<u>Investigation of Broadleaf Perennial Herbicide Fusilade on Velvet Grass *Holcus lanatus.*</u>

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by,
ESM 485: Ecology and Management of Bio-Invasions
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Acknowledgements,
We would like to thank Dr. Catherine de Rivera and David Thompson for coordination and guidance with this project.

December 7th, 2023

Executive Summary

The Oregon Silverspot Butterfly (*Speyeria zerene hippolyta*) is a federally listed butterfly inhabiting Coastal prairies in Oregon. The Nestucca Bay Wildlife refuge has restored over 30 acres of upland coastal prairie, providing habitat for the endangered species. The butterfly species relies on the Early Blue Violet (*Viola adunca)* for caterpillar development. Currently, Velvet grass (*Holcus lanatus)*, a non-native species that promotes succession into late seral coastal prairies threatens to exclude *V. adunca* through preemptive and competitive mechanisms. In this study, herbicide applications of the grass-specific pesticide, Fusilade dx are studied for effective application timing and concentration rates. The results of the study did not show any strong effects based on timing or concentration. However, herbicide application has shown to be effective with repeated application in the early and late spring, with the highest plant mortality rate observed in early November. We recommend further herbicide studies include fall herbicide application, spot spraying, and seedhead harvesting. We also recommend that all mortality observations be carried out in November or later.

Coastal Prairies, Velvet Grass, and the Oregon Silverspot Butterfly

The Nestucca Bay National Wildlife Refuge (NBNWR) is the largest of Oregon's six wildlife refuges. Founded in 1991, the then 370 acres of short grass pastures along the Nestucca and Little Nestucca Rivers provided wintering habitat for white-cheeked geese. Now at just over 1,200 acres, the refuge hosts a mosaic of ecosystems ranging from marshland, coastal bog, forested wetlands and upland meadow. These ecosystems form a diverse habitat of native plants and animals. *(U.S. Fish and Wildlife, n.d.).* Most importantly, the prairie is home to the Oregon Silverspot Butterfly (*Speyeria zerene hippolyta*), a federally threatened butterfly species. The butterfly, whose population is incredibly sensitive and in decline, can only be found in five locations throughout the world, one being the Nestucca Bay National Wildlife Refuge. *(Hays & Stinston, 2019)* In 2017, the Oregon Silverspot Butterfly was reintroduced to the refuge after facing extinction in the 1970's (*U.S. Fish and Wildlife, 2018).* The Early Blue Violet, *Viola adunca,* plays a vital role in the Oregon Silverspot's life cycle; it acts as a place for female butterflies to lay their eggs and as a food source for the caterpillar. Although Early blue violets aren't rare, the coastal prairies they grow in within the Oregon Silverspot's range are diminishing, leading to a decline in suitable butterfly habitat *(Oregonshores.org,2015).* Currently less than 1% of Oregon's coastal prairies still exist due to fire suppression, the establishment of invasive species and residential development *(U.S. Fish and Wildlife, n.d.).* The main focus of our study and one of the main issues facing the NBNWR prairie ecosystem is the establishment of invasive grasses such as Velvet Grass, *Holcus lanatus;* and their indirect effect on the endangered Oregon Silverspot Butterfly by pushing out its native host plant. Site managers are continually developing the best practice for managing the invasive grass populations through prescribed burns and the use of herbicides. Experiments have shown that the combination of multiple control treatments can


Photo via Friends of Kananaskis Country


Photo via Woodland Park Zoo Blog

achieve the best results for invasive plant management. *"the 2000 survey revealed that, mimosa did not increase in plots treated with repeat herbicide applications or combined herbicide and bulldozing treatment as well as fire…the timing of the fire treatment ensured there was insufficient time for seedlings germinating… which can favour biological control."* (Paynter & Flanagan, 2004*)*. The main concern regarding herbicide application within the prairie is its overall effects on the threatened Oregon Silverspot Butterfly, which relies on the early blue violet in order to reproduce. As well as negatively affecting the plant it needs to survive, herbicides can directly harm the butterfly through direct physical contact.

The goal of this study was to understand the concentration of Fusilade and point in the Velvet Grass's lifecycle that prove most effective at decreasing the grass.We were also interested in changes that could be made to herbicide application in order to minimize the effects on non-target species.

