Bringing back flowering plants and pollinators through effective control of invasive winter annual grasses with Esplanade® herbicide

Boulder County Open Space Small Grant Report

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December 6, 2018

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EXECUTIVE SUMMARY

Invasive species pose one of the largest threats to the biodiversity native to a region, including impacting important pollinator species. Currently the state of Colorado's list of noxious species includes 105 species, 9 of which are grasses. Cheatgrass, or downy brome, is one of the most problematic exotic grass species in the American West, and the most widespread of the Colorado listed species, occurring in every county in the state. Control of invasive grasses has largely depended on grazing, prescribed burning, or use of herbicides such as imazapic, glyphosate and rimsulfuron. However, control is challenged by the dependence of livestock on forage grass species and the nonselective nature of many herbicides. Indaziflam (Esplanade), an herbicide recently approved for use in controlling invasive annual grasses on rangelands and open spaces, shows promise to eliminate or limit spread of those invasive grasses. As invasive species are controlled and eventually eliminated, it is expected that the areas previously occupied by the invasive species will be amenable for growth of flowering forbs and in turn support pollinators that depend on these flora.

To test this, we conducted surveys to observe and record pollinator visitation, flowering forb richness and diversity at 3 sets of paired plots in Boulder County, CO. A preliminary study in 2017 demonstrated an increase in forb cover in plots treated with indaziflam, as well as a greater number of insects visiting flowers in treated plots vs. flowers in control plots. The current study was extended to cover more of the flowering season and expanded to include timed observations on flowering plants that were not on the standard transects. One plot in each pair had been treated with indaziflam while the other served as an untreated control. Timed observations were conducted along transects covering the entire plot. Our results in the 2018 study concurred with the results in the preliminary 2017 study. Interestingly, while they did occur in the plots, bees and butterflies were not recorded as frequently during the timed observations as were visitors from other insect orders, notably hemipterans. In order to obtain a good idea of the pollinator diversity in the area, it would be necessary to conduct destructive sampling using blue vane traps or net sampling.

Our study aimed to minimize removal and killing of insects and hence we relied on visual observations of pollinators at flowers which was restricted to the periods of observations during the study. Many of the forbs reported in our study are dependent on pollinators for successful reproduction. Further study of seed and fruit production of the native flora in the plots will provide conclusive evidence for the activity of pollinators. Improved fruit and seed production in native flora imply better reseeding potential and increased success of restoration in these reclaimed areas. Application of Esplanade could thus pave the way to restore native biodiversity. The results of our study demonstrate some of the positive impacts of Esplanade on the restoration of flowering plants. These analyses of floral resources will help describe the benefits of Esplanade application in supporting and sustaining pollinator diversity in Boulder County.

INTRODUCTION

Invasive winter annual grasses, particularly downy brome or cheatgrass (*Bromus tectorum*) and Japanese brome (*Bromus arvensis*), have had devastating consequences across the rangeland habitat of western United States. They are highly competitive exotics that effectively displace native vegetation by depleting soil moisture and nutrients. Downy brome infests a combined area estimated at over 54 million acres (Sebastian et al. 2017), disrupting the ecosystem by restructuring historic fire regimes, displacing native flora and rendering the land less fit for grazing and other human purposes. Recently, indaziflam (marketed as Esplanade by Bayer CropScience) has been approved for use on rangeland and open spaces infested by invasive annual grasses. Esplanade, acting immediately following germination of cheatgrass seeds, provides a mode of action different from previously used herbicides (Sebastian et al. 2017). Beginning in 2015 Esplanade has been applied in areas of Boulder County Open Space lands and the initial applications have shown effective control of invasive winter annual grasses and a resulting increase in broadleaved forbs (Sebastian et al. 2017). Many of these forbs promote and sustain important ecosystem services including pollination as they provide food and shelter for pollinating bees, butterflies and flies. Several of these pollinators are native to this region of Colorado, and the prevalent bee fauna of Boulder County and their importance have been particularly well-documented (Kearns and Oliveros 2009a. Kearns and Oliveros 2009b, Goldstein and Scott 2015). Pollinating insects in general are facing debilitating challenges due to widespread loss of habitats. Thus, the use of Esplanade has the potential to serve as a tool in restoring biodiversity in degraded lands.

Increased habitat loss and degradation has serious negative impacts on biodiversity and ecosystem functions, notably that of pollination. Pollinating insects are not only important for production of food crops, they are critical for the reproductive success of many native plant species, as over 70% of flowering plants depend on pollinators for successful seed production (Blitzer et al. 2012). Ongoing challenges faced by pollinators are directly related to increasing habitat loss and habitat degradation. Loss of plant diversity also compromises pollinator nutrition as these beneficial insects are restricted to a few available plant species for nectar and pollen. While the impact of invasive species on the native vegetation is well known, few studies explore the relation between herbicide use for invasive plant control and the subsequent impact on pollinators.

