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MS ENVIRONMENTAL BIOLOGY CAPSTONE PROJECT

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

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CHAPTER 1. LITERATURE REVIEW

Invasive Plant Considerations in Alpine Restoration Project Planning

Restoration project managers and land managers are increasingly concerned about invasive plant species. Next to habitat destruction, invasive species most jeopardize biodiversity (Sheley, James, Rinella, Blumenthal, & Ditomaso, 2011). An invasion refers to the uncontrolled distribution of a plant species outside of its native range and is likely to cause harmful unintended consequences (Beck et al., 2008). Non-native plant invasions can have severe ecological consequences and should be managed to minimize impacts (Hanley & Roberts, 2019). Restoration is crucial in supporting ecological function and biodiversity of native habitats (Funk, Cleland, Suding, & Zavaleta, 2008). Restoration projects come from the need to recover a damaged ecosystem from some type of disturbance (Clewell & Aronson, 2013). These disturbances can either be unintended, such as fire or flood damage, or intended, such as an attempt to control invasive species (Brown et al., 2008). Restoring habitats that have been invaded by non-native species is often unsuccessful and can be economically wasteful (Davies & Johnson, 2011). Considering the amount of effort and resources that go into restoration projects, it is important to understand how these species affect the success of a completed restoration project. There have not been many studies regarding invasive species in the alpine or their effects on alpine restoration projects. My purpose is to fill the knowledge gap between these specific areas of study in order to inform restoration project managers and land managers. In addition, more research is needed to determine how invasive plant species affect the success of an alpine restoration project and managers need to weigh the costs and benefits of using invasive species in their restoration plan, such as the conditions of the restoration site or consequences of non-native species becoming invasive.

Early successional plants are the first species to establish themselves after a disturbance and are the plants that most commonly become invasive (Brandon, Gibson, & Middleton, 2004; Johnson, Litvaitis, Lee, & Frey, 2006). They are typically characterized by having a high seed output, high ability to disperse seeds, high tolerance to stress, and fast growth (Huston & Smith, 1987). In this literature review, I will explore how invasive plant species can influence the recovery of a recently disturbed alpine habitat.

Alpine ecosystems are some of the most difficult to restore after a disturbance has occurred (Chambers, 1997). The alpine environment is defined as a mountainous area higher in altitude than timberline. Alpine ecosystems are generally characterized as having rocky soils, low atmospheric pressure, low temperatures, and short and unpredictable growing seasons (Billings, 1974). With this harsh environment, plants must have special adaptations that allow them to survive in these conditions, and successful alpine restoration projects are, therefore, difficult to achieve. There might be low survival rates when reseeding or planting plugs grown off-site because of the shortened growing season or lack of protection from cold winds by more established plants. Frost also limits reproductive success of plant species (Marcante, Sierra-Almeida, Spindelböck, Erschbamer, & Neuner, 2012). Alpine plants typically require little water and get most of their water from the spring snowmelt. To increase plant establishment success, restoration techniques include using mulch or erosion control matting as cover to protect plants as they become established (Colorado Department of Natural Resources, 1998). It is, therefore, reasonable to assume that seedlings might have a higher survival rate when they are sheltered by more established plants, rocks, or snow drifts.

Invasive plant species are aggressive competitors of native plant species (Moles, Gruber, & Bosner, 2008). The likelihood of a plant species becoming invasive at a restoration site is determined by the specific characteristics of that species and the lack of a native seedbank. Before invasive species enter an ecosystem, unoccupied areas are most likely filled by neighboring native species that have similar traits to those species that previously occupied the space. In an ecosystem containing invasive species, there is greater potential for non-native invasive species to occupy the vacant area (Moles et al., 2008). Due to the high fecundity of invasive species, these plants likely act as early successional species in a recently disturbed or unoccupied area. If invasives establish early, the competitive traits of these species, such as high seed output, rapid growth (Huston & Smith, 1987), and early establishment (Brandon et al., 2004; Johnson et al., 2006) might not allow for native species to colonize a disturbed area. In an alpine ecosystem, native plants are pitted against short growing seasons, harsh temperature changes, and erosion (Andel & Aronson, 2006). It is crucial for managers to understand that invasive species could additionally constrain native species growth in alpine sites. Non-native species in a landscape may become invasive; however, not all non-native species will become invasive in every environment. Having a native seedbank in a disturbed area is important to control for the competition of invasive species. Areas without an established native seedbank do not recover as quickly as areas with a large seedbank present (Erskine Ogden & Rejmánek, 2005). It is important for restoration project managers to take into consideration both the traits of neighboring invasive species and also determine the presence of a native seed bank at the restoration site in order to ensure the success of their project. If a non-native species does not have the potential to become invasive in a restoration site, then it might be of little concern to managers.

If not monitored and controlled during and after the restoration project, the characteristics of non-native species make them likely to establish with little competition from native species and have the potential to become numerically dominant in a newly restored area (Erskine Ogden & Rejmánek, 2005). Invasive species can be detrimental to newly restored areas because they establish quickly and the more mature non-native plants often outcompete later germinating native seedlings. If native seedlings must compete with older, invasive plants in this delicate stage of growth, then they are more susceptible to dying leaving only the invasive species in the restoration area (Huston & Smith, 1987). Restorationists and land managers should consider this if they are thinking about introducing invasive species in their restoration plan, or if a large number of invasive species already exist at their restoration site.

Non-native invasive species might have some benefit to restoration-project managers if they are able to act as nurse plants for developing native seedlings. The nurse effect is described as abiotic or biotic material providing protection for establishing vegetation (Roberts & Seastedt, 2019). Nurse plant syndrome is when a young plant is more successful due to the proximity of an established adult plant. Adult nurse plants may provide shade for vulnerable young seedlings, and may protect them from harsh wind and predators (Padilla & Pugnaire, 2006). Restoration-project managers sometimes incorporate older plants in the form of plugs into their restoration plan rather than rely solely on reseeding. Nurse plants often increase the establishment success and species richness in harsh environmental conditions (Badano, Bustamante, Villarroel, Marquet, & Cavieres, 2015). Seeds that end up near already established plants or larger plants that are planted during the restoration project may have a higher chance of survival, which might increase the overall success of seedling reestablishment in a restoration area. Therefore, restoration-project managers could include already established non-native plants at an alpine restoration site. It is possible that these adult plants could protect young seedlings which is critical to reestablishment of native species in a harsh alpine habitat. Managers should weigh the costs and benefits of using non-native species as nurse plants because if they outcompete the young native plants, then they might cause more harm to the project than benefit.

