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ORIGINAL PAPER

Characterizing a contentious management tool: the effects of a grass-specific herbicide on the silvery blue butterfly

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Abstract Selective herbicides are a conservation tool employed to reduce invasive vegetation and improve habitat for native plants and animals. However, herbicides may negatively affect non-target organisms such as butterflies through direct chemical exposure or by altering plant community composition and structure. We evaluate the effect of the grass-specific herbicide fluazifop-*p*-butyl on behavior and demographic responses of the silvery blue butterfly (*Glaucopsyche lygdamus*) in the field and also quantify effects on reproductive behavior in the greenhouse. We find that in the first few months after an early spring application, herbicide decreases vertical grass structure but does not have a positive or negative net effect on adult behavior, egg deposition, larval density, pupal weight, or ant-tending association for the silvery blue. Our greenhouse oviposition choice trials corroborate field findings and indicate that females do not show preference for unsprayed host plants. Selective herbicides create a vegetative structure preferred by butterflies and do not negatively affect the silvery blue when applied in the early spring. Appropriate timing of herbicide application is likely the key to avoiding adverse effects on vulnerable butterfly life stages. Depending on the longevity of the vegetative reduction, strategic herbicide application may be useful for restoring prairie communities in concert with other restoration tools; however, further testing on additional butterfly species is an imperative precursor to large-scale spraying.

Keywords Butterfly · Lycaenidae · Grass-specific herbicide · Fluazifop-*p*-butyl · Prairie management · Oviposition choice trial

Introduction

Rachel Carson's 1962 book *Silent Spring* first brought the environmental hazards of pesticides under the purview of a wide audience. Although numerous pesticides from that era have been banned, new formulations are broadly used and effects on non-target species are still poorly characterized. Despite the potential for unwanted effects, selective herbicides are an increasingly appealing tool for eradicating pernicious, invasive plants. Invasive vegetation alters communities and is implicated in species extirpation, e.g. butterfly extirpation (Weiss 1999). While herbicides may curb problematic vegetation and improve habitat quality, they may also have unintended consequences for non-target butterflies (Agnello et al. 1986b; Stark et al. 2012). Herbicides are suspected to affect behavior and demography through direct chemically-mediated effects as well as through the indirect effect of altered vegetation structure and composition (Taylor et al. 2006). Butterfly conservation and recovery efforts in the United States commonly recommend action to control invasive plants; thus, characterizing the net effects of herbicides on butterflies is a pressing imperative (LaBar and Schultz 2012).

Studies assessing the non-target effects of herbicide usually occur in agricultural systems and focus on broad-spectrum herbicides and arthropods of economic

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importance (Agnello et al. 1986b; Chiverton and Sotherton 1991; Freemark and Boutin 1995; Haughton et al. 2001b; Hawes et al. 2003; Taylor et al. 2006). Work in wildlands is limited and studies predominantly evaluate broad-spectrum and broad-leaf herbicides, chemicals that target a wide variety of plants (Fuhlendorf et al. 2002; Crone et al. 2009; Bramble et al. 1999). For butterflies, one concern is that using broad-spectrum or broad-leaf chemicals to control invasive plants reduces adult nectar and larval host plant abundance (Smallidge and Donald 1997; Longley and Sotherton 1997; Feber et al. 1996; Pleasants and Oberhauser 2013). However, grass-specific herbicides may promote forb resources and create more favorable vegetation structure for certain butterfly species (Blake et al. 2011).

Vegetation structure is an important component of butterfly habitat and can influence resource use and reproductive behavior (Wiklund 1984). For butterflies residing in relatively open habitats with short vegetation, a shift to taller vegetation decreases host-plant visitation by reducing host plant apparency (Severns 2011). Taller vegetation also alters thermal microclimate, which impacts adult reproductive behavior and ant-tending mutualists (Eichel and Fartmann 2008; Thomas et al. 2009). In Europe, the mire-associated Common Ringlet butterfly (*Coenonympha tullia*) selects oviposition sites with low cover of herbaceous material (Weking et al. 2013). The endangered large blue (*Phengaris arion*) of Great Britain suffered a massive population decline when increased grass structure created an unacceptable thermal environment for its obligate ant mutualist (Thomas et al. 2009). Similarly, North American prairie-dependent butterflies respond to changes in grass structure. For example, the endangered Fender's blue (*Plebejus = Icaricia icarioides fenderi*), Taylor's checkerspot (*Euphydryas editha taylori*), and great copper (*Lycaena xanthoides*) butterflies lay more eggs on host plants in areas where tall grasses are removed or absent (Severns 2008; Severns and Warren 2008; Severns 2011). Likewise, Mardon skipper (*Polites mardon*) selects oviposition sites in open areas with low density of vertical vegetation (Henry and Schultz 2012).

Using herbicides to reduce vegetation structure can have unintended negative consequences for butterflies. In laboratory studies, directly sprayed cabbage white (*Pieris rapae*) and Behr's metalmark (*Apodemia virgulti*) larvae experience up to 30 % lower survivorship (Russell and Schultz 2010; Stark et al. 2012). In addition to measuring lethality, several studies demonstrate sub-lethal effects such as increased development time, reduced pupal weight, and altered pheromone production for sprayed larvae (Russell and Schultz 2010; Eliyahu et al. 2003; Hicks unpublished data). However, no laboratory studies address the possible sub-lethal, direct chemical effects of herbicide on butterfly oviposition preference. Field trials suggest a chemically-mediated effect on butterfly behavior; males of four butterfly species spend less time in plots sprayed with herbicide than in control plots when there is no observable difference in vegetation structure (LaBar and Schultz 2012). In combination, field and laboratory work can elucidate both the toxicity of a chemical and the likelihood of exposure for an organism. Only a few studies investigate the non-target effects of herbicides in both the field and laboratory setting (Chakravarty and Chatarpaul 1990; Haughton et al. 2001a, b) and there are no such studies for the effects of selective herbicides on butterflies.