Herbicide Application Study and Monitoring

Study Location

This study was conducted on a mostly uniform hillside in the coastal prairie of the Nestucca Bay National Wildlife Refuge. The prairie is a part of the coastal upland ecoregion categorized as a location adjacent the coastline and lowlands. These prairies are interspersed with the upland forests present within the wildlife refuge. The climate is characterized by warm dry summers and moderate wet winters. Historic average temperatures in the area range from 55℉ to 60℉, while average monthly precipitation ranges from 2 to 4 inches in the summer months. Winter temperatures range from 38 ℉ to 41℉, and average precipitation ranges from 12 to 13 inches. *(Oregon Department of Environmental quality, 2002)*. Specifics of the area show wetter summers along the coast than farther inland, due to the continuous presence of fog, providing moisture. The lack of vegetal moisture stress and seasonal extremes benefits the native grasses as well as the invasive vegetation.

Herbicide Application

The study site consists of a 55 meter by 96 meter plot on the coastal prairie hillside of the Nestucca Bay National Wildlife Refuge. The plot is divided into 6 transects, with a 4 meter buffer zone on all sides of each transect (Figure 1). These transects are divided by high or low treatment concentrations with corresponding application intervals during early or late spring as shown in Figure 1.

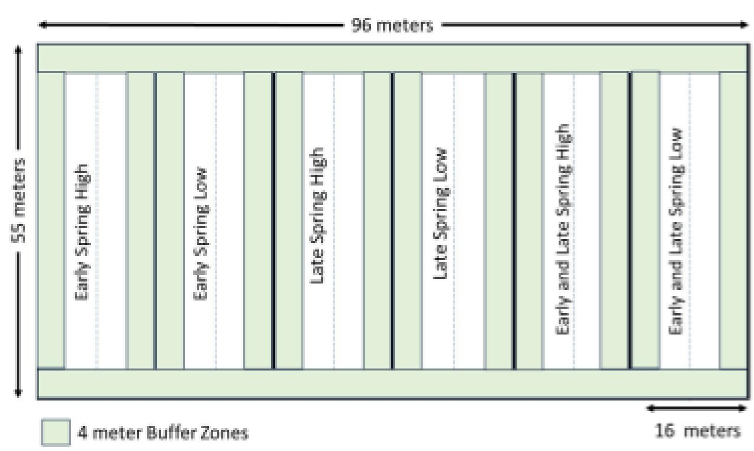


Figure 1. Illustrates the study site for Fusilade DX treatment in the Nestucca Bay Wildlife Refuge coastal prairie. Six treatment plots of early and late spring application with high concentrations of 0.5 oz per gallon of Fusilade DX, and low concentrations of 0.25 oz per gallon of Fusilade DX. Each treatment includes the surfactant NuFilm, and blue dye.

Twenty individuals of a single *H. lanatus* were chosen at random (twice as many as needed) within each of the transects for all the treatments, leaving a 4 meter buffer width to account for overspray or drift. Each *H. lanatus* plant was tagged with a plate by a stake near the single plant, with the treatment and treatment number, and yellow flag for monitoring access. To avoid disturbance by the stake, each stake was driven as close to the specimen as possible without disturbing the foliage or roots system.

Figure 2. Plant marker with a tag identifying marker number and plot location of H. Lanatus at the Nestucca Wildlife Refuge coastal prairie fusilade treatment site.

Application of the Fusilade needed to be on a low wind day after a dry period. This was to help reduce overspray/patch spraying, ensure the mixture could adhere to the plants and better adsorption; as well as to reduce the buffer zone to ensure size of each transect.Early spring applications were applied on the 5th of March, and late spring applications were applied on the 30th of March.

Since high concentration rates have the possibility of killing the upper structure of the plant, low concentrations are also applied to the experimental plots. The herbicide mixture was set for the high treatments to be 0.24 oz per gallon of Fusilade. and low treatments to be 0.10 oz per gallon of Fusilade. High treatments are the maximum allowed by the label and low treatments are the manufacturer's recommendation for perennial grasses. Added was the surfactant NuFilm at the max rate as set by the label to reduce the likelihood of herbicide perching on the hairy blades of *H. lanatus* from surface tension. A blue dye for observation of spray coverage was also added to the mixture. The boomless sprayer was calibrated and set for the lowest droplet size, and each transect was applied down the center line to reduce the spray bleeding past the buffer zone, effectively covering a 16.76 meter spray width.