Using non-native species might not have significant negative effects on an alpine restoration project. If these species do not become invasive, they could increase the overall biodiversity of the site and provide shelter that allows native species establishment. These fast-establishing species might also stabilize soil that is susceptible to erosion until native species are able to develop to provide the same service (Vince, 2011). This might help quickly control soil erosion for alpine projects in which the main concern from managers is increased erosion due to lack of vegetative cover. Since invasive species have traits that allow them to establish and reproduce quickly (Moles et al., 2008), they could be useful in this situation because they could quickly cover an area, stabilize soils, and prevent further damage to the alpine restoration site from erosion. Restoration project managers could incorporate this consideration into their project plan because the quickly establishing plants could save them money and time that would otherwise be spent restoring a larger and more degraded site.

Managers should keep in mind some potential future implications of introducing invasive species to an alpine restoration project. With alpine temperatures increasing globally, the urgency of invasive species management in the alpine is increasing. The temperature increase has the potential to allow many species to become invasive in areas that they would not normally be able to survive the harsh alpine conditions (Becker, Dietz, Billeter, Buschmann, & Edwards, 2005). Managers should consider any climate changes that may occur at the site after the restoration project is complete before making species-introduction decisions. If they choose to incorporate non-natives into their project plan or leave pre-existing non-native species, they should consider the potential of those species becoming invasive if there are any expected environmental changes that allow the species to better adapt to the new conditions and move up the altitudinal gradient.

Another consideration for restoration-project managers is the site's recreation potential. Human recreation is anticipated to rise within the alpine environment (Evju, Hagen, & Hofgaard, 2012) probably due to increased access to once difficult to reach areas and increased outdoor-based tourism activities. An increase in human activity can increase non-native plant species along the altitudinal gradient (Bear, Hill, & Pickering, 2006). Invasion from non-native species is dependent on the density of humans occupying an area (Marini, Gaston, Prosser, & Hulme, 2009). The more visitors that an alpine site receives, the higher the chance for non-native species to occur and consequently, non-native plant invasions. Growing human populations and more accessibility to alpine environments is a concern for restoration-project managers and invasive-species managers. Planning for these changes is essential for the success of alpine restoration projects.

With an increase in recreation in sensitive alpine areas, public education is essential in determining the success of restoration projects and possibly reducing the need for some projects altogether. Restoration project managers that use volunteers to complete restoration work could also benefit the community of invasive weed managers by promoting education about the concerns discussed in this paper. Using volunteers can mitigate the costs of invasive-species removal if restoration managers choose to manage non-native invasive species on the project site. Involving the public in projects increases awareness of invasive species issues and can help to gain public support (Daab & Flint, 2010), which might increase funding for projects as well as their success. Increasing public participation in projects is a positive way for restoration-project managers and land managers to involve public stakeholders in the restoration of areas for their own recreational benefit.

There are many considerations for restoration-project managers and land managers regarding invasive species in alpine restoration projects. When developing a restoration project plan, managers need to weigh the costs and benefits of either incorporating potentially invasive species into the project plan or allowing existing non-natives before starting the project. Managers should also consider the conditions of their restoration site and choose a plan that works best for their site. These decisions need to be made by considering the potential consequences of the non-native species becoming persistently invasive. If a restoration site has a history of invasions, high potential for neighboring species to become invasive, or rare and sensitive native species present, then managers should take measures to exclude non-native plant species from their project plan. If a project site does not have a high potential for invasion, then managers can incorporate non-native species to increase the success of acquiring native vegetation or if the non-natives are determined to be nonthreatening and the removal would be too costly. A need for more research still exists to determine the impacts of invasive species on alpine restoration sites and how invasive species impact the community composition of alpine habitats; but the effects of invasive species on other restoration projects can, at minimum, provide managers with general guidelines on whether or not to incorporate non-natives into their alpine restoration projects.

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CHAPTER 2. GRANT PROPOSAL

Success Rates of Native See Germination on Esplanade-Treated Plots

Abstract

Cheatgrass (*Bromus tectorum*) is a winter annual invasive weed species that increases fire frequency on the landscape. Controlling cheatgrass with typical weed control methods can be difficult. Prescribed burning can exacerbate the abundance of cheatgrass and mechanical removal is often ineffective due to an existing soil seedbank. Boulder County Parks and Open Space (BCPOS) is interested in using Esplanade, a newly developed herbicide, for cheatgrass control on their properties, but its effects on native seed germination are unknown. This study will use two methods to determine how germination success rates vary with different seed mix applications and with different Esplanade application rates. The first method will compare native seed germination across plots treated with different species mixes of native seeds after Esplanade has been applied. The second method will compare native seed germination across plots treated with different application rates of Esplanade. The results of this experiment will provide BCPOS and other land managers with information about Esplanade's effectiveness for cheatgrass control and how the herbicide impacts germination of native seed species that are applied after herbicide application.

Introduction

Objectives

I propose to evaluate the germination success rate of native grass and forb seeds on Esplanade-treated plots with the purpose of providing Boulder County Parks and Open Space (BCPOS) with valuable guidelines for further invasive weed management and restoration. My aim is to conduct a field experiment comparing multiple vegetation plots treated with Esplanade herbicide and then restored using various combinations of native grass and forb seed mixes. The goal of this study is to determine the best practice for restoring herbicide-treated areas with native vegetation.

Questions and Hypotheses

Question 1: How does the germination success rate differ across various seed species mixes in Esplanade-treated plots?

Hypothesis 1: Seed mixes containing a higher percentage of native perennial grass species will contribute to a higher germination success rate in Esplanade-treated plots because Esplanade is designed to inhibit annual grass and forb emergence.

Question 2: How does the application rate of Esplanade affect the germination success rate of native grass and forb seed application?

Hypothesis 2: Plots with higher rates of Esplanade application will experience a lower germination success rate of native species than in plots treated with lower rates of Esplanade application because more seedlings will be inhibited by the increased amount of herbicide applied.

Anticipated Value

In accordance with the Boulder County Noxious Weed Management Plan, BCPOS needs to assess the effectiveness of various weed management strategies for controlling undesired plant species and promoting desired species. Noxious weeds present a threat to the economic and environmental value of BCPOS lands. With the goal of employing the least environmentally damaging control method, it is important for BCPOS to know how Esplanade herbicide affects seed germination rates of native grasses and fobs. This will ensure that control methods and post-control restoration of treated areas is as successful as possible.