In western Oregon and Washington, land managers would like to use herbicides to control tall invasive perennial grasses (Dunwiddie and Delvin 2006; Schultz et al. 2011; Stanley et al. 2011). One regime currently in practice is to apply post-emergent, grass-specific herbicides such as fluazifop-*p*-butyl and sethoxydim (Fusilade® and Poast®, respectively) in the early spring (Dennehy et al. 2011). These herbicides delay the bolt of target grasses, providing a short-term reprieve from grass dominance (Blakeley-Smith 2006; Dunwiddie and Delvin 2006). This decrease in vegetative structure could benefit several Northwest prairie-dependent butterfly species such as the endangered Fender's blue and the co-occurring silvery blue (*Glaucopsyche lygdamus columbia*). However, previous research has not characterized both the chemically-mediated and altered-vegetation effects of selective herbicides on these butterflies. To the learn about effects of fluazifop-*p*-butyl on behavior and demographic indicators of the silvery blue butterfly, we sprayed plots in an Oregon prairie during the early spring and also conducted a complementary controlled laboratory experiment to test for oviposition preference.

Methods

Study species, field site set up, and herbicide application

The Columbia silvery blue (*Glaucopsyche lygdamus columbia*) is the Oregon and Washington subspecies of *Glaucopsyche lygdamus*, a Lycaenid that ranges across North America (Langston 1969; Pelham 2008). In Oregon, univoltine adults emerge in early spring to summer, fly for several weeks and lay eggs singly on multiple species in the pea family (Fabaceae). Larvae hatch and feed during the late spring and summer and then diapause during the winter as pupae. Larvae primarily feed on flowers, fruits and seeds and they secrete a carbohydrate-rich substance, which is collected by multiple species of tending ants (Nunnallee and James 2011).

Baskett Slough National Wildlife Refuge (BSNWR) is located in the Willamette Valley, 16 km west of Salem, Oregon (44°58′3″N, 123°15′24″W) and managed by the U.S. Fish and Wildlife Service. The refuge encompasses 1,000 ha of cropland, wetland, and oak-prairie woodland. Our survey area contained approximately 14 ha of silvery blue habitat: open upland prairie comprised of native and exotic grasses and forbs.

We established 32 plots (20 m × 20 m) at BSNWR in March 2013. We selected plots that contained at least 15 m^2 of the host plant Kincaid's lupine (*Lupinus oreganus* = *L. sulphureus kincaidii*) and paired plots based on equal lupine cover and spatial proximity. We then randomly assigned one plot in each pair to either the herbicide or control group. For egg and larva surveys, we generated three random points within each plot, found the nearest lupine with at least one developing flower raceme and set up a quadrat (60 cm × 60 cm) centered on that lupine. On the morning of March 25, 2013, U.S. Fish and Wildlife Service employees applied a mixture of Fusilade DX® (24.5 % fluazifop-*p*-butyl, Syngenta Crop Protection, LLC), a grass-specific herbicide, and Nufilm® (96 % Poly-1-*p*-Menthene, Miller Chemical and Fertilizer Corporation), a non-ionic surfactant, to the treatment plots using a 2-m wide boom sprayer hitched to an ATV. Herbicide was applied at 326 g active ingredient per hectare (concentration: 5.86 mL/L) and the surfactant was applied at 425 g active ingredient per hectare (concentration: 1.95 mL/L).

Lupine and vegetation assessment

We quantified vegetation structure and nectar abundance in four weekly surveys starting in early May. In each plot, we measured vegetation height and density using the Robel pole visual obstruction method (Robel et al. 1970). Robel cover is a measure of vegetation height and density. We established eight evenly spaced permanent points within each plot and randomly selected a plot corner for sighting the Robel pole. At these eight points we also measured and identified the tallest grass within a 15 cm radius. We then calculated the mean Robel cover and mean tallest grass height for each plot. To quantify nectar availability in each plot we counted open flowering units of several important nectar species: *Calochortus tolmiei*, *Eriophyllum lanatum*, *Linum bienne*, *Lupinus oreganus*, *Vicia americana*, *Vicia cracca,* and *Vicia sativa*. We calculated number of flowers for each species using flowers/unit estimates from Schultz and Dlugosch (1999). To assess lupine reproductive phenology, we classified each raceme in a quadrat as predominantly in bud, flower, or fruit in mid May. We recorded lupine isolation by measuring the distance between the center of a quadrat and its three closest lupine ramets (each with a minimum of 5 leaves).

Adult behavior assessment

To ensure we were conducting observations on a sufficiently large population, we estimated a minimum silvery blue population size for 2013 at our site. Four times during the survey season, we counted male silvery blues in our study area using the distance sampling method and used those counts to estimate a minimum silvery blue population size (Isaac et al. 2011; Schultz and Dlugosch 1999). Distance sampling involves counting butterflies encountered along transects in a study area. We calculated butterflies (assuming a 1:1 male–female ratio) per meter squared based on the total area of the transects and then estimated a density for our 14 ha site. In early May 2013, we observed adult silvery blue butterflies in 30 plots. We conducted 15-min observations for each plot during periods of low cloud cover, low wind (Beaufort of 2 or less) and warm temperature (>16, <27 °C, LaBar and Schultz 2012). We determined sex and distinguished silvery blue from Fender's blue using binoculars or by netting exiting individuals. We identified the sex and species of each butterfly as well as manually recorded entrance and exit times and whether individuals landed within the plot.

