive grass species in Fort Lewis prairies are tall oatgrass (*Arrhenatherum elatius* [L.] J. Presl and C. Presl) and velvet grass (*Holcus lanatus* L.). Grass-specific herbicides and combinations of herbicides and mowing or burning are most successful in controlling these invasive grasses (Fitzpatrick 2001, Stanley et al. 2009). These herbicides are applied when most butterflies are in the larval stage, their most vulnerable life stage due to potential ingestion and dermal absorption of chemicals. Understanding how herbicides affect

these butterflies could have substantial implications for which habitat restoration strategies are appropriate when recovery of rare butterflies is also a priority.

Puget blue butterfly (*Icaricia icarioides blackmorei* Barnes and McDunnough, state species of concern, WDFW 2008) is a subspecies related to the federally endangered Fender's blue. Puget blue utilizes similar habitat to other at-risk prairie butterflies, which makes it an ideal species for investigating herbicide effects on these butterflies. We investigated the effects of the grass-specific herbicide sethoxydim on Puget blue and three common native butterfly species: silvery blue (*Glaucopsyche lygdamus* Doubleday), ochre ringlet (*Coenonympha tullia* Müller) and wood nymph (*Cercyonis pegala* Fabricius), in Johnson Prairie, at Fort Lewis. We selected an area with high densities of these four species, but low densities of invasive perennial grasses to test direct effects of sethoxydim on butterflies rather than indirect effects through reduction of grass cover. Reduction of invasive grasses decreases competition with native plant species for available resources, as well as increases visibility of nectar flowers and host plants. For example, Hays et al. (2000) report that Mardon skipper and Puget blue primarily utilize areas with high percent cover of native forbs and grasses and low invasive grass cover.

We assessed sethoxydim impacts on vegetation (nectar species and lupine host plants), adult butterfly residence time, Puget blue larval survival, and Puget blue oviposition. As sethoxydim does not target forbs, we expected it would not significantly change nectar species abundance or lupine cover. Laboratory tests found that sethoxydim reduced survival, wing size, and pupal weight of cabbage white butterflies (*Pieris rapae* L.) and reduced development time of Puget blues (Russell and Schultz

2009). However, there are no field studies of sethoxydim effects on Puget blues; therefore, we assessed herbicide effects on larval survival in the field. Many studies show that female butterflies are highly selective of host plants during oviposition (e.g. Scott 1986, Bergman 1999, Bergström 2005), and often select for specific chemicals (Honda 1995, Nakayama et al. 2002, Reudler Talsma et al. 2008). It is unknown if female Puget blues can also detect herbicide chemicals in host lupines, therefore we also tested for possible avoidance of sprayed lupines. Some studies indicate butterflies are attracted to nectar species by certain visual or olfactory cues (Honda et al. 1998, Andersson et al. 2002, Andersson 2003). It is unknown whether sethoxydim interferes with these cues, thereby causing a change in butterfly behavior, such as avoidance or decreased residence time in sprayed areas. We investigated any avoidance or altered residence time of adult Puget and silvery blue, ochre ringlet, and wood nymph butterflies in sprayed areas.

METHODS

Study species and field site

Johnson Prairie is a 90-ha glacial outwash prairie located in the southern part of the Fort Lewis Military Installation (46.9° N, 122.7° W). Fort Lewis contains a majority of the largest and highest quality patches of prairie and oak habitat in the South Puget Trough area of Washington, USA (Morgenweck and Dunn 2003). Large grassy swales dominate Johnson Prairie, along with a high diversity of forb species and an edge of evergreen forests and oak woodlands. Morgenweck and Dunn (2003) found the highest

abundance (52% of individuals) and diversity (66% of species) of butterflies at Johnson Prairie compared to other Fort Lewis prairies.

The Puget blue is a small butterfly found in glacial outwash prairies in the Puget Trough of Washington (Pyle 2002). It is a Washington State species of concern, whose decline is largely attributed to habitat loss caused by development, fire suppression and Scotch broom (*Cytisus scoparius* L.) invasion (Pyle 2002). Johnson Prairie supports a population of at least 1,500-2,500 Puget blues (Schultz, unpublished data). The silvery blue is morphologically similar to the Puget blue, although it is more common and widespread. The flight season for both silvery and Puget blues occurs from mid-May to late-June. Both species are sexually dimorphic: the male dorsal color is blue, while the female is brown. Larvae of both species feed on sickle-keeled lupine (*Lupinus albicaulis* Douglas) at Johnson Prairie. Female Puget blues oviposit singly on the underside of leaves, while female silvery blues oviposit singly on developing flowers. Puget blues overwinter as young larvae, emerging from diapause in early spring and pupate in late-April or early-May. Silvery blues overwinter as pupae. Both ochre ringlets and wood nymphs use grasses as their larval hosts. Roemer's fescue (*Festuca roemeri* Pavlick) is the only observed host of wood nymphs and ochre ringlets at Johnson Prairie, although other grass species occur there that wood nymphs and ringlets are recorded using elsewhere. Both species overwinter as larvae. Ochre ringlets have two broods per year; adults peak in late-May to early-June and mid- to late-July. Wood nymphs have one brood and adults peak in late-July. Ochre ringlets are not sexually dimorphic. In contrast, female wood nymphs, compared to males, have larger eyespots surrounded by a light patch on their forewing. Both species are

common and widespread, and together with Puget and silvery blues, they constitute the four most abundant species at Johnson Prairie.

Experimental design

This study had two primary goals. The first was to determine potential demographic effects of sethoxydim on Puget blue butterflies by assessing larval survival and oviposition. The second goal was to determine the ecological effects of sethoxydim on butterfly habitat by assessing lupine cover, nectar species abundance, and habitat use of adult Puget blue, silvery blue, ochre ringlet, and wood nymph butterflies. We established control and experimental plots in June 2006. We used forty small plots (2 x 2 m) to assess Puget blue larval survival, female oviposition behavior, and lupine flower density and cover. We assessed abundance of twelve nectar species, lupine cover, and adult butterfly behavior in twenty large plots (20 x 20 m). We placed the small plots in an area of the prairie with relatively high lupine density, and distributed the large plots across the prairie. Each large plot contained at least ten lupine ramets, a variety of nectar species, and grass cover was primarily Roemer's fescue. To minimize spatial variance across the prairie, we paired control and experimental plots for a total of twenty small plot and ten large plot pairs, and randomly selected one plot in each pair as the control.