Literature Review

Because invasive plant species can negatively impact the economic and ecological value of the environment (Hanley & Roberts, 2019), it is important for land managers to control these species. When using herbicides, managers need to consider their effects on non-target species. After an area has been treated for invasive species, it is common for managers to restore the treated areas by reseeding with desired native species. Managers most often use a seed mix of only native grasses and rarely a combination of grasses, forbs, and shrubs (Barr, Jonas, & Paschke, 2017) probably due to the high costs of species-rich seed mixtures.

Esplanade herbicide is a relatively new herbicide on the market (Sebastian, Sebastian, Nissen, & Beck, 2016) and it is critical for managers to understand how it can affect the success of post-treatment restoration by seeding. This herbicide works by hindering seedlings from extending their roots (Sebastian et al., 2016). Esplanade controls pre-emergent annual grasses and broadleaf plant species and persists in the soil for up to eight months (Bayer Environmental Science, n.d.). Since Esplanade is not effective on post-emergent species or dormant seeds, it can be used only to address the germinating seedbank of undesired non-native species. However, if managers are interested in restoring an area that has been treated for invasive species by reseeding with native seed mixes, they should understand how Esplanade may inhibit the seed germination of desirable native species. Since some desirable native species of BCPOS are considered to be tolerant of Esplanade, such as blue grama (*Bouteloua gracilis*), fringed sage (*Artemisia frigida*), and prickly pear (*Opuntia* spp.), it is reasonable to assume that seed mixes containing these tolerant species will have higher germination rates after herbicide application and seeding restoration. This study will focus on a site that is high in cheatgrass (*Bromus tectorum*) abundance. Cheatgrass is an invasive winter annual grass that increases fire frequency. This increased fire frequency can lower the abundance of native species, especially perennials and shrubs, and can decrease species diversity (Reid, Goodrich, & Bowns, 2008). Due to its severe ecological impacts, cheatgrass is an increasing concern for managers along the Front Range.

Current restoration practices often involve using seeding mixes with five to 50 different species (Barr et al., 2017). Seed mixes consisting of many species that are more densely applied produce a higher percent cover and higher species richness of native plant species than seed mixes with fewer species (Carter & Blair, 2012). For grassland restoration projects, the optimal seed mix diversity is 35 species at a rate of 1,366 pure live seed per m^2 (Barr et al., 2017). A seed mix with a higher seed species richness more successfully resists invasion of non-native species than applying a seed mix with lower species richness at a higher density (Nemec, Allen, Helzer, & Wedin, 2013). Seed mixes that have a combination of different species, either various grasses or both grasses and forbs, are more likely to have higher germination success rates (Tinsley, Simmons, & Windhager, 2006).

Varying Esplanade application rates during treatment can have different germination success rates post-restoration. A high application rate between 5 to 7 fluid ounces per acre during herbicide treatment will negatively impact the seedling germination post-treatment (Bayer Environmental Science, n.d.). A lower application rate between 3.5 to 5 fluid ounces per acre will least affect seedling germination of non-tolerant species; however, the control of invasive species will not be as effective (Bayer Environmental Science, n.d.). Thus, it is crucial for managers to determine the application rate that will most effectively control for non-native species, while also being least harmful to native seed germination post-restoration.

Methods

I will conduct my research at Walker Ranch, near the southeast corner of the property (Figure 3). This site was chosen due to the high abundance of cheatgrass and the far distance from trails and water to minimize the impact of herbicide use to the surrounding environment and to the public. To address both research questions, I will set 16 quarter-acre plots and each treatment will be randomly assigned to a plot in order to minimize the effects of plot proximity. I will quantify the plant species composition pre-treatment by randomly choosing 30 one-meter quadrats within each plot. In each quadrat I will estimate percent cover of each species. Then, I will mow the plots with a bagging mower so that there are no residual seeds from the present plant species.

Question 1 Treatment: In eight quarter-acre plots, I will spray each plot at an application rate of five fluid ounces per acre of Esplanade. Then, I will apply seeding mixtures with the lowest seeding diversity of five species and will increase the treatment of each plot by five species until the last plot receives the highest mixture of 40 species (Figure 1).

Question 2 Treatment: In the remaining set of eight quarter-acre plots, I will spray each quarter-acre plot with a pre-determined herbicide application rate. The plot with the lowest application rate will have 3.5 fluid ounces per acre and I will increase the treatment of each plot by 0.5 fluid ounces per acre until the highest plot receives an application rate of 7 fluid ounces per acre (Figure 2). Then I will seed each plot with a seed mixture containing 10 species (70% native grasses and 30% native forbs). This seeding mixture was chosen due to the availability from seed suppliers and to fit the budget requirements.

Sampling After Treatment: I will determine the seed germination rate for both methods by sampling the same 30 one-meter quadrats in the before treatment every month during the growing season (May-September). In these quadrats, I will quantify the percent cover of germinating seedlings and all mature live plants by species.

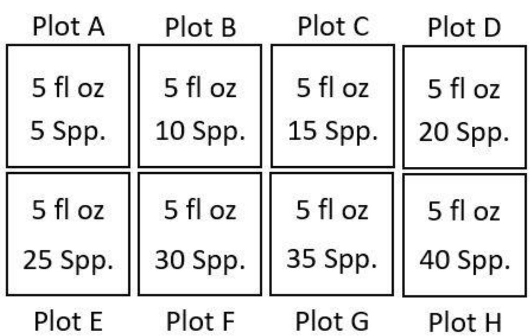

Figure 1. Seed mixture species richness of each quarter-acre plot. Plots will be randomly selected for each treatment type.

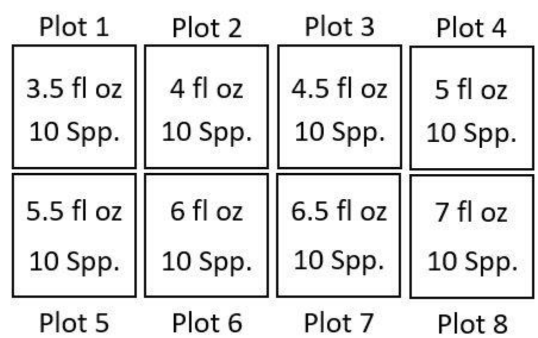

Figure 2. Esplanade herbicide application rate of each quarter-acre plot. Plots will be randomly selected for each treatment type.

Data Analysis: I will use a Pearson's Correlation Coefficient to determine the strength and direction of (1) the relationship between the seed mix diversity and the percent cover of germinated species and (2) the relationship between the Esplanade application rate and the percent cover of germinated species.