Egg deposition and larva assessment

To assess silvery blue oviposition, we conducted five surveys of hatched and unhatched eggs within the quadrats at 4–5 day intervals starting at the end of April. During each sampling occasion, we also counted the number of racemes in each quadrat and measured the height (at least 1 cm high) of up to five racemes selected haphazardly. In the quadrats, we collected larval data on the same dates as egg data with three additional assessments at the end of May and beginning of June. We recorded the number and size of larvae, as well as the presence of ants and number of ants tending the larvae. Larval length was used to determine instar stage according to Nunnallee and James (2011). We collected 46 (23 per treatment), late instar larvae (>8 mm) from within 23 plots in late May and early June. We also recorded the number of ants tending and collected one ant per larva for identification. We placed each larva in a glass pint jar with a mesh top and fed them ornamental *Lupinus polyphyllus* and *Vicia sativa* flowers until they either reached pupation or died. We collected any parasitoids that emerged from the larvae and recorded pupal mass.

Oviposition choice trials

To test for an effect of herbicide on oviposition preference, we collected 48 mated silvery blue females from a single population located at Cook Meadow east of Mt. Hood, Oregon (45°24′15″N, 121°32′33″W, elevation: 1,300 m).

Collection took place during three trips from mid June to early July. The host plant for this population was most likely *Lupinus latifolius*. To account for differences in oviposition effort as butterflies age, we classified butterflies into three age groups using wing wear and damage as an age proxy (Karlsson 1994). We housed females in 60 × 60 × 60 cm insect rearing tents (BugDorm, Taichung, Taiwan), fed them Gatorade "Fierce Melon"® from soaked sponges, and misted them several times per day with water.

We prepared and treated 38 *Lupinus micranthus* = *polycarpus* plants grown from seed (Heritage Seedlings; Salem, Oregon) in a single bay in the greenhouse at WSU Vancouver. Plants were started in March 2013, and butterfly testing commenced in late June. We paired plants by similar size and structure and paired racemes within each plant pair by phenology. We randomly selected one plant in each pair to be sprayed with Fusilade DX® and Nufilm®; the other plant was sprayed with water as a procedural control. We applied treatments using an R&D Precision CO_2 powered backpack sprayer (R&D Sprayers, Opelousas, Louisiana) with a hand held wand and flat fan 8002VS nozzle set at a spray pressure of 276 kPa. We used the maximum labeled spot spray concentration for herbicide (5.86 mL/L fluazifop-*p*-butyl) and surfactant (1.95 mL/L Nufilm®). The rate of application was similar to the field application.

We followed a protocol that measures post-alighting oviposition preference in staged encounters where a female "accepts" a plant by pressing her extruded ovipositor against a plant but she is removed before laying an egg (Singer 1982; Singer et al. 1992b). Silvery blue females were tested 3–5 days after spray. For the tests, we used 30 raceme pairs from 12 plant pairs. Some females were tested on multiple raceme pairs and plant pairs because raceme phenology limited the number of available racemes. To test one female, we placed her on a raceme on the first plant in a pair for 5 min or until she displayed oviposition behavior. If she tried to oviposit, we contained her in a plastic vial for 3 min before placing her on the paired raceme from the other plant. We presented each of the 48 females with a pair of racemes so that each female was exposed five times to each raceme in the pair. Testing occurred between the hours of 9:00 and 18:00.

Statistical analysis

All analyses were conducted in R 3.0.2 (R Core Team 2013). We first created model sets for the following response variables: residence time, landing rate, egg and larval density. Candidate models consisted of combinations of explanatory variables representing specific *a priori* hypotheses (Electronic Supplementary Material 1). For sets of generalized linear mixed models (GLMMs), we used Gauss-Hermite quadrature to fit models by maximum likelihood, and AIC for model selection (packages lme4, Bates 2013; MuMIn, Barton 2013; Burnham and Anderson 2002; Bolker et al. 2009). Covariates were standardized (divided by standard deviation and centered) prior to analysis. To reduce the influence of extreme values, the variable *third closest individual*, a measure of lupine isolation, was log transformed and *total ants tending* was square root transformed prior to model selection. All GLMMs for field data included plot as a random effect. We used the best model (according to AIC) for subsequent hypothesis testing. We decided not to include model averaging of the top models (ΔAIC <2) in our results because of the difficulties in estimating AIC values when including random effects (Grueber et al. 2011; Bolker et al. 2009) as well as the problem of combining models with the correlated variables of "Robel pole cover" and "tallest grass height". Despite these concerns, when we did average the top models, we reached the same qualitative conclusions about the effect of herbicide and the other covariates (unpublished analyses).

We modeled differences in vegetation structure (measured by Robel pole cover and tallest grass) between control and treatment plots using a repeated measures, linear mixed effects model with normal errors that included treatment as a fixed effect and plot as a random effect. Because data across sampling occasions was heteroskedastic, we estimated separate variances for each sampling occasion. Butterfly response variables measured as counts were modeled as either Poisson (not overdispersed), Poisson-lognormal (overdispersed), or negative binomial (overdispersed) random variables (Gelman et al. 2004). Butterfly entrances into the plots were modeled as a negative binomial random variable using a generalized linear model (GLM) with a log link (MASS, Venables and Ripley 2002). We modeled cumulative egg and larva counts with a Poisson-lognormal distribution, which is compatible with the glmer command for GLMMs in the R package lme4 and similar to the negative binomial distribution. We used a GLMM with a log link for eggs and a GLMM with a square root link for larva. We modeled the infrequently-occurring late instar larvae using a zero-inflated Poisson GLMM (glmmADMB, Skaug et al. 2013) and treatment as the only fixed effect. Ant tending counts between control and herbicide plots were modeled with a Poisson distribution using a GLMM with log link. The continuous variable residence time was modeled as a Gamma-distributed random variable using a GLMM with a log link. We modeled the proportion of butterflies landing in plots as a binomial random variable using a GLMM with a logit link. We calculated larval survivorship by dividing the number of late instar larvae (>8 mm) per quadrat by cumulative eggs per quadrat. We compared pupal weights between control and herbicide plots with a two-tailed Student's *t* test.