***Preliminary study*:** In collaboration with Bayer CropScience and county agents, we conducted a preliminary study on Boulder County Open Space lands from June-September 2017. Three study plots were treated with Esplanade between Dec 2016 and Feb 2017, while three plots were established in similar habitats as untreated controls. Esplanade is known to have residual effects for up to 3 years after application (Sebastian et al. 2017). Our preliminary observations conducted only 120 – 180 days after application, demonstrated an increase in flowering plants in treated plots as compared to untreated sites. Treated plots also showed higher proportions of native plants and the treated sites had more unique species. Our preliminary study began in June which mean early flowering plants and pollinators were not recorded in the 2017 study. To effectively demonstrate the benefits and efficacy of Esplanade, in 2018 we expanded our study in time and scope.

Objectives:

1. Monitor treated and untreated plots demarcated in the 2017 pilot study (Fig. 1), from late April 2018 to Sep 2018, and document plant and beneficial insect species richness, diversity and abundance.
2. Perform timed observations of insects visiting the flowers and capture representative pollinators if they could not be identified visually in the field.

We hypothesized that:

1) Flowering species would be more abundant in treated plots as compared to untreated control plots.
2) We would document a wider range of insect species foraging on the flowers in treated plots.
3) Treated plots would provide a wider resource profile for pollinators as a result of increased flowering plant diversity.

METHODS

Timed observations of pollinators: Beginning in mid-April and continuing through mid-September we conducted timed pollinator surveys, at biweekly intervals (depending on weather), in the plots shown in Figure 1, following accepted protocols (Kearns and Inouye 1993). Plots were selected with the guidance of Boulder County staff and were of different sizes, rhomboidal in shape, encompassing areas that had been previously sprayed with herbicide (treated plots) and untreated control sites that were in similar habitat types on the landscape.

Transect sampling: Eight permanent transects perpendicular to each other were established at each survey plot, spaced evenly across the vertical and horizontal axes of the plots (Fig. 2). Transects ranged from 30 m to 135 m in length, depending on the size and shape of the plot. A meter tape was stretched between the ends of the transects and a $1m^2$ frame placed a meter away from the tape at 10m intervals, on alternating sides of the transect line. The directions in which transect lines were run and the order in which plots were surveyed were changed on alternate visits in an effort to eliminate bias in timing of pollinator activity and flowering phenology. On each visit the date, time, and weather conditions were recorded. Observations were generally conducted between 7:50 a.m. and 1:30 p.m., and towards the later parts of the sampling season, we were forced to begin later due to cooler morning temperatures resulting in observations being completed a little after 3:30 p.m.

Pollinator observations: Using a timer, flowers inside the frame were observed for 2 minutes. Following protocols described in Mason et al. (2018), the data recorded included: the species of flowering plant, number of flowers of that species occurring inside the frame, and the number and morphospecies of insects that contacted reproductive structures of the flowers.

Random walk sampling: As our transects were permanent, flowering plants that did not fall within the sampling regions of the transects would not be recorded during our study. To avoid consistently missing plants and hence under-reporting the information on flowering plants in the plots, we conducted random walks in the same plots. We walked through the plot in a haphazard fashion with a goal of targeting all plants that did not fall into our quadrats and recorded all blooming species on that day. Together with the transects, we were thus able to quantify all the plants in flower in the experimental plots. We conducted focal plant samplings by selecting flowering plants that were seen during the random walk in the plot. Plant species were selected based on their non-occurrence on the transects, and/or the number of flowers in bloom. These were conducted by placing a sampling frame around a patch of a particular forb species and observing pollinator visitations during a 2-minute period, as done along the transects. In addition, any pollinators that were incidentally observed on flowers while walking through the plots were recorded, along with the flowering species they were visiting.

Data analyses: Standard ecological indices for plant and pollinator species diversity, richness and abundance were calculated for the treated and untreated plots for each sample event (Nielsen et al 2011). The values were compared between treated and untreated plots by a one-way ANOVA followed by post-hoc mean comparison. As needed, transformations were performed for non-normal data.

RESULTS AND DISCUSSION

Timed observations of pollinators: Between April 27 and September 20, 2018 we documented a total of 116 species of entomophilous forbs blooming in the plots (Table 1). This compares to 60 species observed in the 2017 preliminary study that began a little later in the season (June 22 2017). Our results demonstrate the high level of biodiversity that occurs in the grasslands and shrublands in this area of Boulder County and the northern Front Range of Colorado. During the timed observations we recorded visitations by 13 morphospecies of pollinators, such as bees and butterflies (Table 2). We also observed other insects including small hemipterans, tiny beetles, ants and green lacewings, that were flower visitors and contacted the reproductive structures of flowers and/or fed on nectar but did not actively move from one plant to another carrying pollen and hence were not classified under pollinators.