To test the before and after treatments of each plot throughout time, I will perform a two-way ANOVA to determine which treatment was the most successful for reducing the presence of cheatgrass and which treatment had the highest germination rate at which point in time after Esplanade treatment.

Project Requirements and Logistics: I will collaborate with BCPOS to gain any required access to open space properties and I will post "herbicide in use" signs to notify the public of any occurring herbicide activities.

Potential Negative Impacts: Possible impacts to natural resources might include negative effects on the native vegetation within the treatment plots; however, the selected area has a very high abundance of cheatgrass and will be reseeded with native vegetation. The possibility of negative impacts is minimal and the results are expected to have a positive influence on native vegetation within the plots.

Timeline

Table 1. Proposed project timeline

Dates (2020)	**Activities**	**Deliverables**
Apr. 27-May 8	• Mark 16 quarter-acre plots • Collect pre-treatment quadrat data	• Raw data & plot coordinates • Before photos of plots
May 20-May 31 (or after last frost)	• Mow and bag present vegetation • Treat plots with Esplanade & seed mix	• Photos immediately after treatment
Jun. 20-Jun. 30	• Collect post-treatment quadrat data	• Raw data • Photos at 1-month growth
Jul. 20-Jul. 30	• Collect post-treatment quadrat data • Begin first draft of report	• Raw data & first report draft • Photos at 2-months growth
Aug. 20-Aug. 30	• Collect post-treatment quadrat data	• Raw data • Photos at 3-months growth
Sept. 20-Sept. 30	• Collect post-treatment quadrat data	• Raw data • Photos at 4-months growth
Oct. 1-Oct. 15	• Data analysis	• Data analysis for H1 & H2
Oct. 15-Oct. 31	• Edit, revise, and finalize report	• Final report & data analysis

Budget

Table 2. Proposed project budget

Item	**Justification**	**Unit Cost (Source)**	**Quantity**	**Total Cost**
Esplanade 200 SC Herbicide	Treatment of plots	$400/quart (ChemicalWarehouse.com)	1	$400
Bagging Mower	Vegetation removal	Provided by BCPOS	1	$0
Backpack Herbicide Sprayer	Apply herbicide for treatment	$96 (Amazon)	2	$192
Gas for vehicles	Trips to site	0.545/mile	560 miles	$305.20
Field Technician Stipend	Stipend for 2 people	$15/hour	60 hours/ person	$1,800
Measuring Wheel	Measure plots	$21 (Amazon)	1	$21
65 Gallon Water Tank	Provide water to mix with herbicides	$150 (Amazon)	1	$150
Seed Mixes	Reseeding of treated plots	Varies per mix (Western Native Seed)	Varies per mix	$4,500
Marking Flags	Marking plot corners	$12 (Amazon)	1	$12
Ratchet Straps	Water tank tie down	$30 (Amazon)	1	$30
Gas for mower	Vegetation removal	$3/gallon	5 gallons	$15
TOTAL PROPOSAL REQUEST				**$7,425.20**

Qualifications of Researchers (See attached resume)

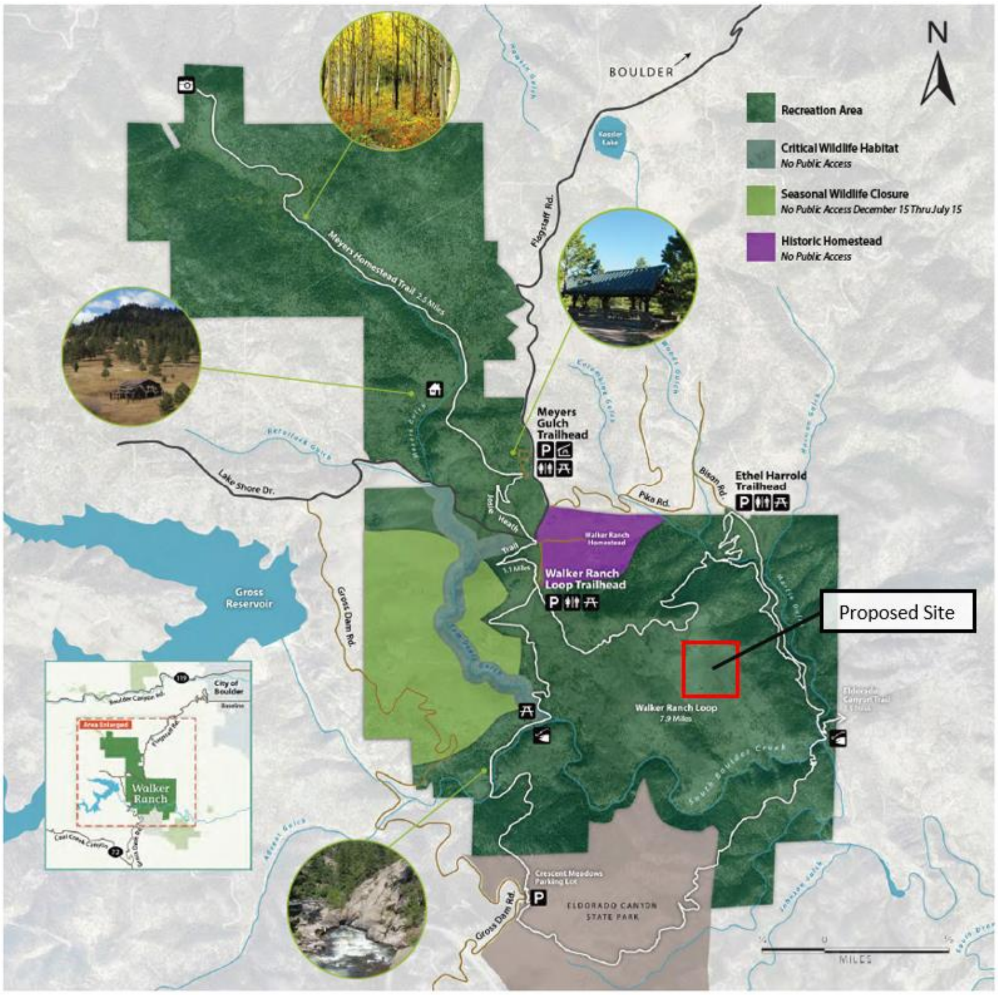

Figure 3. Map of proposed sampling area

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CHAPTER 3. JOURNAL MANUSCRIPT

Meta-Analysis of Cheatgrass (*Bromus Tectorum*) Control with Three Commonly Used Herbicides

Abstract

Cheatgrass (*Bromus tectorum*) is a prolific and aggressive invasive grass species in the western United States. Cheatgrass is a major concern for land managers due to an increased risk of fire and competition between native plant species. Various herbicides have been used to treat cheatgrass to various levels of effectiveness. This meta-analysis examined the effects of three of the most commonly used herbicides; imazapic, glyphosate, and indaziflam. The results of this analysis show that glyphosate best controlled cheatgrass. Additional research following this meta-analysis, should focus on differences in herbicide application timing, application rate, reapplication, and geographic location.