We collected pre-treatment vegetation data in the large plots and counted Puget blue eggs in the small plots in July and August 2006. We conducted herbicide treatments on April 26, 2007, followed by field assessment of vegetation, Puget blue larvae and oviposition, and adult butterfly behavior in the spring and summer of 2007.

On April 17, 2008, we repeated the sethoxydim treatment in the small plots, and counted larvae and assessed feeding damage between April 24 and May 16, 2008. Assessments began one week after spraying to allow time for the herbicide to take effect. We left the control plots untreated, and sprayed the experimental plots with sethoxydim (Poast[®]) and a nonionic surfactant (Agridex[®]). Sethoxydim was applied at 526 grams a.i. per hectare (2.5 pints per acre), and the surfactant at 1028 grams a.i. per hectare (1 pint per acre). Large plots were sprayed from a tractor. Small plots were sprayed with a backpack filled from the tractor and sprayed with a jet tip making a 1-meter wide swath at 9 kilograms pressure to replicate the tractor pace. To minimize potential edge effects, we sprayed the large plots at least 0.5 meters past the boundary, and only assessed the interior 1.5 x 1.5 meters of the small plots. Cliff Chapman and Casey Dennehy (The Nature Conservancy) applied the herbicide based on consultations with Roderick Gilbert (Fort Lewis), Ann Potter and Dave Hays (Washington Department of Fish and Wildlife).

Vegetation assessment

To assess vegetation impacts, we surveyed seven native and five nonnative forb species (Table 2) in the large plots in late July of 2006 and 2007. The species we chose are either known or potential nectar sources for butterflies, and most are documented nectar sources for Puget blues (Table 2). We also assessed cover of sickle-keeled lupine in both small and large plots, and density of lupine racemes in the small plots. The twelve species were surveyed in the large plots using a stratified

random design with two 0.5 x 2 meter quadrats randomly placed along each of ten transects. We analyzed the data as frequencies for each species, calculated as the number of quadrats per plot containing at least one flower of each species. To assess lupine cover in small plots, we measured the longest axis and perpendicular width of lupine ramets in each plot, calculated the square meter cover, and divided by the total area of the plot. We estimated percent cover in the large plots using the line-intercept method, by measuring the width of lupine ramets intersecting ten random transects, and dividing by the total length of the transects. We assessed lupine flower production in the small plots by counting the number of racemes in each plot.

Larval survival

We searched lupines in each small plot for Puget blue eggs during the end of June and beginning of July in both 2006 and 2007 (pre-treatment). Survival of eggs to post-diapause larvae for Puget blue is unknown; however, survival is 5-10% for Fender's blue (Schultz and Crone 1998). Based on these data, we calculated that plots need no fewer than 20 eggs or pre-diapause larvae per plot to have a statistically detectable effect. After counting the hatched and unhatched eggs in each plot, we supplemented them with pre-diapause larvae from outside the plots. In 2006, we supplemented 38 plots with an average of nine larvae per plot for a total of 20-25 eggs or pre-diapause larvae per plot. Egg numbers were higher in 2007, so only 28 plots needed supplementing with an average of seven larvae for a total of 25 eggs or pre-diapause larvae per plot.

The spring of 2007 was early, and most individuals had already pupated prior to the sethoxydim application, thus we found few post-diapause larvae in this assessment. We repeated the experiment in 2008, and closely examined the base of each lupine plant searched in the field, as well as the entire plant, and although the larvae had not pupated at the time of the surveys, they were difficult to find. Instead, we assessed larval feeding damage to estimate differences between control and sprayed plots. Through previous observations of larval feeding in the field and greenhouse, we were able to differentiate between Puget blue larval feeding signs and leaf and stem damage from other insects and small herbivores. Larval feeding damage was defined as stems, whole leaves, or individual leaflets that were chewed through by larvae, or windows in leaves where larvae mined inside the plant tissue. We counted the number of observations of each type of feeding damage.

Oviposition assessment

The forelegs of all female butterflies have a combination of spines and olfactory hairs that the females use to puncture and taste leaves to choose suitable host plants prior to oviposition (Scott 1986). Some studies indicate that female butterflies select for specific chemicals (Honda 1995, Nakayama et al. 2002, Reudler Talsma et al. 2008). Based on this information, we tested for possible avoidance of sprayed lupines by female Puget blue butterflies by comparing the total number of eggs laid in control and sprayed small plots.

Behavior observations

We observed adult Puget blue, silvery blue, ochre ringlet and wood nymph butterflies in the large plots to determine if sethoxydim affects their residence time. We observed plot pairs on the same day to reduce effects of weather on behavior. For each plot, two or three people stationed around the plot edge recorded observations for one-half hour. One person entered observations into a portable computer that recorded the duration in seconds a butterfly spent in the plot. Another person caught butterflies exiting the plot to determine sex and species. We counted all butterflies entering the plot and observed the residence time for as many butterflies as possible. Butterflies in the plot at the beginning of each one-half hour observation were not included in the assessment. No time limit was set for how long we observed an individual, as most spent between 3 seconds and 8 minutes in a plot. We observed each plot in May, June, and July 2007 to sample across the butterfly flight season at Johnson Prairie. Because the adult lifespan of these four species is usually less than ten days (Scott 1986), we believe the three samples were independent. We did not sample two plots in May and eight in July because of time restrictions. There were no observations of female ochre ringlets or wood nymphs.

Statistical analyses

Statistical analyses were conducted using Minitab[®] version 15.1 and SAS[®] version 9.1 software. To achieve normality and homogeneous variances, data were natural-log and arcsine-square root transformed for continuous and percent cover data,

respectively. We used a paired t-test with a before-after control-impact (BACI) design to test for differences between years and herbicide treatments for lupine cover in both small and large plots, and lupine flower density in small plots. We assessed nectar species frequency with discriminant function analysis (DFA), using an analysis similar to that of Alhamad et al. (2008). We performed the DFA using the PROC CANDISC procedure in SAS[®] with a BACI design to test for differences between pre-treatment (2006) and post-treatment (2007) frequency and between control and experimental plots.