Effect of Esplanade treatment on plant species diversity measures: Univariate General Linear Models Analysis of Variance (GLM ANOVA) was used to compare plant species diversity measures with treatments (Esplanade treated and Untreated) and sites (Rabbit Mountain 1, Rabbit Mountain 2 and Colp) are fixed effects, week as the covariate and species richness, Shannon diversity Index, Simpson's index of abundance and Evenness index as dependent variables. Table 3 provides results of the GLM ANOVA. Esplanade treatment showed significant effect on the variance of all the four diversity measures. Weeks had a significant effect only on species richness, Shannon diversity Index and Simpson's index of abundance. There was no effect of sites on any of the diversity indices implying that the observed effects of esplanade treatment were uniform across all the three sites. Post-hoc comparison of means indicated significant differences between treated and control treatments for species richness (Fig.3), Shannon diversity Index (Fig. 4) and Simpson's index of abundance (Fig. 5). There was no significant difference between treatment means for evenness index (Fig. 6).

Our results indicate that controlling cheat grass with the treatment of Indaziflam significantly increases flowering plant richness, diversity and abundance. Detailed statistical analyses and weekly patterns of differences between species diversity values are still under progress and we expect to be able to demonstrate stronger effects of cheatgrass control. Our observational studies were limited to a few hours each day resulting in low recordings of pollinator visitation rates. However, with the demonstration that flowering plants have returned into these areas in increased diversity and abundance we expect that these resources will support pollinator populations. Continued monitoring of these locations could provide strong data showing increase in pollinator populations.

We proposed to conduct studies on floral display size which is a strong predictor of pollinator activity and is strongly influenced by the effects of below ground competition by other plants. However due to paucity of resources and time we were unable to complete the analyses of floral size measurements. Another indicator of the resource availability for pollinators is the presence and quantity of nectar and pollen. For the future, it would be interesting to explore the nectar and pollen content and determine differences in nutritional quality and quantity in treated and control plots. We expect that under reduced competition from cheatgrass, pollen quantity and quality were likely to be higher in treated plots.

While the results being reported here are based on preliminary analyses of the data collected, we will continue to perform correlation and regression analyses of pollinator activity were conducted as a function of floral resources (number of flowers per plant) available at the time of observation. Out study is one of the first to demonstrate the effects of invasive grass control on restoring pollinator habitats and hence bring back pollinators to the area. We do hope that control measure to eliminate cheat grass will continue to be implemented in these rangeland ecosystems helping the protection of our fragile pollinator populations.

Pending funding, in the future, we aim to characterize pollen grains of all the flowering species in these locations and compare size, quantity and quality of pollen under differential competition regimes (with and without cheat grass).

LITERATURE

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FIG. 1. Study locations were all located on Boulder County Parks and Open Space lands west of the city of Loveland. RM plots were at the Rabbit Mountain Open Space and the Colp sites were located 3.5 miles apart along U.S. Hwy 36 on open space land not currently open to the public.