Introduction

Invasive species are of increasing concern to land managers and, next to habitat degradation, invasive species are the largest threat to ecosystem biodiversity (Sheley, James, Rinella, Blumenthal, & Ditomaso, 2011). Non-native plant species are often aggressive competitors with native plant species and can be invasive, quickly progressing as the dominant species in an area (Barak, Fant, Kramer, & Skogen, 2015). An invasion refers to an unrestrained infestation of a plant species outside of its native range (Beck et al., 2008). It is important to note that not all non-native plants are considered invasive, but uncontrolled spreading makes non-native invasions a concern for managers. Restoring areas that have been invaded is often expensive and can be difficult or unsuccessful (Davies & Johnson, 2011). In order to avoid or mitigate the ecological and economic damages caused by non-native plant invasions, managers need to focus resources on invasive species management (Hanley & Roberts, 2019).

Cheatgrass (*Bromus tectorum*) is a winter annual grass originating in southwestern Asia (Menalled, Mangold, Orloff, & Davis, 2017) and has become one of the most aggressive invasive plant species in the United States (Bradley, Curtis, Fusco, Abatzoglou, & Balch, 2017). The first occurrence in North America was discovered in Denver, Colorado and was likely transported in shipping material (Whitson et al., 1992). Cheatgrass spread throughout the western United States as a contaminant in grain seed and continued to spread via railroad and highway transport (Billings, 1994). Cheatgrass was also planted by the United States Department of Agriculture as a hardy grazing species for rangelands that have been degraded (Mealor et al., 2013). Today, in the United States, cheatgrass has been reported in all 50 states ("Cheatgrass (*Bromus tectorum*) - EDDMapS State Distribution - EDDMapS," n.d.). Cheatgrass is often associated with areas of disturbance such as roads, trails, construction areas, or livestock grazing pastures (Reid, Goodrich, & Bowns, 2008).

Cheatgrass is characterized by long, drooping awns on its seeds and turns purple-red in the summer (Mealor et al., 2013). This species matures in the early spring; and due to this trait, it competes for early moisture with desirable native species (Whitson et al., 1992). Drying out in May and June (Billings, 1994), cheatgrass can leave generous expanses of fine fuels that can easily ignite and carry a wildfire quickly (Colorado State University Extension, 2012). The increased fire risk that is associated with cheatgrass leads to a shorter fire return interval and can negatively impact native vegetation by not allowing native shrubs and perennial plants to use the early summer for growth and reproduction (Reid et al., 2008). A single cheatgrass plant can produce up to 500 seeds that may remain viable in the soil for up to three years (Menalled et al., 2017). Because of the shortened fire return interval harming potentially competitive native plants and the other competitive characteristics of cheatgrass, it easily proliferates following a fire (Bradley et al., 2017).

Herbicides are a common method of cheatgrass treatment and are applied either by hand-sprayers or larger vehicle sprayers, such as specially equipped ATVs or tractors. Herbicides are sometimes used in an integrated management approach with other methods of treatment, such as grazing or mowing (Lehnhoff, Rew, Mangold, Seipel, & Ragen, 2019), but often herbicides are used as the only method, with either a single chemical or a tank mix consisting of multiple chemicals. Herbicides are advantageous for cheatgrass treatment because they do not disturb the soil as severely as other methods, they are less labor-intensive, and they offer flexibility in the amount of control (Mealor et al., 2013). The most common herbicides used are imazapic and glyphosate, but after the recent registration of a new herbicide, indaziflam, many management agencies are beginning to incorporate this chemical into their cheatgrass management plans (Sebastian, Fleming, Patterson, Sebastian, & Nissen, 2017).

Plants absorb imazapic through the leaves, stems, and roots which inhibits the synthesis of several amino acids (Mealor et al., 2013). Imazapic can be selective for annual grasses when used at a low application rate and can allow for the growth of more desirable plant species by suppressing competitive invasives (Mangold et al., 2013). Imazapic is widely used for controlling cheatgrass; however, control can be limited by the large soil seedbank that is usually associated with cheatgrass (Ehlert, Mangold, & Engel, 2014). Glyphosate inhibits the synthesis of proteins. Because it is non-selective, it has the potential to harm a wide range of non-target plant species (Mealor et al., 2013). Glyphosate does not exhibit soil residual activity (Kyser, Wilson, Zhang, & Ditomaso, 2013). Indaziflam is a cellulose biosynthesis inhibitor that works by targeting pre-emergent seedlings (Sebastian, Sebastian, Nissen, & Beck, 2016). This herbicide also has a substantial residual effect in the soil after application with a reported soil half-life of greater than 150 days (Brosnan, Breeden, Mccullough, & Henry, 2012).

This meta-analysis will compare imazapic, glyphosate, and indaziflam as control agents and attempt to answer the following question: Which herbicide is the most effective at reducing the presence of cheatgrass? I hypothesize that the most effective method of treatment will be indaziflam because it affects the cheatgrass seedlings prior to their emergence and it continues to affect cheatgrass longer than other treatments due to its soil residual characteristics. If my hypothesis is correct, then I will observe a higher reduction in cheatgrass presence from pre-treatment to post-treatment.

Methods

Literature Search

I used Google Scholar and Academic Search Premier databases to exhaustively search for all papers related to cheatgrass herbicide treatment. I chose to compare only studies that used indaziflam, glyphosate, or imazapic herbicides because of their wide use. I used Boolean phrases to search various terms related to cheatgrass and herbicide. I read through the paper titles to determine if the study fit the scope of my question. I created a database with the papers that were returned in the search and that fit the scope of my question based on my basic search by title. Gray literature was not included in this search, primarily due to the scope of the project. Papers included in the database were further evaluated to determine if the criteria of the meta-analysis were met. These criteria included relevance to the question and reported statistics that could be used to find a Hedges' *d* standardized difference metric. The first search of this study found 38 papers, five papers were immediately excluded from a scan of the abstracts due to lack of relevance to the question, 27 additional papers were excluded upon further reading because they did not meet the criteria stated above, and six were included in the final analysis, with some papers contributing more than one effect size. This literature search resulted in an analysis of nine effect sizes (Table 1).