For butterfly response variables that had a statistically significant association with vegetation structure, we used a mediation model in which the effect of herbicide on the butterfly response is mediated by vegetation structure (Preacher and Hayes 2004; Shipley 2009). For these models, we defined the direct effect of herbicide on vegetation structure as a linear relationship between herbicide and either Robel cover or tallest grass. We defined the direct effects of herbicide and vegetation on the butterfly response as a change in the response after accounting for all other covariates in our GLMMs. We estimated herbicide's indirect effect by first simulating values of herbicide's direct effect on vegetation and vegetation's direct effect on the response. We simulated these values from a multivariate normal distribution with the maximum likelihood estimates for the regression coefficients as the mean and standard errors used to calculate variance. We used the product of these samples to estimate the distribution of the indirect effect (Preacher and Selig 2012). We estimated the overall effect of herbicide on the response variables by summing its direct and indirect effects.

For the oviposition choice trials, we modeled rates of acceptance and rejection as a binomial random variable using a GLMM with logit link. Models included random effects of plant pair, individual plant, raceme pair and female. We used AIC model selection to select the best random effects structure and then used likelihood ratio tests ($\alpha = 0.05$) to compare candidate models with different fixed effects. For hypothesis testing, we used the Wald test to evaluate fixed effect significance and Tukey post hoc tests to compare categorical fixed effects.

Results

Vegetation response

We found that herbicide decreased grass height and density compared to control plots and that the difference increased over time (Fig. 1). Robel cover and tallest grass height were correlated in each sampling occasion (0.733 in the first sampling occasion and over 0.90 in the following sampling occasions). Tall oat grass (*Arrhenatherum elatius*) was the most frequently occurring tall grass (40 % of observations), followed by tall fescue (*Festuca arundinacea*, 11 % of observations).

Adult behavior

Within our 14 ha study area, we estimated a minimum silvery blue population of 2,000 butterflies for the 2013 flight season. The male flight period started around April 17 with females beginning a week later. The flight period lasted to the end of May. We observed 310 silvery blue butterflies (47 female and 263 male) in 30 plots over the sample period. There were 11.7 (SD = 7.6) butterflies entering the control plots and 10.3 (SD = 7.4) entering the herbicide plots. Entrance rates were similar between plot types (z = −0.466; $P = 0.641$). Butterflies spent between 1 s and 14 min in plots with females spending 98 s (SD = 120) in control plots and 154 s (SD = 212) in herbicide plots. Males spent 40 s (SD = 67) in control plots and 34 s (SD = 48) in herbicide plots (Fig. 2a). Out of 15 considered models, the best model contained the treatment effect as well as three other covariates (Table 1). Closely ranked models had similar covariate coefficient estimates and the herbicide treatment effect was not significant in any closely ranked models (Electronic Supplementary Material 1). Fluazifop-*p*-butyl did not directly affect the residence time of butterflies in treatment plots (estimated treatment effect and bootstrapped 95 % CI = −0.5840 <0.4305 <0.8539 on the log scale, $P = 0.7364$). On the scale of the response, the herbicide treatment did not substantially increase residence time (an average increase of 44.7 s, bootstrapped 95 % CI −61.9 to 88.2). Females on average spent more time in both plot types than

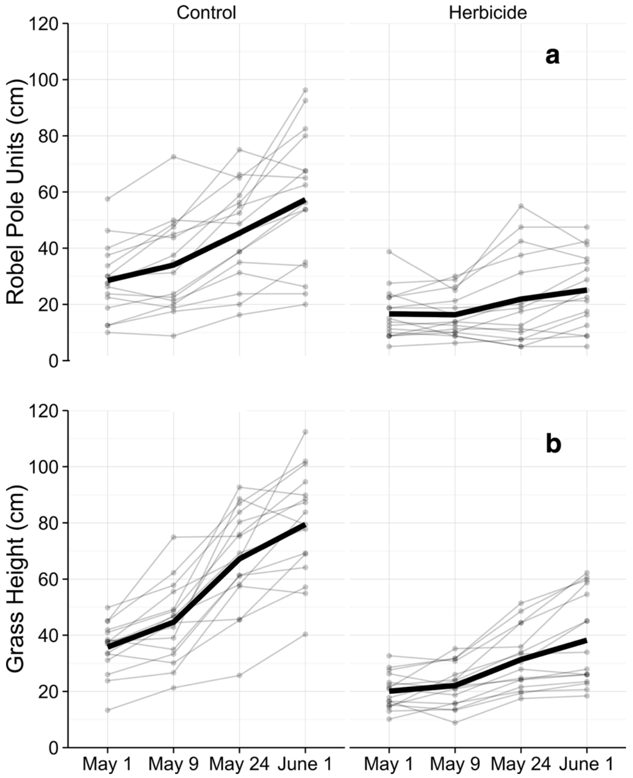

Fig. 1 Mean value for **a** Robel cover (measured in Robel units where 1 unit = 10 cm) and **b** tallest grass height (cm) over the silvery blue adult flight and larval development period. *Light grey lines* show values for each plot

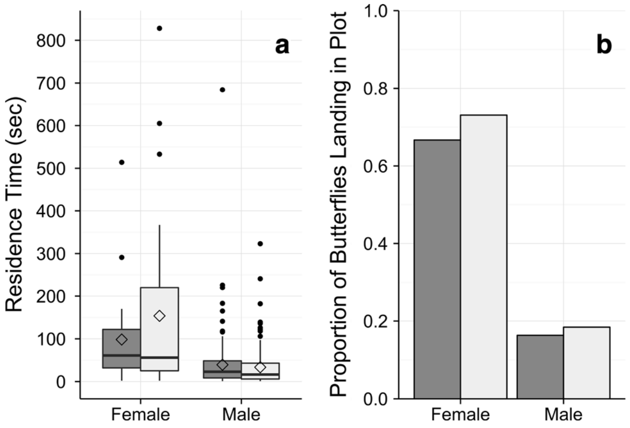

Fig. 2 **a** Silvery blue residence time in seconds for control (*dark grey*) and herbicide (*light grey*) plots. *Boxplots* show the distribution of observations. The median time is denoted by the *black horizontal line* and the mean time is denoted by a *black diamond*. **b** Proportion of butterflies landing in control (*dark grey*) and herbicide (*light grey*) plots