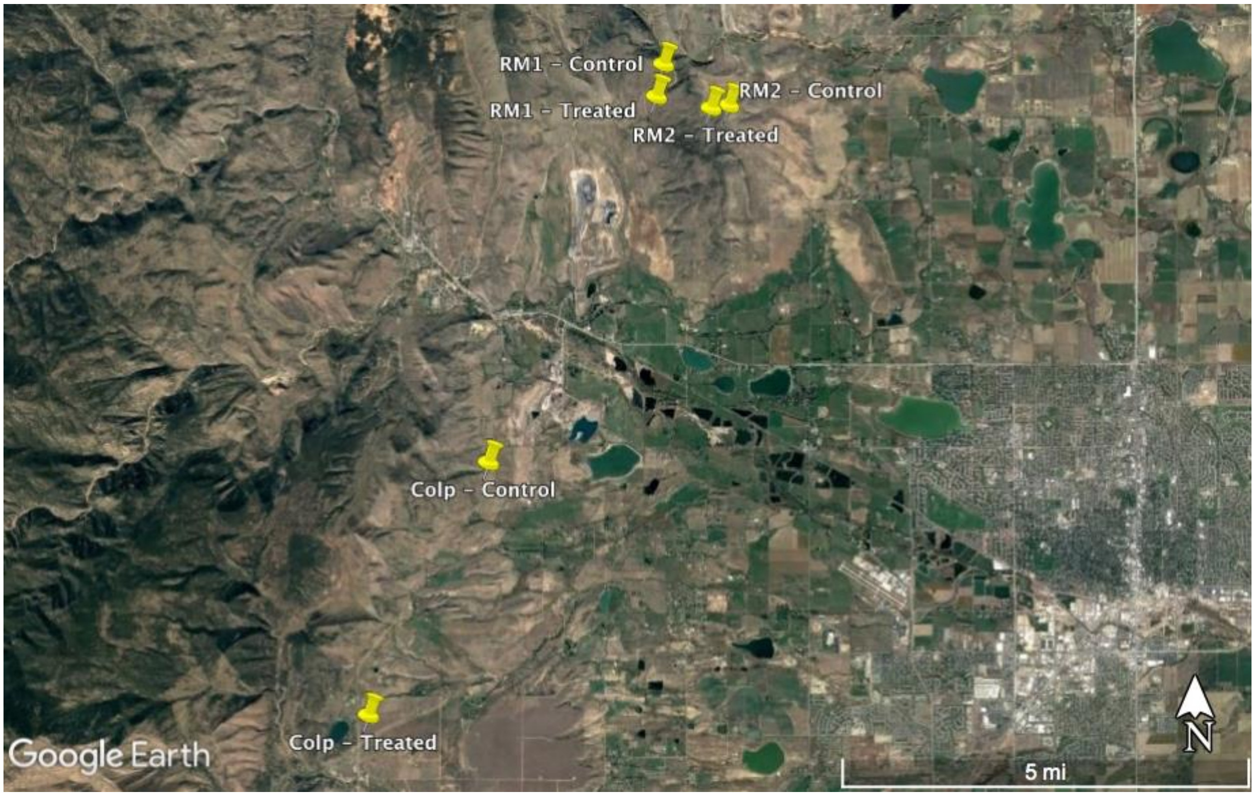

FIG. 2. Transect layout at each study plot.

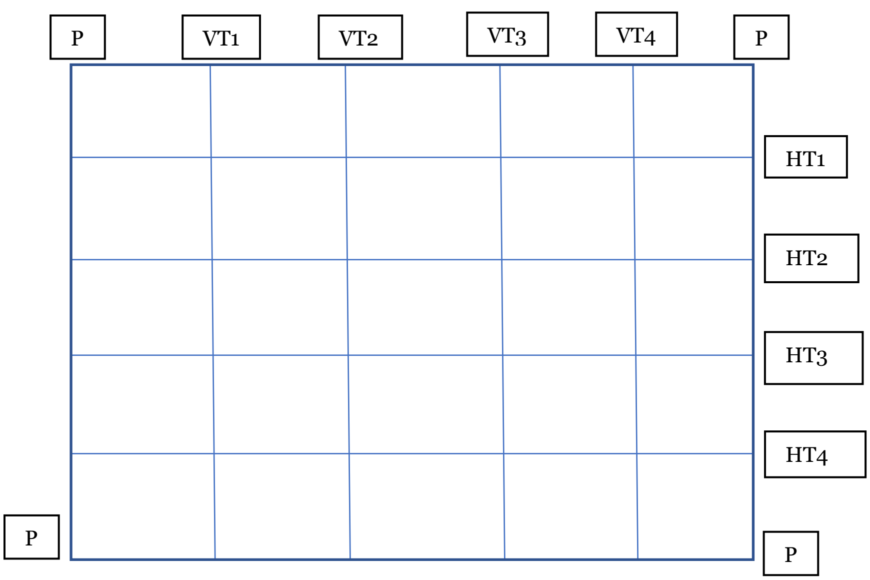

Figure 3: Mean ± SE of species richness values between control and Esplanade treated plots in the three locations.