Although herbicide application was done on days with light wind, the technique and droplet side can allow for greater drift. In some cases wind direction is to be blamed for overspray; air moisture should also be noted as the herbicide absorption can be affected depending on the relative humidity.

Figure 3. Researchers monitoring H. lanatus herbicide treatment plots at the Nestucca Bay Wildlife Refuge coastal prairie restoration site. Researchers from left to right are, David Thompson, Ariel Myton, and Haley Cohn.

Data Collection

We observed the plant condition to gather data on the effect of the Fusilade spray. All the data was collected from each of the six transects on the same day. We started at the bottom of the hillside of the study site and went up and down each of the treatments, observing the effects on the 20 marked plants (for some treatments, we were unable to find all 20).With each tag found, we noted the number and treatment applied, we also took note of the plant stress levels—if it appeared alive or dead, had some browning or was growing fine—as well as plant size. In some cases, the tagged plant would be completely gone but there were possible offshoots of a new plant. Due to the choice to include twice as many sample plants as necessary within each plot, small complications such as not locating all plants, did not render the data irrelevant.

Data Analysis Methodology

Overall plot data collected was collated by marker number, treatment, stress and size. All data collection was qualitative in the field, with each marked plant observed to have "a bit of stress", "some stress", "a lot of stress", "dead" or "none". For analysis each marker observation was given a percentage and evaluated for biological or ecological trends by treatment transect. "No stress" was the lowest ranking given a value of 0%, this was characterized by a healthy green plant, "a bit of stress" was given a value of 25% this was characterized by a plant that was mostly green but did have some browning.The other categories were "some stress" which was characterized by half healthy and browning and a was given a value of 50%; and "a lot of stress" was a plant where more than half of its blades had browned and was given a value of 75%, and "dead" was a plant that was all brown and dead or completely gone and was given a value of 100%. All figures were produced in R using "vegan" packages.

Findings From the Study

Timing and application

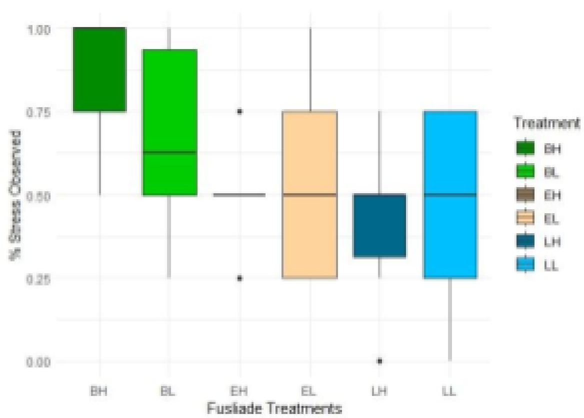


Figure 4. Box and whisker plots of fusilade dx treatments during the November survey. Box and whisker plots show median values with upper and lower quartiles. Whiskers indicate minimum and maximum values, while dots represent outliers. Treatment plots are; bothe and late spring treatment with high concentrations (BH), both early spring and late spring application with low concentrations (BL), early spring high concentrations (EH), early spring low concentrations (EL), late spring high concentrations (LH), and late spring low concentrations (LL). All single treatment applications show the greatest survival in November, while both double treatment applications showing lower survival. In both double treatment plots of high and low concentrations survival decreases in variation as concentrations increase.

Analysis indicated high concentrations of Fusilade in both early spring and late spring showed the greatest *H. lanatus* mortality with ten of the eighteen observed species mortality events by early November. Of the remaining eight, all were observed to have some or more apparent stress. Lower concentrations applied over the same period caused some plant mortality with 5 of 18 individual mortality events while the remaining 13 show variations in observed stress(figure 4).