Table 1 Coefficient estimates for explanatory variables and 95 % CI from parametric bootstrapping for best candidate models. Bold values indicate covariate coefficients estimates significantly different from zero ($P < 0.05$)

Model	Estimate	SE	2.5 % CI	97.5 % CI	*P* value
Residence time					
(Intercept)	4.419	0.272	3.998	5.079	<0.0005
Herbicide	0.431	0.362	−0.584	0.854	0.7364
Sex—males	−0.724	0.283	−1.348	−0.230	**0.0060**
Robel cover	−0.212	0.081	−0.452	−0.136	**0.0010**
Herbicide*sex	−0.789	0.379	−1.443	0.060	0.0790
Landing rate					
(Intercept)	1.164	0.502	0.245	2.211	0.0110
Herbicide	−0.719	0.637	−2.005	0.462	0.2511
Sex—males	−2.590	0.434	−3.504	−1.826	**<0.0005**
Tall grass	−0.680	0.328	−1.362	−0.071	**0.0310**
EGG					
(Intercept)	0.793	0.178	0.428	1.133	<0.0005
Herbicide	0.056	0.238	−0.431	0.524	0.8474
Racemes	0.320	0.082	0.153	0.470	**<0.0005**
Raceme height	0.598	0.100	0.403	0.789	**<0.0005**
Third closest individual	0.285	0.084	0.126	0.439	**0.0030**
Percent bud	0.182	0.092	0.003	0.359	**0.0490**
LARVA					
(Intercept)	0.879	0.086	0.681	1.024	<0.0005
Eggs	0.429	0.071	0.300	0.579	**<0.0005**
Herbicide	0.156	0.136	−0.105	0.429	0.2331
Raceme height	0.088	0.063	−0.033	0.212	0.1451
Ants	0.212	0.061	0.088	0.322	**<0.0005**
Robel cover	0.117	0.069	−0.013	0.251	0.0800

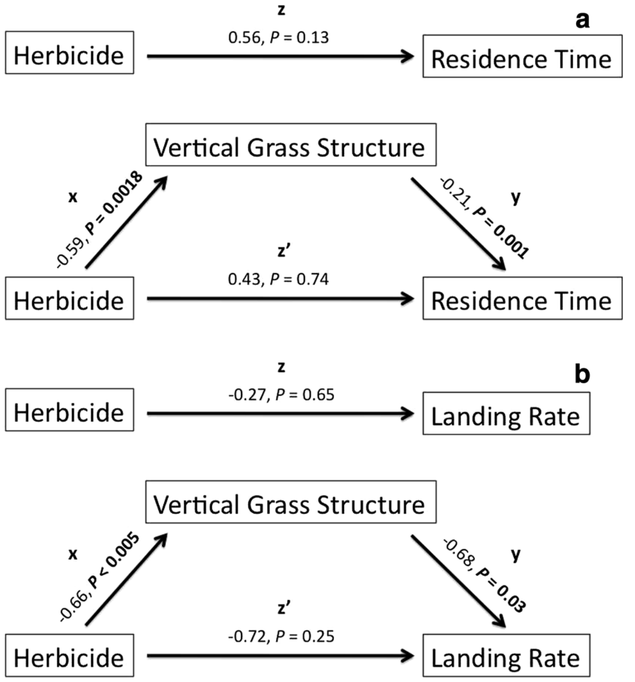

Fig. 3 Mediation model in which the effect of Herbicide on silvery blue **a** Residence Time and **b** Landing Rate is mediated by Vertical Grass Structure. Direct effects are estimated from GLMMs (**y** and **z′**) or linear mixed effects models (**x**). The indirect effect of Herbicide as mediated by Vertical Grass Structure $= \mathbf{x} * \mathbf{y}$. The total effect of Herbicide, $\mathbf{z} = (\mathbf{x} * \mathbf{y}) + \mathbf{z}'$

males. Also, higher Robel cover was associated with decreased residence time (Table 1; Fig. 3a).

Because the herbicide had a significant effect on vegetation structure and vegetation structure affected residence time, we also calculated the indirect effect of herbicide on residence time through altered vegetation structure. There was a positive indirect effect of herbicide on residence time (estimated effect and Monte Carlo (MC) 95 % CI = 0.012 <0.125 <0.297 on the log scale, $P = 0.0266$). However, the overall effect of herbicide (direct + indirect effect) was positive but not significant (estimated effect and MC 95 % CI = −0.160 <0.555 <1.287 on the log scale, $P = 0.133$, Fig. 3a).