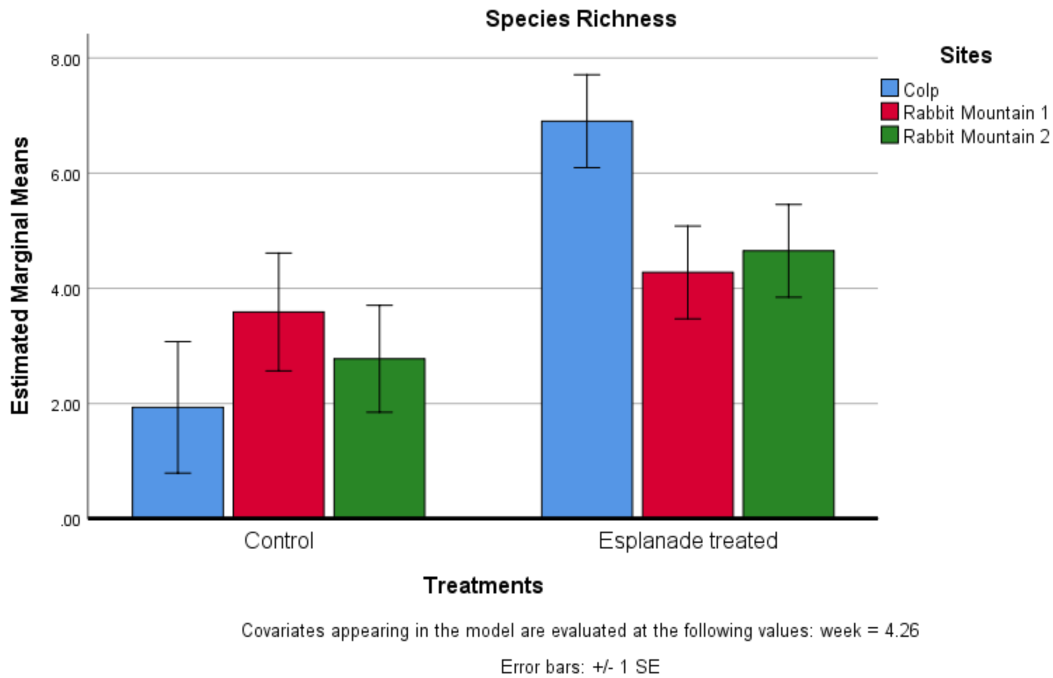

Figure 4: Mean ± SE of Shannon Diversity Index values between control and Esplanade treated plots in the three locations.

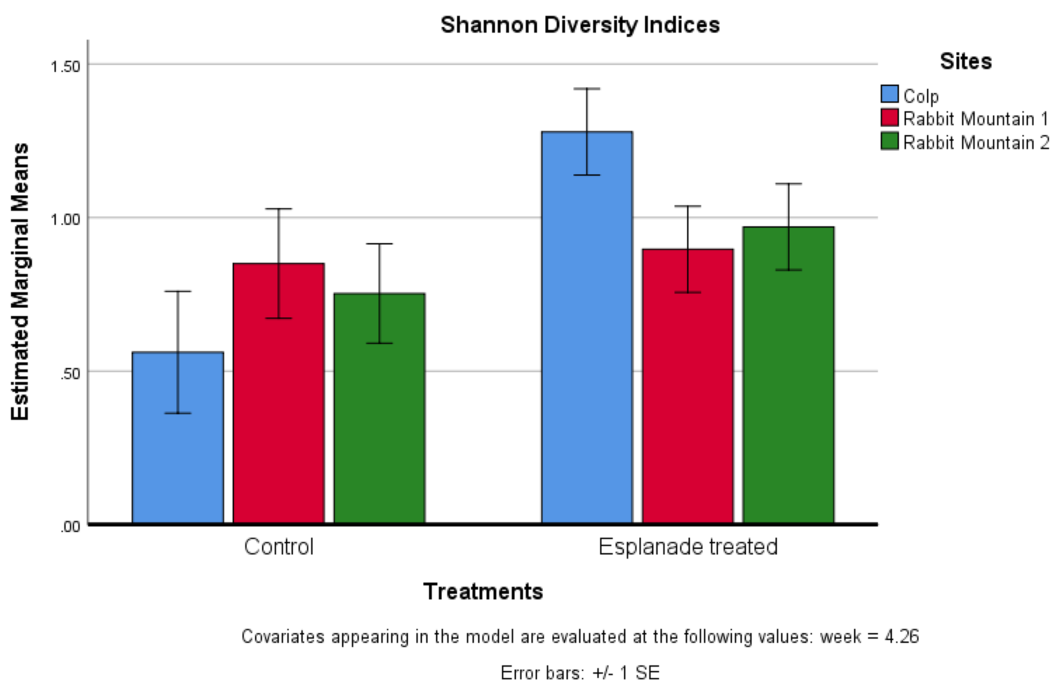

Figure 5: Mean ± SE of Simpson’s Index values for abundance between control and Esplanade treated plots in the three locations.