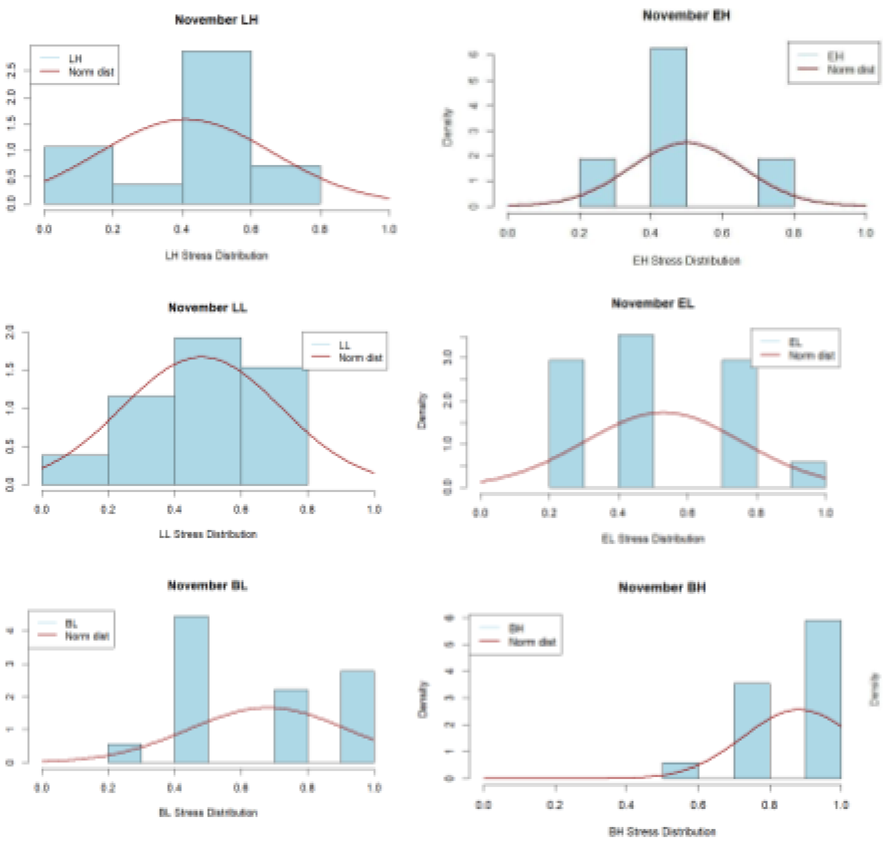
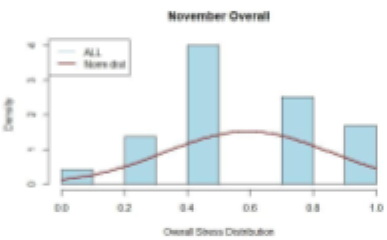


Figure 2. Normal density distribution plots over the study period for % stress in each treatment. All treatments show normal distributions. Both early and late spring show population shift toward greater stress. Over all treatments, stress distribution is normal. The curve represents probability density, width represents the standard standard deviation from the mean. (LH n=17, EH n=18, LL n=14, EL n =16, BL n=18, BH n = 18 November Overall = 96).



While all other treatments show some stress, no other mortality events were observed in the study. However, the early high treatments and the late high treatments show less variation than their low concentration counterparts (figure 4 & 5). Early high treatments show no variation with 10 plants showing "some" stress, 3 plants having "lots" of stress and 3 having "a bit" of stress. Utilizing R to generate a normal distribution plot shows normal distribution throughout the study. Evaluating height of the density bins, and distribution from 0 to 1 of the distribution probability plot among the treatments, we can see how likely a subsample may occur in the treatment. All single treatments of both high and low concentration are normally distributed around 0.5. However, treatment populations of high and low concentrations shift the observed stress toward higher stress levels of 0.75 in low concentrations and 0.9 in high concentration treatments. Average percent survival similarly shows high survival rates in late and early applications, while repeated applications have lower survival rates (figure 5) .

Table 1. Average percent survival rates per Fusilade DX treatment timing and concentration plots over the summer and fall of 2023. All treatment applications show some survivors throughout the study period.

Average Percent Survival Over the Study Period			
	Average	min	max
Late spring low concentration	100%	100%	100%
Late spring high concentration	100%	100%	100%
Early spring low Concentration	100%	100%	100%
Early spring high concentration	92%	75%	100%
Both early and late spring low concentrations	88%	62%	100%
Both early and late spring high concentrations	59%	17%	100%

Discussion

Although we cannot infer any statistical significance as that would be pseudoreplication, a pattern in the data does appear. Timing of the application of Fusilade in the study site in either late or early spring was observed to have little effect on plant mortality over the entire study period. Conversely, the combination of late and early spring treatments had a greater effect on mortality whether applied at high or low concentrations. This would indicate that treatment timing is not the most important factor, mortality in plots with both application times regardless of Fusilade concentration was greater than single application of high or low concentration. This shows that repeated application further stresses *H. lanatus* throughout the growing season, leading to greater plant mortality in late fall as illustrated in figure 2. The seasonal trend in mortality events shows that mortality observations should be carried out later than August and have been shown to be greatest in November.