We used a paired t-test to test for differences between post-treatment control and experimental plots for the oviposition assessment. We analyzed the number of larval feeding signs for each of four types (chewed leaves, leaflets, and stems, and mined leaves) with MANOVA. The total number of butterflies that entered each plot was compared between treatments using a paired t-test. We analyzed the residence time of adult butterflies using general linear models (GLMs) in Minitab[®]. Because male and female Puget and silvery blues differ in behavior (LaBar, Schultz, personal observations), we first tested for a difference in residence time between male and female blues. We then used a GLM with a two-way design to assess differences between treatment and species for female blues, and a two-way GLM and post-hoc Tukey test to assess differences between treatment and species for males of all four species. Interactions between treatment and species were not significant and were pooled in the final model. All results are presented as means and standard error.

RESULTS

Vegetation assessment

Sethoxydim did not affect lupine cover or flower density. Percent cover of lupine in both small and large plots was similar between control and experimental plots before and after treatment (small plots: $t_{39} = -0.01$, $P = 0.990$; large plots: $t_{19} = -0.73$, $P = 0.483$; Table 3). Percent lupine cover was higher in small plots compared to large plots, as we selected the small plots for lupine. Lupine flower density in small plots was also similar between control and experimental plots before or after treatment ($t_{39} = -0.07$, $P = 0.949$; Table 3).

Discriminant function analysis of the twelve flower species found no effect of sethoxydim on flower frequency (Figure 1). Eighty-seven percent of the variation on the first (x) axis (eigenvalue = 6.92, $F_{36, 75} = 2.41$, $P = 0.0007$) was explained by differences between years (Figure 1). Treatment effects were aligned along the second (y) axis, but these were not significant and explained only 15% of the variation along this axis (eigenvalue = 0.18, $F_{22, 52} = 0.26$, $P = 0.9995$, Figure 1). Control plots generally exhibited higher flower frequencies than experimental plots both before and after treatment, and nearly all control and experimental plots sampled post-treatment (2007) were associated with an increase in species frequency from the year before (Figures 1 and 2, Table 4).

Larval survival

We found only eleven and ten post-diapause larvae in 2007 and 2008, respectively. Assessment of leaf damage, used as an index of larval abundance, found no significant difference between controls and treatments ($F_{4,35} = 0.55$, $P = 0.702$; Table 3). Assuming that feeding damage corresponds with larval numbers, this indicates no effect of sethoxydim on the number of larvae that survived.

Oviposition assessment

Female Puget blue oviposition did not appear to be influenced by the sethoxydim treatment ($t_{39} = -0.27$, $P = 0.787$), as indicated by a similar number of eggs laid in control and sprayed plots (Table 3).

Behavior observations

During the three observation periods, we counted 742 and 709 butterflies entering control and experimental plots, respectively. The numbers of butterflies observed for residence time were 123 Puget blues (106 males and 17 females), 101 silvery blues (69 males and 32 females), 77 wood nymphs (all male), and 30 ochre ringlets (all male). The total number of butterflies that entered each plot during ½ hour observations was similar between control and experimental plots ($t_{49} = 0.49$, $P = 0.631$; Table 3). Female Puget and silvery blues had significantly longer residence time than male blues ($F_{1,222} = 7.99$; $P = 0.005$; Table 3, Figures 3a and 3b). The sethoxydim

treatment significantly influenced the average residence time of males of all four species ($F_{1,277} = 8.71$; $P = 0.003$; Figure 3a), and suggest that females spend greater time in controls than experimental plots ($F_{1,46} = 3.48$; $P = 0.068$; Figure 3b). Adult males and females of all four species spent more time in control plots than in experimental plots (Table 3, Figures 3a and 3b). Differences in residence time between species were significant for males of all species ($F_{3,277} = 4.40$; $P = 0.005$; Table 3) but not for female blues ($F_{1,46} = 2.58$; $P = 0.115$; Table 3). A post-hoc Tukey test found that Puget blue males had higher residence time than male silvery blues ($P = 0.011$), ochre ringlets ($P = 0.051$), and wood nymphs ($P = 0.098$), while the latter three species had similar residence times (pairwise comparisons $P > 0.80$).

DISCUSSION

Invasive grasses, such as tall oatgrass, are a significant problem in prairie systems. It is well known that management of invasive grass species is needed to restore and conserve habitat for prairie-dependant butterflies (Hayes et al. 2000, Schultz et al. 2003, Severns 2008). However, managers should take care in how they control invasive species, because methods such as herbicide use can negatively affect nontarget species (Brown 1987, Jepson 1989, Kutlesa and Caveney 2001, Russell and Schultz 2009). In our study, we investigated potential direct effects of the grass-specific herbicide sethoxydim on four prairie butterflies. Sethoxydim had very little to no impact on nectar species abundance, lupine cover and flower abundance, larval survival, and Puget blue oviposition, while adult Puget and silvery blues, ochre ringlets, and wood nymphs spent less time in sprayed plots than in controls.

Because sethoxydim is grass-specific, we did not expect it to alter the abundance or cover of lupine and nectar species. Lupine cover and flower abundance remained similar between control and experimental plots after treatment with sethoxydim, and most nectar species increased in abundance from 2006 to 2007. Lemerle and Hinkley (1991) also found that sethoxydim did not affect lupine cultivars in Australia, although crop diseases such as brown leaf spot increased under fluazifop-p-butyl and other herbicide treatments. Stanley et al. (2009) evaluated combinations of mowing, burning, and herbicide treatments over three years in prairies throughout the Pacific Northwest. They found that spring application of sethoxydim, followed by fall burning and a post-fire glyphosate treatment, resulted in the best control of invasive grasses and forbs without reducing native species abundance. Our results and these studies indicate no short-term visible effect of sethoxydim on native plants.

We did not find an effect of sethoxydim on Puget blue larval survival, although we did not have a sufficient larval sample size, and were therefore restricted to using leaf damage as an indicator of survival. However, it is well known that herbicides have varying lethal and sublethal effects on Lepidoptera and other invertebrates (Brown 1987, Jepson 1989, Kutlesa and Caveney 2001, Stark and Banks 2003, Russell and Schultz 2009). In a laboratory study, Russell and Schultz (2009) show that sethoxydim and fluazifop-p-butyl both reduce development time of Puget blues from the date of treatment to eclosure, and reduce survival, pupal weight, and wing size of cabbage white butterflies. The lack of field assessments, in addition to our results, and the variability of herbicide effects depending on insect species and herbicide type (e.g.