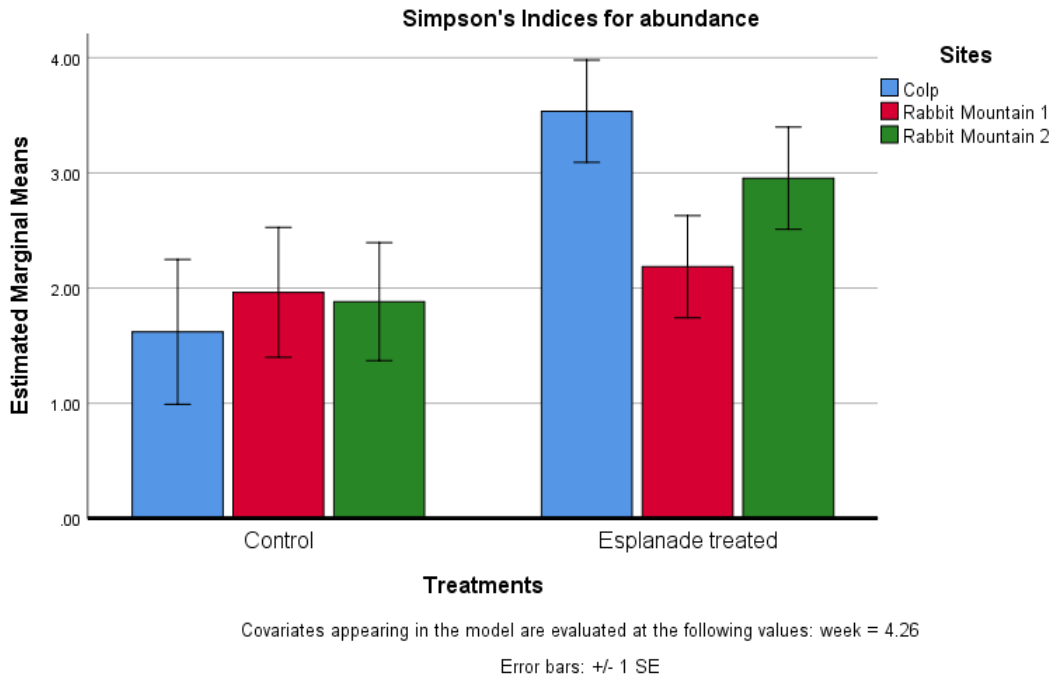

Figure 6: Mean ± SE of Evenness index values for abundance between control and Esplanade treated plots in the three locations.

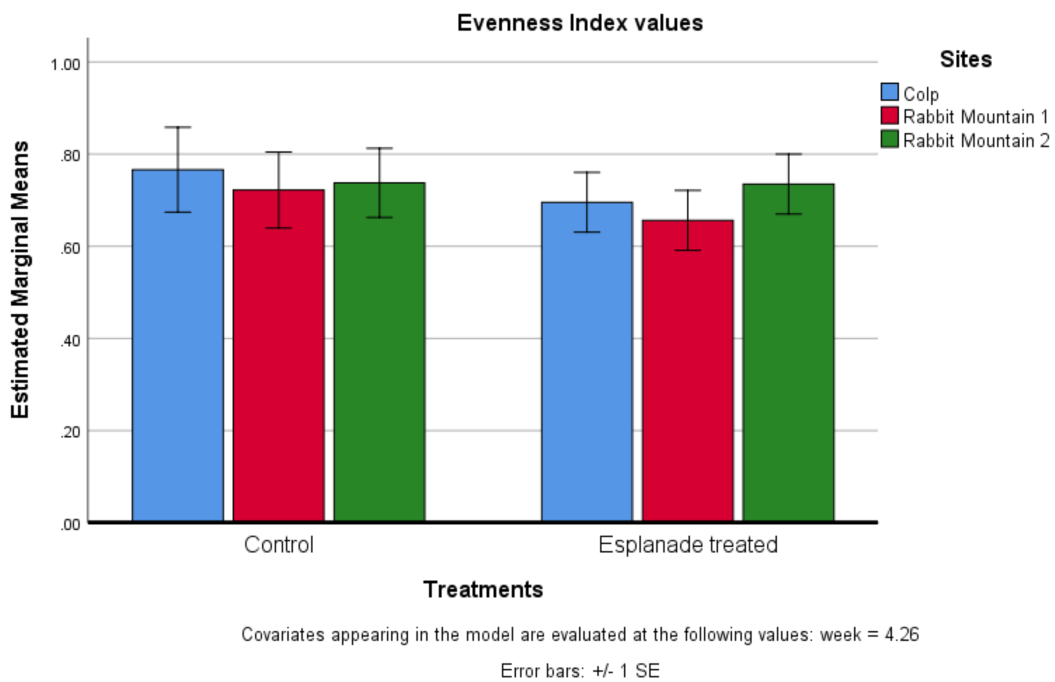

TABLE 1. List of insect-pollinated flowering forb species occurring in study plots in 2018.

Family	**Species (USDA Plant nomenclature)**	**Colp-Control**	**Colp-Treated**	**Rabbit Mountain 1-Control**	**Rabbit Mountain 1-Treated**	**Rabbit Mountain 2-Control**	**Rabbit Mountain 2-Treated**
Liliaceae	*Allium textile*		X	X	X	X	X
Brassicaceae	*Alyssum simplex*	X	X	X		X	X
Asteraceae	*Antennaria parvifolia*						X
Papaveraceae	*Argemone polyanthemos*	X	X				
Asteraceae	*Arnica fulgens*				X		X
Apocynaceae	*Asclepias viridiflora*	X	X				
Fabaceae	*Astragalus agrestis*			X		X	X
Fabaceae	*Astragalus drummondii*						X
Fabaceae	*Astragalus flexuosus*	X			X		X
Fabaceae	*Astragalus shortianus*		X				X
Brassicaceae	*Boechera fendleri*			X			X
Brassicaceae	*Boechera stricta*					X	
Liliaceae	*Calochortus gunnisonii*			X	X		X
Onagraceae	*Calylophus serrulatus*		X		X		
Brassicaceae	*Camelina microcarpa*			X		X	
Asteraceae	*Carduus nutans*			X		X	X
Orobanchaceae	*Castilleja sessiliflora*		X				
Rhamnaceae	*Ceanothus herbaceous*		X				
Asteraceae	*Centaurea diffusa*		X				
Caryophyllaceae	*Cerastium arvense*		X	X	X	X	X
Asteraceae	*Chondrilla juncea*		X				
Asteraceae	*Cirsium undulatum*	X	X		X		X
Convolvulaceae	*Convolvulus arvensis*	X	X	X	X	X	
Asteraceae	*Crepis occidentalis*						X