Data Extraction and Analysis

I fully read the six papers included in this meta-analysis and a single effect size for each herbicide in each study was determined. If a study had a single control and multiple treatments, then I extracted the most extreme treatment in order to simplify the meta-analysis. If a study had multiple years of data, I extracted effect size for the most immediate measurement after the treatment was applied. I performed the analysis in R (version 3.6.2) and I calculated the Hedge's *d* effect size and variance for each different herbicide used in each paper. Then, I analyzed the data using fixed effects models. To account for the heterogeneity between studies, I examined herbicide type as a moderator variable which would explain possible heterogeneity.

Table 1: Literature that was included in the meta-analysis and the herbicides each paper used

Literature Included	Herbicide Used
Brisbin, Thode, Brooks, & Weber, 2013	Imazapic
Burnett & Mealor, 2015	Imazapic
Clark, 2019	Imazapic
Clark, 2019	Indaziflam
Clark, 2019	Glyphosate
Clark, Sebastian, Nissen, & Sebastian, 2019	Indaziflam
Metier, Lehnhoff, Mangold, Rinella, & Rew, 2019	Glyphosate
Morris, Morris, & Surface, 2020	Glyphosate
Owen, Sieg, & Gehring, 2011	Imazapic

Limitations

The interpretations of this meta-analysis are limited by any papers that were not found in my search and the exclusion of gray literature. The specific limitations of each of the individual studies themselves might also be problematic for interpretation. There might be publication bias from papers that were not published due to their negative results or exclusion of papers where statistics were not able to be extracted for analysis.

Results

Glyphosate had a more severe negative effect than both imazapic and indaziflam ($p = 0.03$, Figure 1). After accounting for herbicide type as a moderator, herbicide type is not a significant moderator ($p = 0.34$) and there is still a significant amount of heterogeneity between the studies ($p = 0.0001$, Figure 2).

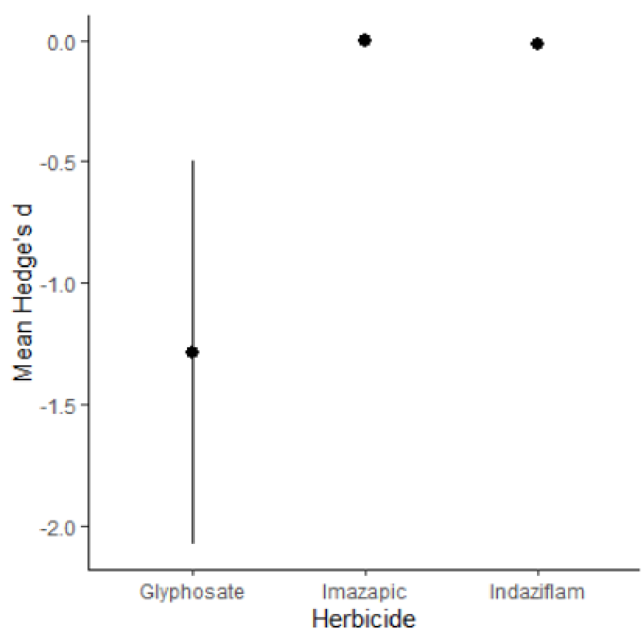

Figure 1: Although all herbicides show a negative effect on cheatgrass, glyphosate was most effective (larger negative effect) at treating cheatgrass across all the examined studies.

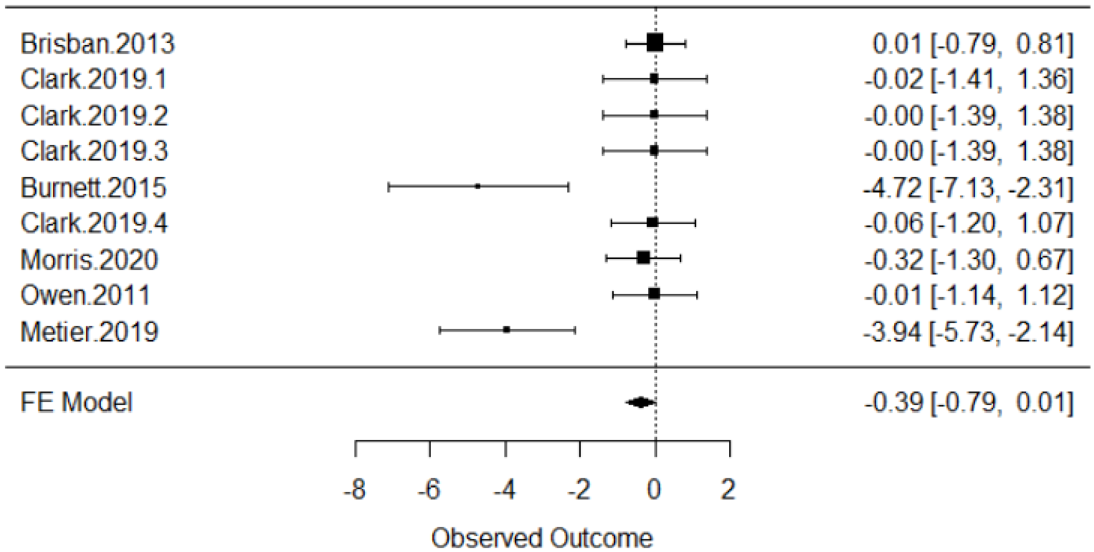

Figure 2: Forest plot showing the effect size of each herbicide used in each study. There was a high amount of variation between the studies.

Discussion

This meta-analysis found that glyphosate best controlled cheatgrass compared to indaziflam and imazapic. There was no significant difference between the effectiveness of indaziflam and imazapic. These results do not support my original hypothesis of indaziflam producing the greatest negative effect on cheatgrass. While the results of this study can help managers to decide if glyphosate is the best treatment for their unique situation, there are many other variables that managers should take into consideration for their management plan. It is not only important to ensure cheatgrass control, but also that the desirable species, such as native forbs and grasses, are not severely damaged by the selected herbicide.