Agnello et al. 1986a, Kjaer and Heimbach 2001, Stark and Banks 2003), warrant further investigation of herbicide effects on butterfly larvae in prairie habitat.

The scarcity of Puget blue larvae compared to the number of adults observed in our study area at Johnson Prairie raises questions about their natural history. Ant tending of butterfly larvae is common for many species in the family Lycaenidae, and its importance for those species is well-documented (Fiedler and Maschwitz 1988, Pierce et al. 2002). In most of these associations, ants provide protection from parasitoids and predators in exchange for a nectar-like secretion high in nitrogen from the larvae (Pierce 1985, Wagner 1993). Nitrogen levels in host plants of Lycaenidae species may affect the number of tending ants per larva (Billick et al. 2005), as well as survival of ant-tended larvae (Baylis and Pierce 1991). Ants frequently tend both Puget and silvery blue larvae in western Washington prairies. Evaluation of the relationship between Puget blue larvae and tending ants may provide answers to why larvae are difficult to find in the field, and whether herbicides disrupt this relationship.

We observed lower adult butterfly residence times in sprayed large plots compared to controls. This difference in behavior has the potential to influence oviposition by reducing the time females spend in an area. Our assessment of oviposition in small plots found that Puget blues laid nearly the same number of eggs in sprayed plots as they did in controls. This is what we would expect with no visible herbicide effects to their host lupines and nectar plants. However, based on our residence time observations, there may be an effect at broader scales that we did not detect in smaller areas. This lower residence time in sprayed large plots could indicate a response either to host plant volatiles or distribution. During flight, female butterflies

may cue to host plant volatiles (Honda 1995), leaf color (Hirota and Kato 2001), or host plant distribution (Webb and Pullin 2000, Singer and Wee 2005). After landing, certain chemicals in the host plant can stimulate oviposition (Honda 1995, Nakayama et al. 2002, Reudler Talsma et al. 2008). Interference with any of these factors can result in reduced oviposition. For example, an invasive mustard (*Alliaria petiolata* Cavara and Grande) in eastern North America interferes with oviposition of two native butterflies (*Pieris napi oleracea* Harris and *Pieris virginiensis* W. H. Edwards) by producing a chemical cue that signals acceptability to the butterflies for ovipositing (Huang et al. 1995). The eggs hatch, but the larvae suffer high mortality because they are unable to metabolize a defense chemical in the plant (Porter 1994, Haribal and Renwick 1998). As female butterfly selectivity affects survival of their offspring, chemical cues are important to consider when managing habitat for species such as the Puget blue; the potential for herbicides to alter these cues needs further investigation.

Adult butterflies did not avoid sprayed plots in our study, although their residence time was lower in sprayed plots than controls, indicating that sethoxydim alters their behavior. Female butterflies spent 50-70% and males spent 80-95% of the observation period in flight in both sprayed and control plots, suggesting that any difference between sprayed and control plots was detected through sensory input, such as chemical cues. Chemical and visual cues affect oviposition behavior and detection and recognition of nectar flowers (e.g., Honda et al. 1998, Andersson and Dobson 2003). Although visual cues are important in close range flower recognition, it is not known how butterflies respond to these cues at distances greater than a few meters (Omura et al. 1999, Rutowski 2003). The extent to which chemical cues, such as floral scent, affect butterfly

behavior is also relatively unknown (Honda et al. 1998, Andersson et al. 2002, Andersson and Dobson 2003).

If sethoxydim alters or inhibits floral volatiles, it could have an effect on butterflies by creating unattractive nectar resources. However, an extensive search of the literature found no studies of the influence of herbicides on butterfly behavior. Studies that investigate herbicide effects on butterflies largely focus on changes in the butterfly community, such as abundance and diversity (e.g., de Snoo and de Leeuw 1996, Feber et al. 1996, Longley and Sotherton 1997). These changes are primarily attributed to the loss of food plants and changes in the overall vegetation community, and do not reflect direct chemical effects on butterfly species. Because we selected a site with low invasive grass density, we believe the butterflies in our study responded to sethoxydim directly, rather than being influenced by reduction of grass cover. Sethoxydim also had no effect on the frequency of the twelve flower species surveyed. These results accentuate the need for further investigation into how herbicides affect butterfly behavior. Future behavioral assessments, as well as chemical assays of native plants, may reveal why butterflies spend less time in sprayed areas.

Next steps

Invasion of exotic plants is of concern worldwide (Andow et al. 1990, Cronk and Fuller 1995, Randall 1996, Culliney 2005), with invasive grasses being of particular concern in prairies (Sinclair et al. 2006, Severns 2008). To avoid habitat degradation and species decline, prevention and early detection of invasive grasses and other invasive species into natural areas is imperative (Randall 1996, Culliney 2005). Early

detection is also crucial in limiting potential herbicide use that is harmful to native species. Once invasive grasses have spread into natural areas, management practices focus on restoring these habitats. Given the limited knowledge of herbicide effects on at-risk butterflies, and the necessity of reducing invasive grasses, we recommend several strategies to minimize any potential negative impacts on butterflies.

First, spot spraying or hand pulling in small areas containing few invasive grasses will minimize impacts on butterflies and other native plants and animals (Daehler and Goergen 2005, Evans 2008). A second strategy is rotating sprayed areas at a scale that allows adult butterflies the option of moving between treated and untreated habitat patches. If the distance between patches is not too great, butterflies are capable of moving to or between other patches when their habitat becomes unsuitable or lacks an essential resource (Quin et al. 2004, Schultz and Crone 2005, Schtickzelle et al. 2007). This is important if herbicides make habitat unattractive, resulting in behavioral changes of butterflies, as seen in our study. Although rotating sprayed areas is appropriate only at sites where density of invasive grasses and the probability of invasive grass recolonization of treated areas are low, it may reduce herbicide impacts on butterfly populations if behaviors such as residence time and oviposition are affected.

When intensive management of sites with a high density of invasive plants is necessary, a combination of chemical and mechanical control is often required, such as the combined use of herbicides and fire to control invasive grasses (Fitzpatrick 2001, Barnes 2004, Evans 2008, Stanley et al. 2009). However, this combination multiplies harmful effects on nontarget butterflies. In the Pacific Northwest, herbicides are often

applied in the spring when many sensitive butterfly species are in early or mid instar larval growth stages. Burns are primarily conducted in the fall, at a time when these species are in diapause as eggs and early instar larvae on plants or in duff, and are susceptible to fire. Similarly, the standard recommendation for invasive tall fescue (*Festuca arundinacea* Schreb.) control in tall grass prairies of the eastern United States is to burn the fescue in late winter, followed one month later by an application of imazapic, a grass and broadleaf herbicide (Barnes 2004). This too is potentially harmful to butterflies, both from reduced food plants and direct mortality.