Boraginaceae	*Cryptantha virgata*		X				
Fabaceae	*Dalea purpurea*		X		X		X
Ranunculaceae	*Delphinium carolinianum*	X	X		X		X
Brassicaceae	*Descurainia sp.*						X
Brassicaceae	*Draba nemorosa*					X	
Cactaceae	*Echinocereus viridiflorus*		X		X		
Asteraceae	*Ericameria nauseosa*			X		X	X
Asteraceae	*Erigeron divergens*	X	X	X	X	X	
Asteraceae	*Erigeron flagellaris*	X	X	X	X	X	X
Asteraceae	*Erigeron pumilus*		X				
Asteraceae	*Erigeron sp.*			X	X	X	X
Polygonaceae	*Eriogonum alatum*		X		X		X
Polygonaceae	*Eriogonum effusum*		X			X	
Polygonaceae	*Eriogonum umbellatum*			X	X	X	X
Geraniaceae	*Erodium cicutarium*	X	X	X		X	
Brassicaceae	*Erysimum asperum*		X		X	X	X
Euphorbiaceae	*Euphorbia brachycera*						X
Euphorbiaceae	*Euphorbia dentata*	X					
Convolvulaceae	*Evolvulus nuttallianus*		X				
Asteraceae	*Gaillardia aristata*		X	X	X	X	X
Geraniaceae	*Geranium caespitosum*			X	X		
Verbenaceae	*Glandularia bipinnatifida*	X				X	
Asteraceae	*Grindelia squarrosa*	X	X	X	X	X	X
Asteraceae	*Gutierrezia sarothrae*	X	X			X	X
Asteraceae	*Helianthus annuus*				X		X
Asteraceae	*Helianthus pumilus*		X				X
Asteraceae	*Heterotheca villosa*	X	X	X	X	X	X
Asteraceae	*Hymenopappus filifolius*		X		X	X	X

Clusiaceae	*Hypericum perforatum*	X		X	X	X	X
Asteraceae	*Lactuca serriola*	X	X			X	
Boraginaceae	*Lappula occidentalis*	X	X		X		
Brassicaceae	*Lepidium campestre*		X				X
Brassicaceae	*Lepidium perfoliatum*			X			
Brassicaceae	*Lesquerella montana*		X		X	X	X
Liliaceae	*Leucocrinum montanum*						X
Asteraceae	*Liatris punctata*		X	X	X	X	X
Plantaginaceae	*Linaria dalmatica*	X	X	X	X	X	X
Linaceae	*Linum lewisii*		X				X
Linaceae	*Linum pratense*	X					
Boraginaceae	*Lithospermum incisum*			X	X	X	X
Apiaceae	*Lomatium orientale*		X		X		X
Asteraceae	*Lygodesmia juncea*					X	
Asteraceae	*Machaeranthera bipinnatifida*					X	
Fabaceae	*Medicago sativa*	X					
Boraginaceae	*Mertensia lanceolata*		X		X		X
Nyctaginaceae	*Mirabilis linearis*		X		X		
Lamiaceae	*Monarda pectinata*	X	X				
Lamiaceae	*Nepeta cataria*						X
Asteraceae	*Nothocalais cuspidata*		X		X		X
Onagraceae	*Oenothera howardii*						X
Onagraceae	*Oenothera suffrutescens*	X	X		X	X	X
Boraginaceae	*Onosmodium molle*	X	X				X
Cactaceae	*Opuntia phaeacantha*	X	X	X	X		X
Cactaceae	*Opuntia polyacantha*	X			X	X	X
Orobanchaceae	*Orobanche fasciculatum*		X		X		X
Oxalidaceae	*Oxalis dillenii*	X					