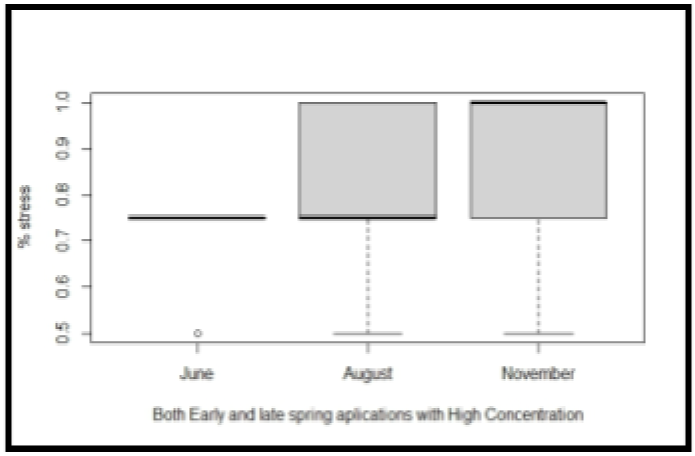


Figure 6. Box and whisker plots showing percent observed stress of both early and late spring treatments with high concentration applications of 0.5 oz per gallon of fusilade. Boxes show the median value with upper and lower quartiles, while whiskers show minimum and maximum values. Circles indicate outliers in data. Over the course of the study period, observed stress increased throughout the growing season leading to plant mortality in August and November.

Timing

Herbicide timing applications in butterfly restoration sites have been shown to be the most effective outside of larval emergence throughout diapause (Russel et al., 2010; Glaeser & Schultz, 2014). Spring herbicide label recommendations often coincide with larval emergence, increasing the likelihood of herbicide interactions. When considering timing, chemical degradation intervals of at least one month should be considered to break down herbicides. (Russel et. al, 2010).

Seed bank considerations

Small plants were the most often questionable resprouting events. Determining the impact of resprouting was an overall challenge throughout the study. Difficulty in observing differences in small resprouting plants and the original marked plantings created difficulties in differentiation of clustered groups of *H. lanatus*. Small observed plants without any stress may have been resprouts, indicating top kill without impacting the root systems. Adjacent plots with higher stress and mortality may also influence adjacent *H. lanatus,* such as seeding events and density dependent colonization efforts.

Monitoring strategies such as bordering the original plant with a small pin during observation to help identify previous size or stress over time in the field. Attributing the sub-samples to a compass azimuth to the sample centerpoint may also relieve some complication without interference. While these strategies can reduce complications, a garden study could reduce environmental and design variability by reducing interference and spatial environmental complications.

Future research and management considerations
Although we did see plant mortality, every treatment plot witnessed survivors. Whether these live plants were from resprouting and seeding events, or overall survival, none of the treatments were one-hundred percent effective. But, repeated spraying efforts did show further progress toward some suppression. Since plant stress was observed to increase during the growing season and into fall, a third application in the fall could be warranted for successive studies. Integrated management studies in the Clatsop Plains, and the Thurston County South Puget Sound Prairie lands have also shown burn sites or topsoil removal reduce survival of non-native species in their respective habitat systems (Muzychko 2023; Petix et al., 2018; Hamman, 2011). Burn sites in the late fall may eliminate the need for successive fall herbicide application while effectively utilizing traditional ecological knowledge in conjunction with conventional pesticide-based management.

Trends in herbicide application timing provide avenues for further research. If timing is not a factor, and both early and late low concentrations show similar stress, we pose a question; If herbicide shows some stress from repeated application, what is the best time to spray? Since seed production is of great importance in the management of *H. lanatus*, when would it be most effective to suppress seeding? Furthermore, since *H. lanatus* is limited to approximately ten percent coverage, this site can benefit from spot spraying, rather than broadcast spraying. This approach could lend to more effective management and minimize the unnecessary use of herbicides while accommodating further research. Spot spraying can incorporate experimental designs with smaller plot size and replication allowing for the inference of statistically significant findings. This approach has the benefits of reducing any edge effects in spraying application or ecologic interference, and allowing for easier monitoring of seed harvest studies. Overall future research combined with seed head collection monitoring could give a greater understanding into management of *H. lanatus.*

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