Even when herbicides are applied during egg or pupal stages, when butterflies are not feeding, chemicals can persist in the system, potentially causing problems once larvae or adults have emerged. Sethoxydim and fluazifop-p-butyl have half-lives in soil of five and fifteen days, respectively, and are readily absorbed from the soil by plants, with sethoxydim fully metabolized in plants within two days and fluazifop-p-butyl persisting in an acidic form up to 45 days (Tu et al. 2001). Glyphosate, another commonly used herbicide, is absorbed only through foliage, but can persist in soil from three to 249 days and is metabolized by only a few plant species (USFS 1997). Though these herbicides may only persist for a week or two, they can significantly affect sensitive species during that time. The flight period of many butterfly species is only three to six weeks. If herbicides persist throughout this period, causing butterflies to spend less time in parts of their habitat, as seen in our study, it may lead to long-term negative effects on the population, especially if herbicides are applied over multiple years. We therefore recommend monitoring of host plants and nectar species for multiple years to assess long-term herbicide effects on butterfly food sources, as well as

performing chemical assays of these plants to detect persisting effects. Additional assessments of butterfly residence time are recommended to separate direct chemical effects from effects caused by changes in vegetation structure. We also recommend larger-scale oviposition assessments, such as in 20 x 20 m plots, to identify if female butterflies select for or against host plants sprayed with herbicide.

Balancing the benefits of invasive plant management with negative impacts on nontarget plants and animals is challenging, especially when the effects of herbicides and other management practices on nontarget species are not well known. Because controlling invasive plants in or near sites with at-risk butterfly populations often involves herbicide use (Table 1), assessments of herbicide effects on these butterflies are necessary to avoid further population declines. Continued evaluations of herbicide effects on at-risk butterflies will aid managers in developing habitat restoration plans and herbicide use guidelines.

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Table 1. Herbicides used in or near the habitat of sixteen threatened (T), endangered (E), and candidate (C) butterfly species in the United States.

Data based on personal communications with natural area managers and agency personnel.

Butterfly species	Status	Habitat	Herbicide target(s)	Herbicides used
Lange's metalmark (<i>Apodemia mormo langei</i> Comstock) ^{a, b}	E	Coastal dunes	Primarily gorse (<i>Ulex europaeus</i> L.), scotch broom, and <i>Eucalyptus</i> sp. Also fennel (<i>Foeniculum vulgare</i> Mill.), Himalayan blackberry (<i>Rubus armeniacus</i> Focke), pampas grass (<i>Cortaderia selloana</i> ([Schult. & Schult. f.] Asch. & Graebn.), and <i>Oxalis</i> L. species	Triclopyr (Garlon 4), glyphosate (Roundup)
Pawnee montane skipper (<i>Hesperia leonardus montana</i> Skinner) ^c	T	Pine woodlands with blue grama grass (<i>Bouteloua gracilis</i>)	Leafy spurge (<i>Euphorbia esula</i> L.), diffuse and spotted knapweed (<i>Centaurea diffusa</i> Lam., <i>C. maculosa</i> Lam.), Canada thistle (<i>Cirsium arvense</i> L.), musk and plumeless thistle (<i>Carduus</i> sp. L.), and orange hawkweed (<i>Pilosella aurantiaca</i> L.)	Imazapic (Plateau), aminopyralid (Milestone), chlorsulfuron (Telar), 2,4D
Mardon skipper (<i>Polites mardon</i> Edwards) ^d	C	Prairies, mountain meadows	Tall oatgrass, bentgrass (<i>Agrostis</i> sp.), cat's ear dandelion, scotch broom, others	Fluazifop-p-butyl (Fusilade), sethoxydim, glyphosate, triclopyr (Garlon 4)
Carson wandering skipper (<i>Pseudocopaeodes eunus obscurus</i> Austin and J. Emmel) ^e	E	Saltgrass (<i>Distichlis spicata</i>)	Whitetop (<i>Lepidium latifolium</i> L.)	2,4D and chlorsulfuron (Telar)
San Bruno elfin (<i>Callophrys mossii bayensis</i> Brown) ^a	E	Coastal scrub	Primarily gorse, broom, and eucalyptus. Also fennel, Himalayan blackberry, pampas grass, and oxalis	Triclopyr (Garlon 4), glyphosate
Mission blue (<i>Icaricia icarioides missionensis</i> Hovanitz) ^a	E	Coastal chaparral and grasslands	Primarily gorse, broom, and eucalyptus. Also fennel, Himalayan blackberry, pampas grass, and oxalis	Triclopyr (Garlon 4), glyphosate
Fender's blue (<i>I. icarioides fenderi</i> Macy) ^f	E	Grasslands, lupine habitat	Tall oatgrass, false brome (<i>Brachypodium sylvaticum</i> [Huds.] Beauv.), tall fescue, poison oak (<i>Toxicodendron diversilobum</i> [Torr. & A. Gray] Greene), sprouting oak tree stumps (<i>Quercus</i> sp.), some knapweed (<i>Centaurea</i> sp.)	Glyphosate, sethoxydim, triclopyr (Garlon 3A), potentially aminopyralid (Milestone) on knapweed