Fabaceae	*Oxytropis lambertii*		X		X		X
Fabaceae	*Oxytropis sericeus*		X				
Asteraceae	*Packera fendleri*	X	X		X	X	X
Fabaceae	*Pediomelum esculentum*		X				
Plantaginaceae	*Penstemon secundiflorus*		X		X		X
Plantaginaceae	*Penstemon virens*		X		X		X
Hydrphyllaceae	*Phacelia heterophylla*		X				
Solanaceae	*Physalis hederifolia*					X	X
Solanaceae	*Physalis virginiana*					X	
Plantaginaceae	*Plantago patagonica*					X	
Rosaceae	*Potentilla fissa*		X		X		
Rosaceae	*Prunus virginiana*						X
Fabaceae	*Psoralidium tenuiflorum*	X	X	X	X	X	X
Asteraceae	*Ratibida columnifera*	X	X	X	X	X	X
Rhamnaceae	*Rhus trilobata*		X	X	X		X
Rosaceae	*Rosa woodsii*						X
Asteraceae	*Scorzonera laciniata*		X	X		X	
Lamiaceae	*Scutellaria brittonii*				X		X
Asteraceae	*Senecio spartioides*		X		X	X	X
Caryophyllaceae	*Silene antirrhina*		X				
Brassicaceae	*Sisymbrium altissimum*	X	X			X	
Brassicaceae	*Sisymbrium sp.*			X			
Asteraceae	*Solidago missouriensis*			X			X
Asteraceae	*Solidago nana*		X				
Malvaceae	*Sphaeralcea coccinea*		X			X	X
Asteraceae	*Symphyotrichum ericoides*	X	X	X	X	X	X
Asteraceae	*Symphyotrichum porteri*	X		X	X		X
Asteraceae	*Taraxacum officinale*		X	X	X	X	

Asteraceae	*Tetradymia canescens*			X		X	X
Commelinaceae	*Tradescantia occidentalis*	X	X		X		
Asteraceae	*Tragopogon dubius*	X	X	X	X	X	X
Campanulaceae	*Triodanis perfoliata*	X					
Plantaginaceae	*Verbascum blattaria*			X		X	
Plantaginaceae	*Verbascum thapsus*					X	
Fabaceae	*Vicia americana*			X			
Violaceae	*Viola nuttallii*		X		X		X
Agavaceae	*Yucca glauca*	X	X		X	X	X

TABLE 2. List of bees by morpho-species recorded during Random-Walk sampling in the study plots in 2018. See Mason et al (2018) for detailed protocols on morpho-species categorization

Order	**Species or Morphospecies**	**Colp-Control**	**Colp-Treated**	**Rabbit Mountain 1-Control**	**Rabbit Mountain 1-Treated**	**Rabbit Mountain 2-Control**	**Rabbit Mountain 2-Treated**
Hymenoptera	Tiny dark bee	.	.	.	.	.	2
Hymenoptera	Hairy belly bee	.	.	.	.	.	1
Hymenoptera	Hairy belly bee	.	.	.	.	.	1
Hymenoptera	Hairy belly bee	.	.	.	.	.	1
Hymenoptera	Sweat bee	1	.	.	.	.	.
Hymenoptera	Striped sweat bee	.	.	.	.	.	1
Hymenoptera	Tiny ants	7	.	.	.	.	.
Hymenoptera	Unknown	.	.	.	.	.	2
Lepidoptera	*Euptoieta claudia*	.	.	.	.	.	1
Lepidoptera	*Feltia ducens*	.	.	.	.	1	.
Lepidoptera	*Helicoverpa zea*	.	.	.	.	1	.
Lepidoptera	*Loxostege cerealis*	.	.	.	.	2	.
Lepidoptera	*Pseudanarta crocea*	.	.	.	.	1	.

Table 3: GLM ANOVA results showing the effects of treatments and weeks on the different diversity measures. The model design: Intercept + week + treatment + site + (treatment * site). Bold indicates significant values

Tests of Between-Subjects Effects					
Source	Dependent variables	df	Mean Square	F	Sig.
Corrected Model	Species richness	6	27.147	5.240	**0.001**
	Shannon Index	6	0.670	4.272	**0.003**
	Simpson's Index	6	5.387	3.427	**0.010**
	Evenness Index	6	0.029	0.863	0.533
Intercept	Species richness	1	413.490	79.813	**0.000**
	Shannon Index	1	16.948	107.973	**0.000**
	Simpson's Index	1	118.337	75.273	**0.000**
	Evenness Index	1	6.180	183.111	**0.000**
Week	Species richness	1	84.174	16.247	**0.000**
	Shannon Index	1	2.603	16.584	**0.000**
	Simpson's Index	1	17.634	11.217	**0.002**
	Evenness Index	1	0.113	3.356	0.076
Treatment	Species richness	1	56.113	10.831	**0.002**
	Shannon Index	1	0.953	6.072	**0.019**
	Simpson's Index	1	10.201	6.488	**0.016**
	Evenness Index	1	0.019	0.566	0.457
Site	Species richness	2	1.516	0.293	0.748
	Shannon Index	2	0.011	0.071	0.931
	Simpson's Index	2	0.775	0.493	0.616
	Evenness Index	2	0.008	0.246	0.783
Treatment * site	Species richness	2	13.780	2.660	0.085
	Shannon Index	2	0.342	2.181	0.129
	Simpson's Index	2	2.049	1.304	0.286
	Evenness Index	2	0.005	0.136	0.874