Out of 310 total butterfly observations, we observed 38 landings in the control plots and 40 in the herbicide plots. Females entering a plot landed 67 % of the time (SD = 0.48) in control plots and 73 % of the time (SD = 0.45) in herbicide plots. Males landed 16 % of the time (SD = 0.37) in control plots and 18 % of the time (SD = 0.39) in herbicide plots (Fig. 2b). Out of 15 considered models, the best model contained the treatment effect as well as two other covariates (Table 1). Closely ranked models had similar covariate coefficient estimates and the herbicide treatment effect was not significant in any closely ranked models (Electronic Supplementary Material 1). Fluazifop-*p*-butyl

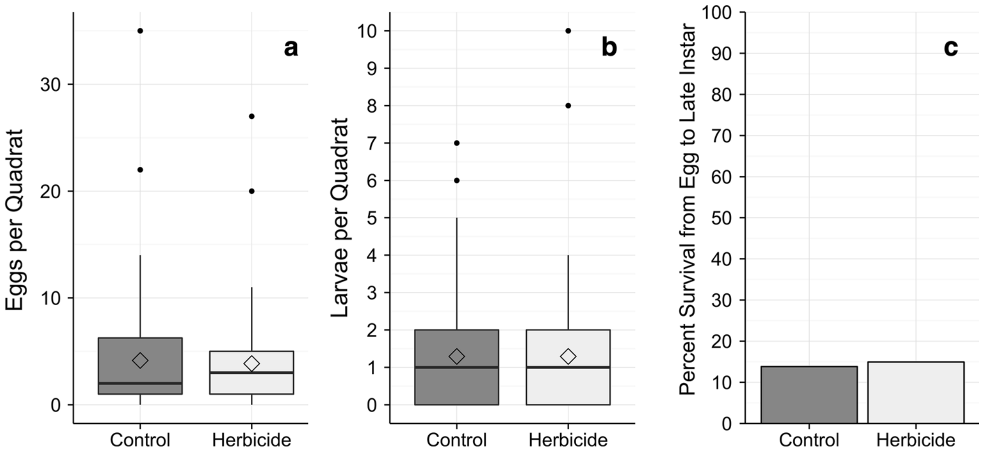

Fig. 4 **a** Silvery blue eggs per quadrat, **b** larvae per quadrat, and **c** Percent survival from egg to late instar larva per quadrat. Boxplots show the distribution of observations. The median density is denoted by the *black horizontal line* and the mean density is denoted by a *black diamond*

did not directly affect the frequency that butterflies landed in treatment plots (estimated treatment effect and bootstrapped 95 % CI = $-2.0055 < -0.7186 < 0.4622$ on the logit scale, $P = 0.2511$). On the scale of the response, the herbicide treatment did not substantially decrease landing rate (an average decrease of 0.153, bootstrapped 95 % CI −0.422 to 0.097). Females on average landed more often in plots than males, and higher grass height was associated with decreased landing rates (Table 1; Fig. 3b). There was a positive indirect effect of herbicide on landing rate (estimated effect and MC 95 % CI = $0.019 < 0.450 < 0.952$ on the logit scale, $P = 0.0396$). However, the overall effect of herbicide (direct + indirect effect) was not significant (estimated effect and MC 95 % CI = $-1.620 < -0.269 < 1.094$ on the logit scale, $P = 0.648$, Fig. 3b).

Adult egg deposition

We counted 384 total eggs in 96 quadrats over the first five sampling occasions. We found an average of 4.1 (SD = 6.2) eggs in control quadrats and 3.9 (SD = 5.0) in herbicide quadrats. Out of 21 considered models, the best model contained the treatment effect as well as four other covariates (Table 1). Closely ranked models had similar covariate coefficient estimates and the herbicide treatment effect was not significant in any closely ranked models (Electronic Supplementary Material 1). Fluazifop-p-butyl did not affect egg density (estimated treatment effect and bootstrapped 95 % CI = $-0.4307 < 0.0555 < 0.5243$ on the log scale, $P = 0.8474$). On the scale of the response, the herbicide treatment did not substantially increase the egg density (an average increase of 0.1261 eggs, bootstrapped 95 % CI −0.9698 to 1.2118). Higher numbers of racemes, taller racemes, more isolated lupine plants, and the percentage of racemes in bud were all associated with higher egg densities (Table 1; Fig. 4a).

Larval density, parasitism, pupal weight, and ant tending

We counted 124 larvae in 96 quadrats over eight sampling occasions. We found an average of 1.3 (SD = 1.6) larvae in control quadrats and 1.3 (SD = 1.9) in herbicide quadrats (Fig. 4b). Out of 16 considered models, the best model contained the treatment effect as well as four other covariates (Table 1). Closely ranked models had similar covariate coefficient estimates and the herbicide treatment effect was not significant in any closely ranked models (Electronic Supplementary Material 1). Fluazifop-p-butyl did not affect larval density in treatment plots (estimated treatment effect and bootstrapped 95 % CI = $-0.1046 < 0.1556 < 0.4287$ on the log scale, $P = 0.2331$). On the scale of the response, the herbicide treatment did not substantially increase larval density (an average increase of 0.4053 larvae, bootstrapped 95 % CI −0.2571 to 1.1183). Increased cumulative eggs and ant tending frequency were associated with greater larvae densities (Table 1). We counted 51 late instar larvae over the eight sampling occasions from 29 out of 96 quadrats. The mean density of late instar larvae was 0.58 (SD = 1.18) in control plots and 0.48 (SD = 0.92) in herbicide plots. Fluazifop-*p*-butyl did not affect the late instar larvae density in treatment plots (estimated effect −0.170 ± 0.439 on the log scale, $z = -0.39$, $P = 0.70$). Survivorship from egg to late instar larvae was 13.8 % in the control plots and 14.9 % in herbicide plots (Fig. 4c).