Smith's blue (<i>Euphilotes enoptes smithi</i> Mattoni) ^g	E	Coastal dunes	Iceplant (<i>Carpobrotus</i> sp.), kikuyu grass (<i>Pennisetum</i> sp.) and beach grass (<i>Ammophila</i> sp.)	Glyphosate, possibly others
Karner blue (<i>Lycaeides melissa samuelis</i> Nabokov) ^h	E	Lupine habitat	Various herbaceous forbs, mostly in red and jack pine forests	Mostly triclopyr (Garlon) and sulfometuron methyl (Oust)
Bay checkerspot (<i>Euphydryas editha bayensis</i> Sternitzky) ^a	T	Serpentine	Primarily gorse, broom, and eucalyptus. Also fennel, Himalayan blackberry, pampas grass, and oxalis	Triclopyr (Garlon 4), glyphosate
Quino checkerspot (<i>E. editha quino</i> Behr) ⁱ	E	Serpentine	Near habitat: agricultural weeds In habitat: invasive annual grasses and red-stem stork's bill (<i>Erodium cicutarium</i> L.)	Near habitat: agricultural herbicides In habitat: fluazifop-p-butyl, glyphosate, possibly others
Taylor's checkerspot (<i>E. editha taylori</i> Edwards) ^{d,i}	C	Grasslands	Tall oatgrass, bentgrass, cat's ear dandelion, scotch broom, others	Fluazifop-p-butyl, sethoxydim, triclopyr (Garlon 3A and 4)
Callippe fritillary (<i>Speyeria callippe callippe</i> Boisduval) ^a	E	Grasslands	Primarily gorse, broom, and eucalyptus. Also fennel, Himalayan blackberry, pampas grass, and oxalis	Triclopyr (Garlon 4), glyphosate
Myrtle's silverspot (<i>S. zerene myrtilleae</i> dos Passos and Grey) ^a	E	Coastal grasslands	Primarily gorse, broom, and eucalyptus. Also fennel, Himalayan blackberry, pampas grass, and oxalis	Triclopyr (Garlon 4), glyphosate
Oregon silverspot (<i>S. zerene hippolyta</i> Edwards) ^k	T	Coastal grasslands	European grasses (primarily velvet grass) and cat's ear dandelion	Sethoxydim and glyphosate (only in test plots outside of habitat)
Mitchell's satyr (<i>Neonympha mitchelli mitchelli</i> French) ^l	E	Fens	Reed (<i>Phragmites australis</i> [Cav.] Trin. ex Steud.), reed canarygrass (<i>Phalaris arundinacea</i> L.)	Various formulations of glyphosate

Sources: ^a San Bruno Mountain HCP, ^b David Kelly (USFWS), ^c Denny Bohon (USFS), ^d Roderick Gilbert (US Army) and Mary Linders (USFWS), ^e Marcy Haworth (USFWS), ^f Jock Beall (USFWS), ^g Jacob Martin (USFWS), ^h Karner blue HCP and Cathy Carnes (USFWS), ⁱ Alison Anderson (USFWS), ^j Al Kitzman (Benton Co. Natural Areas and Parks, Oregon) and David Wilderman (DNR), ^k William Vagt (US Army), ^l John Shuey (TNC).

Table 2. List of known and potential butterfly nectar species (Schultz and LaBar, personal observations) surveyed at Johnson Prairie on Fort Lewis, Washington in 2006 and 2007.

Latin Name	Common Name	Butterfly Nectar Source		Native/ Exotic
		Puget blue	Other species	
<i>Achillea millefolium</i> L.	Yarrow	Possible	Yes	Exotic
<i>Apocynum androsaemifolium</i> L.	Spreading dogbane	Yes	Yes	Native
<i>Balsamorhiza deltoidea</i> Nuttall	Deltoid balsamroot	Yes	Yes	Native
<i>Camassia quamash</i> Pursh	Common camas	Yes	Yes	Native
<i>Crepis capillaris</i> L.	Smooth hawksbeard	No	Possible	Exotic
<i>Eriophyllum lanatum</i> Pursh	Oregon sunshine	Yes	Yes	Native
<i>Hypochaeris radicata</i> L.	Cat's ear dandelion	No	Yes	Exotic
<i>Leucanthemum vulgare</i> Lamarck	Oxeye daisy	Yes	Yes	Exotic
<i>Lomatium triternatum</i> Pursh	Nine-leaved parsley	Yes	Yes	Native
<i>Lupinus albicaulis</i> Douglas	Sickle-keeled lupine	Yes	Yes	Native
<i>Potentilla gracilis</i> Douglas	Slender cinquefoil	Yes	Yes	Native
<i>Vicia sativa</i> L.	Common vetch	Yes	Yes	Exotic

Table 3. Vegetation, larval survival, oviposition, and adult butterfly behavior assessment results (M, males; F, females). Numbers are means \pm standard errors.

Assessment	Plot type	Before (2006)		After (2007)	
		Control	Experiment	Control	Experiment
Lupine cover (%)	Small	33.7 \pm 4.2	36.6 \pm 2.5	39.8 \pm 3.8	45.2 \pm 5.1
Lupine cover (%)	Large	5.8 \pm 1.6	5.2 \pm 0.8	5.6 \pm 1.2	5.1 \pm 1.4
Lupine flowers (stems/m ²)	Small	99.9 \pm 12.7	106.8 \pm 12.2	72.2 \pm 12.3	75.7 \pm 13.4
Larvae – chewed leaves (per plot)	Small	–	–	15.8 \pm 2.3	15.1 \pm 2.4
Larvae – chewed stems (per plot)	Small	–	–	2.1 \pm 0.4	1.4 \pm 0.3
Larvae – mined leaves (per plot)	Small	–	–	5.8 \pm 2.0	2.4 \pm 0.8
Puget blue eggs (per plot)	Small	–	–	18.0 \pm 1.8	20.3 \pm 2.4
Butterflies observed (#/plot/30min)	Large	–	–	29.7 \pm 3.3	38.4 \pm 3.1
Puget blue residence time, F (sec)	Large	–	–	156.9 \pm 106.3	34.1 \pm 8.4
Puget blue residence time, M (sec)	Large	–	–	58.1 \pm 12.4	40.1 \pm 6.9
Silvery blue residence time, F (sec)	Large	–	–	103.4 \pm 19.0	39.3 \pm 7.4
Silvery blue residence time, M (sec)	Large	–	–	32.1 \pm 5.4	17.4 \pm 2.2
Ochre ringlet residence time, M (sec)	Large	–	–	28.3 \pm 4.3	21.8 \pm 4.6
Wood nymph residence time, M (sec)	Large	–	–	33.3 \pm 11.9	24.6 \pm 5.4

Table 4. Plant species frequency loadings for the first two discriminant function axes. Species with a positive loading on the first (x) axis increased in frequency from 2006 to 2007. Species with a positive loading on the second (y) axis had higher frequencies in control plots than experimental plots. Species with a positive loading on the first axis and negative loading on the second indicate a neutral or favorable response to sethoxydim treatment at Johnson Prairie on Fort Lewis, Washington.