Although this meta-analysis showed that glyphosate resulted in the highest amount of control, it is unknown if any of the herbicides exhibit significant damage to desirable species. If so, managers should evaluate their site characteristics and weigh the costs and benefits of using an herbicide that could potentially cause harm to native species. For example, if a site is dominated by cheatgrass and has very few other species, then it would be logical for managers to use the herbicide that most effectively kills cheatgrass. However, if a site has both cheatgrass and a high cover of native species, then managers should consider using a different herbicide or understand that they might have to spend more effort to restore native species after treatment. Glyphosate is a non-selective herbicide (Mealor et al., 2013) and this study suggests that it has a higher degree of effectiveness over other options. So, for an area that is infested with mostly invasive species, glyphosate might prove the most useful in reducing the cover of those invasive plants. Since glyphosate is a non-selective herbicide, it also has the potential to cause damage to perennial species (S. Clark et al., 2019; Sebastian, Sebastian, et al., 2016). In an area with a higher amount of native perennial species, imazapic might be a better option considering it is more selective for annual grasses, such as cheatgrass (Mangold et al., 2013), and does not persist in the soil which could damage native seeds in the soil seedbank (Ehlert et al., 2014). Unlike imazapic and glyphosate, indaziflam does persist in the soil, but other literature suggests that indaziflam does not reduce the species richness or abundance of perennial species while still providing sufficient control of cheatgrass (S. Clark et al., 2019).

Managers also need to consider the amount of effort and cost associated with specific herbicides. This meta-analysis focused on the most immediate effect after treatment, but if a significant cheatgrass seedbank persists in the soil after an herbicide is applied, then it is likely that the long-term control of cheatgrass will be minimal. Lack of long-term control might lead to higher financial costs and more time spent by applicators in the long run if application is required each growing season. Since neither glyphosate nor imazapic persist in the soil (Ehlert et al., 2014; Kyser et al., 2013), indaziflam might be the best option for this situation. Other literature suggests that glyphosate and imazapic are less effective at controlling cheatgrass than indaziflam over a long period of time (Sebastian et al., 2017). Since indaziflam works by affecting cheatgrass seeds, other literature has shown that it is more effective at depleting the seedbank and thus, exhibiting long-term cheatgrass control (Sebastian, Nissen, Sebastian, & Beck, 2016). Unfortunately, these studies could not be included in this meta-analysis because they did not meet the criteria for inclusion, but I leave recommendations for further research that could help to account for these limitations.

In this meta-analysis, there existed a high amount of heterogeneity between studies and it was not reduced after setting herbicide type as a moderator in the models. In addition to the general effectiveness of herbicides on cheatgrass control, future meta-analyses should focus on analyzing variables such as damage to native or other desired species, frequency of herbicide application, amount of herbicide applied, and herbicide persistence in the soil. Other literature suggests that these variables play a key role in determining the effect size of the herbicide on vegetation. Lower application rates of herbicide can cause a plant population to become tolerant to the herbicide and, subsequently, lower the effect of the herbicide. In contrast, higher application rates can lead to higher amounts of bare ground and it can be difficult for desirable plants to re-establish if too much herbicide resides in the soil (Sebastian, Clark, Nissen, & Lauer, 2019). Some herbicides can have both a high amount of control of invasive species and can consequently damage non-target species, such as native forbs (Morris, Monaco, & Rigby, 2009). Commonly used herbicides also vary widely in the frequency of application needed for adequate control, with some requiring multiple applications per year and others only requiring application once every two years (Sebastian et al., 2017). Other possible reasons for this heterogeneity include geographic location of the studies, time passed between herbicide application and measurement of dependent variable, and differences in the measured dependent variable itself. Further meta-analyses should examine these possible moderators for a wide-range of herbicides in addition to the ones compared in this study.

Due to the high amount of heterogeneity in this meta-analysis that was not explained by herbicide alone, I propose that future research could also be directed toward an experimental study to determine the major causes for differences in effectiveness of the three herbicides examined in this paper. This experiment should examine the effects of the herbicide over a long period of time and could account for the limitations previously discussed. Having a clear indication of the long-term effects of these three herbicides can help managers in choosing the most appropriate option for their specific management goals. I argue that this future study should document not only the impact to cheatgrass cover, but also the overall changes to the entire plant community over several years. This information will be more useful to managers who are not just searching for an herbicide that results in the most immediate effect to cheatgrass, but rather, an herbicide that provides an adequate amount of long-term control and has the ability to best promote the growth of desirable native species.

This meta-analysis suggests that glyphosate is the most effective option for immediate cheatgrass control, however, managers still need to determine if it is the most appropriate for their circumstances. These results are useful when considering the most effective herbicide treatment for cheatgrass at a broad scope. Managers can incorporate these results into their management decisions for herbicide treatment, but decisions should ultimately be based on a community-level response for any selected method of treatment and not herbicide effectiveness alone.

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CHAPTER 4. STAKEHOLDER ANALYSIS

Stakeholder Analysis: Recommendation for Herbicide Use at Lippincott Ranch

Invasive species are the second largest threat to ecosystem biodiversity (Sheley, James, Rinella, Blumenthal, & Ditomaso, 2011). Invasive plant species, commonly referred to as invasive weeds, harm protected areas by outcompeting native plant species and altering ecosystem dynamics (Baret & Strasberg, 2005). Restoring natural areas that have been occupied by invasive plant species can be challenging and costly, so it is critical to prevent their establishment in order to protect native plant communities (Davies & Johnson, 2011). In Colorado, the Colorado Department of Agriculture (CDA) issues a hierarchical list of invasive plants, wherein List A Species are designated for eradication and List B Species are designated for suppression to stop their spread (Colorado Department of Agriculture, 2019). Many of these species, if uncontrolled, can alter fire regimes (Bradley, Curtis, Fusco, Abatzoglou, & Balch, 2017), increase runoff and soil erosion (Lacey, Marlow, & Lane, 1989), and can economically impact agricultural lands by outcompeting desired forage or crop species (Olson, 1999). Invasive plants can also displace native vegetation and decrease biological diversity (Colorado Natural Areas Program, 2000). In addition, communities dominated by invasive plants often provide lower quality habitat for wildlife species than those composed primarily of native plant species (Colorado Natural Areas Program, 2000), thereby contributing to the decline of many endangered species (Pimentel, Zuniga, & Morrison, 2005). Vegetation management efforts of local governments should focus on continued control of these species in order to prevent severe ecological and economic consequences in natural areas.

Lippincott Ranch was jointly purchased in 2018 by Jefferson County Open Space (JCOS) and City of Boulder Open Space and Mountain Parks (OSMP) (Great Outdoors Colorado, 2018) with the purpose of land conservation. This property is located on the county line between Jefferson County and Boulder County, just south of Eldorado Springs, Colorado (Figure 1). The majority of the property lies within the boundaries of Jefferson County. The cost of this 424-acre space was evenly split and the property is co-managed by both agencies (K. Duff, personal communication, March 12, 2020). JCOS and OSMP both represent different populations of stakeholders and since this is the first open space property co-managed by these two agencies, it is important that all stakeholders are considered when developing management plans. Management plans for this property are still being discussed between the two agencies, however, plans for Lippincott Ranch should incorporate the use of a wide-range of herbicides in addition to other methods to control the invasive plant species that are presently found on this newly acquired property.