Out of the 46 collected larvae, four did not reach the pupa stage but only one of those four was parasitized. All four were collected from herbicide plots. We found a pupal weight of 62.3 mg (SD = 13.1) for the control plots and 67.9 mg (SD = 13.0) for the herbicide plots. The herbicide treatment did not alter pupal weight ($t_{37} = -1.347$, $P = 0.186$). Ants were found tending 42/46 of the larvae collected at the time of collection. The average number of ants tending each larva was 3.3 (SD = 3.0) in the control

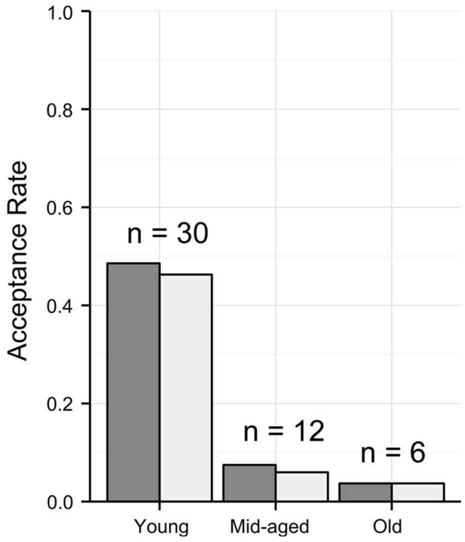

Fig. 5 Oviposition acceptance and rejection rates on control (*dark grey*) and herbicide (*light grey*) plants by butterfly age class

plots and 2.7 (SD = 1.9) in the herbicide plots. The herbicide treatment did not affect the number of ants tending (estimated effect −0.1654 ± 0.3254 on the log scale, $z = -0.508$, $P = 0.611$).

Oviposition choice trials

Out of 48 females tested in the sequential oviposition choice trials, 30 displayed oviposition behavior. The rate of acceptance was 33.8 % on the control plants and 31.2 % on the herbicide plants (Fig. 5). A model with treatment and age was preferred over both a model adding a treatment-age interaction (likelihood ratio test, $X^2 = 0.0335$, $P = 0.9834$) and a model dropping the age effect (likelihood ratio test, $X^2 = 14.661$, $P < 0.0007$). Fluazifop-*p*-butyl did not affect the acceptance rate on treatment plants (estimated treatment effect -0.19 ± 0.27 on the logit scale, Wald test, $z = -0.701$, $P = 0.483$, Fig. 5). Additionally, younger butterflies had higher rates of acceptance than the middle age group (an increase of 3.81 ± 1.12 on the logit scale, Tukey test, $z = 3.414$, $P = 0.002$, Fig. 5) and the old age class (an increase of 4.23 ± 1.70 on the logit scale, Tukey test, $z = 2.480$, $P = 0.034$, Fig. 5). Old butterflies had the same rates of acceptance as the mid-aged group (a difference of −0.42 ± 1.82 on the logit scale, Tukey test, $z = -0.231$, $P = 0.97$, Fig. 5). Plant pair and female individuals explained a substantial amount of variance in acceptance rates.

Discussion

An early spring application of fluazifop-*p*-butyl provides a temporary reprieve from the structural dominance of invasive grasses. As expected, fluazifop-*p*-butyl reduces the height and density of the dominant invasive grasses at our site: tall oat grass (*Arrhenatherum elatius*) and tall fescue (*Festuca arundinacea*). This herbicide delays the bolt of grass flowering stalks resulting in grass height and density that is half that of unsprayed areas throughout the survey period. The magnitude of the difference between plot types increases over time from a relatively minor difference of 10–15 cm in early May to a 30–40 cm difference by June. While the perennial invasive grasses at our site are typically taller than native grasses, the major qualitative difference is that the natives (e.g. Roemer's fescue, *Festuca roemeri*, and California oatgrass, *Danthonia californica*) are bunch grasses that form shorter tufts of foliage, less dense flowering stalks, and more open inflorescences. Invasive grasses alter the historic prairie vegetation structure because they spread quickly, grow tall, have closely-spaced flowering stalks, and increase thatch accumulation (Stanley et al. 2011).

When vegetation reduces host plant apparency and/or alters microclimate, butterflies alter reproductive behavior and lay fewer eggs in a once suitable area (Severns 2008, 2011; Henry and Schultz 2012; Weking et al. 2013). Fluazifop-*p*-butyl application is intended to reduce tall grasses and increase butterfly access to host plants and nectar resources. Silvery blue butterflies alight more often and spend more time in plots with reduced grass structure but, unexpectedly, they do not lay more eggs on host plants in those areas. One explanation is that invasive grasses are not tall enough to obstruct host plant access during the silvery blue flight period. In early May, invasive grasses did not exceed *Lupinus oreganus* height (40–80 cm tall) and the greatest reduction in host apparency was not until the end of the survey season after the peak of the silvery blue flight period. Our results suggest that for early-season silvery blue, invasive grasses do not affect host apparency and oviposition effort. In contrast, for the co-occurring Fender's blue (*Icarica icarioides fenderi*), increased vegetation structure reduces host plant apparency and results in decreased egg deposition (Severns 2008). Fender's blue may be more vulnerable to tall grass interference because they are specialists on *Lupinus oreganus*, lay eggs on foliage rather than inflorescences, and fly up to several weeks later than silvery blue. Compared with other butterfly species, increased grass structure has a similar impact on behavior for silvery blue but lesser impact on oviposition site selection. The Common Ringlet (*Coenonympha tullia*) oviposits at a higher location on host plants when the surrounding herbaceous material is dense (Weking et al. 2013). Tall grasses indirectly harm the large blue (*Phengaris arion*) by creating an unfavorable microclimate for its obligate ant mutualist, which results in drastically increased larval mortality (Thomas et al. 2009). Taylor's checkerspot (*Euphydryas editha taylori*) and Mardon skipper (*Polites mardon*) exhibit behavioral modification in response to elevated grass height and density (Severns and Warren 2008; Henry and Schultz 2012). Whether invasive plants or altered disturbance regimes are the cause, prairie-dependent butterflies can be quite sensitive to the timing and magnitude of changes in vegetative structure.