Species	First axis	Second axis
Common camas	0.926	-0.089
Smooth hawkbeard	-0.387	0.016
Oregon sunshine	0.244	-0.178
Cat's ear dandelion	0.242	-0.250
Spreading dogbane	0.198	0.184
Slender cinquefoil	0.140	0.019
Deltoid balsamroot	0.116	-0.098
Yarrow	0.111	0.278
Common vetch	-0.089	0.034
Nine-leaved parsley	-0.069	-0.276
Oxeye daisy	-0.049	0.495
Sickle-keeled lupine	0.009	0.309

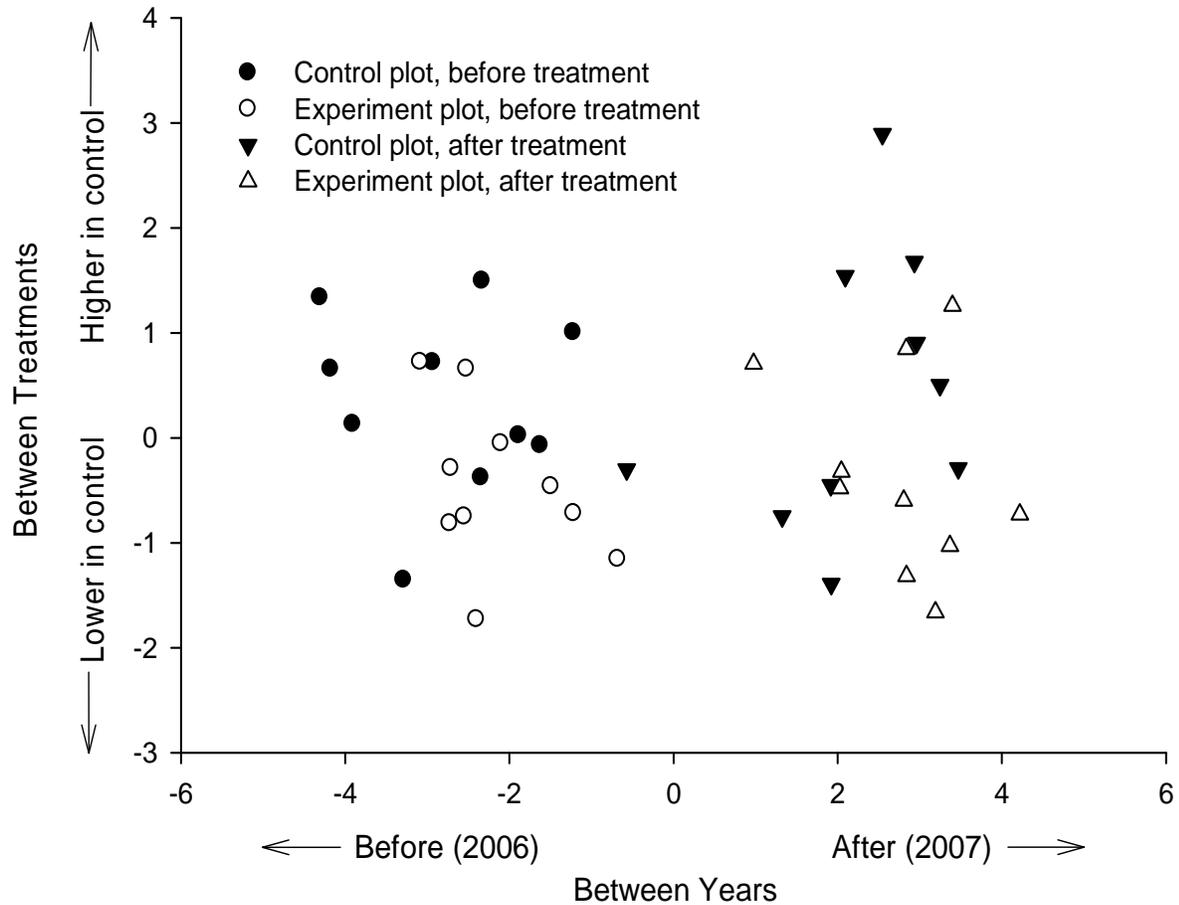


Figure 1. Discriminant function analysis of flower species frequency between control and experimental plots at Johnson Prairie on Fort Lewis, Washington, before (2006) and after (2007) herbicide treatment. Species that increased or decreased in frequency from 2006 to 2007 contribute strongly to the first canonical (x) axis. Species that had higher or lower frequencies in controls compared to experimental plots contribute to the second canonical (y) axis. There is no significant treatment effect.