Although herbicide indirectly affects butterflies through altered vegetation, we find no direct, chemically-mediated effects on the silvery blue. Butterfly species in grassy field margins in the UK also respond more to the indirect rather than direct effects of selective herbicides: butterfly species richness increases with wildflower diversity even when herbicides are used to control grasses (Blake et al. 2011). Field studies comparing direct and indirect effects of herbicide on biological control agents such as carabid beetles and spiders in an agricultural setting demonstrate strong effects of altered vegetation and do not detect direct negative chemical effects (Brust 1990; Haughton et al. 2001b; Taylor et al. 2006). In contrast, LaBar and Schultz (2012) studied the effect of the grass specific herbicide sethoxydim on silvery blue in a prairie with negligible tall grasses. Results indicate that silvery blue males are deterred by the herbicide because there was no measureable change in vegetation structure. Sethoxydim may be more of a deterrent than fluazifop-*p*-butyl because it is more toxic than fluazifop-p-butyl to larvae in greenhouse trials (Hicks, unpublished data). Field trials on the bean leaf beetle (*Cerotoma trifurcata*) also demonstrate that fluazifop-*p*-butyl and sethoxydim have different effects on non-target insects (Agnello et al. 1986b).

Overall, we find no positive or negative total effects of fluazifop-p-butyl on silvery blue butterflies. It is likely that timing the application to avoid exposing more sensitive life stages such as larvae reduces the negative impacts of herbicides on butterflies (Russell and Schultz 2010; Stark et al. 2012). Herbicides do not persist in the environment indefinitely so application timing should take into account the period of chemical degradation. Non-target plants can metabolize fluazifop-*p*-butyl in 2–4 weeks and microorganisms help breakdown the chemical in soils in approximately 1 month (Kulshrestha et al. 1995; El-Metwally and Shaiby 2007). During our field experiment, silvery blue were in the pupal stage during herbicide application and likely avoided direct exposure because they diapause beneath the thatch and soil. Because *L. oreganus* host plants were sprayed before developing any floral tissue, adults and larvae were not exposed to chemical residues on the lupine surface. Our larval results from the field differ from the decreased survivorship and lowered pupal weight observed in laboratory work (Russell and Schultz 2010; Stark et al. 2012), perhaps because larvae in our study were only exposed to a food source sprayed 6–8 weeks previously. Herbicide did not negatively impact silvery blue larva numbers, but we remain uncertain as to whether herbicide affects larvae that are directly exposed in the field. If herbicide alters plant nutritional quality, our findings suggest that larvae are able to compensate for decreased host plant nutritional content as seen in other species such as the monarch butterfly (*Danaus plexippus*, Lavoie and Oberhauser 2004). Lycaenid larvae also depend on ant mutualists for survival because ant tending confers protection from predators (Pierce and Easteal 1986; Fiedler 1991; Stadler et al. 2001). Our results substantiate the importance of ants for Lycaenid survival as well as suggest that herbicide application does not alter this important mutualism. Timing of exposure can moderate the negative effects of herbicide on other insects. The Mexican bean beetle (*Epilachna varivestis*), initially avoids eating a sprayed host plant, but after several days, stops discriminating between food sources (Agnello et al. 1986a). For weevils and gallflies used in biocontrol, herbicides reduce survival the most when applied during immature stages of insect development (Messersmith and Adkins 1995). Seasonal timing of herbicide spray is important in the control of spotted knapweed (*Centaurea stoebe*) because a fall spray adversely affects root-feeding biocontrol beetles and moths more than a spring spray (Story and Stougaard 2006). Herbicide application timing even supersedes choice of herbicide formulation for minimizing survival impacts on the Mimosa Stem-Mining Moth (*Neurostrota gunniella*) used as a biocontrol agent; in this system, avoiding larval exposure is critical (Paynter 2003).

Our greenhouse findings indicate that butterflies do not discriminate against recently sprayed host plants and that herbicide residues on the surface of the host plant do not deter silvery blue females. Under both the more extreme exposure conditions in the greenhouse and more realistic conditions in the field, female silvery blues equally accept sprayed and unsprayed hosts, suggesting that fluazifop-*p*-butyl does not directly or indirectly interfere with silvery blue reproductive effort.

Land managers must take action to curtail the spread of invasive plants. Although there are several methods available to combat perennial invasive grasses, grass-specific herbicides have the highest selectivity. For managers hoping to control invasive grass while minimizing impacts on butterflies, the combination of fluazifop-*p*-butyl and Nufilm® applied in the early spring may be a useful tool. However, before specific application recommendations can be made, there are several remaining knowledge gaps to address. Grass-specific herbicides rarely kill target plants with one spring application (Stanley et al. 2011). Permanently reducing the cover of non-native plants requires multiple years of application, application in the summer or fall, or combination with other management tools such as mowing and burning (Stanley et al. 2011). Also, butterfly emergence and timing of grass bolt can change with annual weather variation and this interaction could alter the effects of herbicide and vegetation structure on butterflies (Severns 2008). Therefore, we need to conduct field trials that span several years at multiple sites and reflect realistic combinations of tools and application timing. Additionally, investigations should quantify the effects on multiple butterfly species with differing life history characteristics and phenology, especially for species that are exposed during the larval stage such as Fender's blue. When possible, studies should attempt to quantify the net effects of herbicides at the population level (Kohler and Triebskorn 2013). Other grass-specific herbicides showing low toxicity to butterfly larvae in laboratory trials such as clethodim (Envoy®) should be tested in field trials (Zemaitis, unpublished data). Finally, managers should be judicious with applications to avoid producing resistant plant populations (Hidayat and Preston 1997). Our study demonstrates a net neutral effect of a grass-specific herbicide on the silvery blue butterfly when sensitive stages are not sprayed and a reduction in vertical grass structure in the season of application. Depending on the responses of additional species to realistic field trials, prairie-dependent butterfly species could benefit from the grass reduction produced by strategic herbicide application. However, this herbicide remains untested with regard to many non-target butterflies and managers should exercise caution when considering this tool for invasive grass control.

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