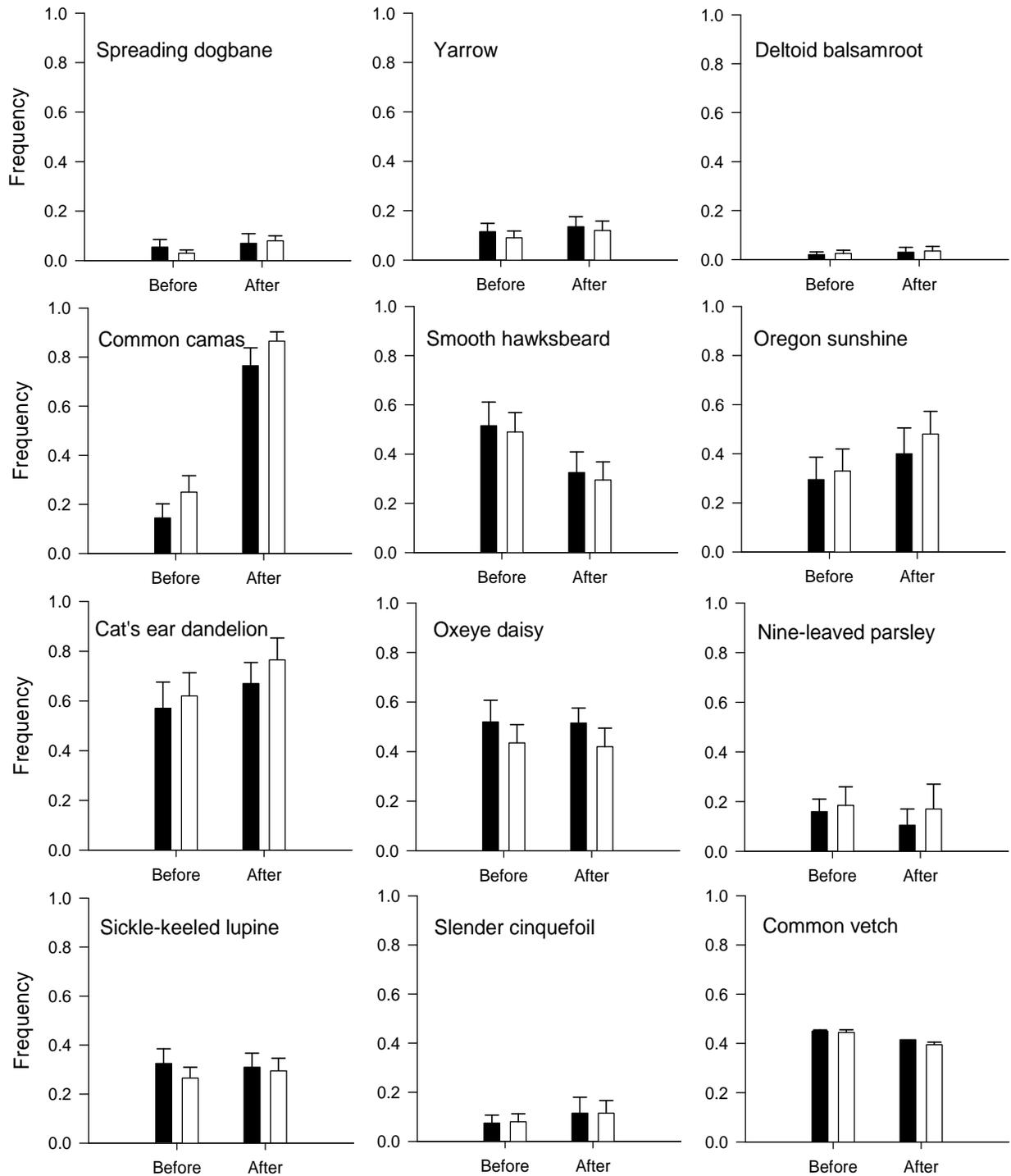


Figure 2. Mean frequency (quadrats per plot) of flower species in control (black) and experimental (white) plots at Johnson Prairie on Fort Lewis, Washington before (2006) and after (2007) treatment. Error bars show standard error.

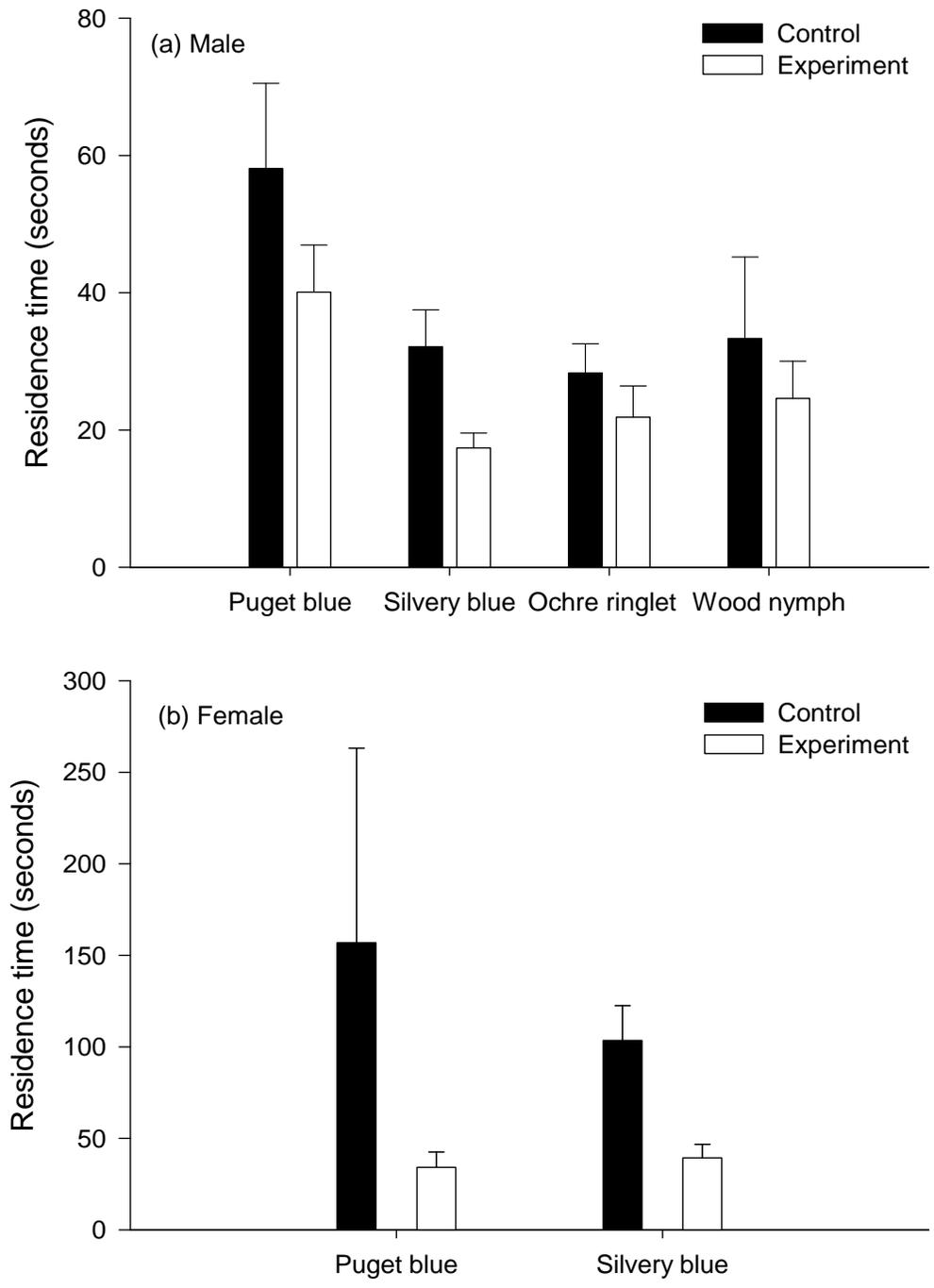


Figure 3. Average residence time of adult Puget and silvery blue, ochre ringlet, and wood nymph butterflies in control and experimental plots during one-half hour observation periods, (a) males, (b) females. Error bars show standard error.