In general, JCOS and OSMP both use an integrated pest management (IPM) approach for treating invasive vegetation (Jefferson County, n.d.). This approach includes using a combination of mechanical, chemical, and sometimes biological control techniques to meet the state requirements for listed invasive weeds. While they both use IPM, they differ in their policies on herbicide use. OSMP mostly uses mechanical treatments and herbicide use is limited to a specific list of city-approved herbicides (M. Bowes, personal communication, February 7, 2020). In accordance with the City of Boulder Integrated Pest Management Policy, pesticides are only used after all other alternatives have been exhausted. Furthermore, the City of Boulder actively encourages their citizens to eliminate the use of pesticides on private property (City of Boulder, 2020). Although the site is still being assessed for vegetation, various List B Species are

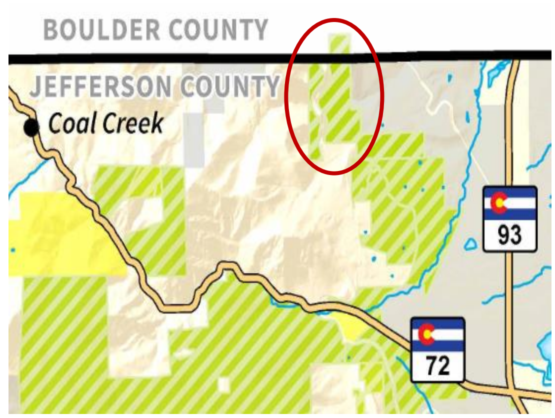

Figure 1: Lippincott Ranch shown in red outline

widespread throughout this property. List A Species may also be present, but their exact prevalence is unknown. The sole use of weed control alternatives, such as mowing or hand-pulling, are not feasible for the size of this property and are not appropriate to control certain species because roots can fracture in the soil and exacerbate the issue (Tu, Hurd, Randall, & Rice, 2001) or a large seedbank can persist in the soil (Sebastian, Sebastian, Nissen, & Beck, 2016) . Although the City of Boulder's policies indicate that only a limited list of herbicides are legal to use within city limits and all city-owned property, special considerations should be given to Lippincott since the cost was evenly split, the majority of the space lies within Jefferson County limits, and the consequences of poorly controlled invasive plant species are greater than the small potential of harm to humans or the environment by herbicide use on this specific property.

The main stakeholders are the populations that both governments represent. While most citizens in the City of Boulder are urban residents, more citizens in Jefferson County live in rural areas. (K. Duff, personal communication, March 12, 2020). OSMP likely avoids herbicide use in response to public concerns for negative health and ecological impacts. Although this property is currently not accessible to the public, future access is likely so it is important to appeal to the concerns of park users. If trails are constructed, these users might include hikers or dog-walkers. At all park access points, the State requires that applicators inform the public of herbicide application and the herbicide in use. The most frequently used herbicides are fast-drying and applied with a blue dye to indicate which plants have been sprayed. Unless there is a complete infestation of a specific plant, applicators will spot-spray only the listed plants. Therefore, it is unlikely that hikers or their dogs will come into contact with the herbicide unless they are recreating off-trail. Weed control on this property will benefit future park users by maintaining a diverse natural landscape that is aesthetically pleasing. Maintaining biodiversity on this property will also support native habitats for wildlife and will maintain wildlife-viewing opportunities for public users. Since the public is informed of herbicide application, they are able to decide whether to continue recreation on any given day of application and whether the benefits received from recreation are greater than the risk of exposure.

Like hiking, other forms of outdoor recreation, such as rock-climbing draw visitors to natural areas like Lippincott. Therefore, a group that should be considered is rock climbers. Within the rock-climbing community, a known spot called "Mickey Mouse Wall" is located outside of but within two kilometers of the boundaries of Lippincott Ranch. It is possible that climbers illegally trespass on the property to access this wall. This is a concern for managers if herbicides are used, because it might be difficult to inform these users of herbicide application. In this situation, a more detailed evaluation of these access points needs to be conducted in order to determine if illegal access through Lippincott Ranch is feasible. If so, managers should make an effort to inform all access points within reason, but inevitably climbers that are illegally trespassing are taking the risk of herbicide exposure.

Whereas future park users could read signage to make informed decisions about whether to risk occasional herbicide exposure, nearby residents would not have the ability to avoid exposure. Most of the land that borders Lippincott consists of conservation easements managed by either JCOS or OSMP, but there are a few residents whose properties are adjacent to Lippincott Ranch. Although relationships between these residents and JCOS and OSMP managers have not yet been established, concern for these stakeholders might include herbicide drift or undesired plant mortality near the property boundary. To mitigate this concern, managers should implement an herbicide application buffer zone around the Lippincott boundary to ensure that herbicides are not accidentally sprayed on residential property. Herbicide drift is unlikely because JCOS and OSMP have adopted widely-accepted protocols that avoid herbicide application during windy conditions. Residents that own adjacent property will benefit from the herbicide applications on Lippincott that reduce the likelihood that invasive species will encroach onto their own property, which they would otherwise be obligated to control themselves. Not only will neighbors obtain benefits of aesthetic value, but they will also reduce the amount of weed maintenance needed on their own properties, since the alternative of limited weed control would likely be costly and labor-intensive.

Herbicide use might also be a concern for wildlife, so organizations that are concerned about negative impacts to wildlife regarding herbicide use, such as the People and Pollinators Action Network or the Colorado Pollinator Network should be considered in management plans. These groups might worry that herbicide use at Lippincott will decrease pollinator abundance or that the chemicals will biomagnify through the food chain to higher trophic levels. Many commonly applied herbicides are considered safe for pollinators by the Environmental Protection Agency (EPA) because they do not contain neonicotinoids which can harm pollinators (United States Environmental Protection Agency, 2019). In addition, applicators are advised to avoid spraying a plant if there are pollinators on it. Although it is impossible to avoid spraying all pollinators, the careful application of herbicides can reduce the risk to pollinators and subsequently to higher trophic levels. Without weed control, many invasive species will ultimately dominate the plant community, decreasing landscape-level biodiversity (Colorado

Natural Areas Program, 2000). The costs of herbicide use to wildlife are insignificant compared to the costs of habitat degradation and loss of biodiversity that would likely result from inadequately controlled weeds.

In addition to terrestrial resources, several drainages and streams run through Lippincott, such as Bull Gulch, Spring Draw, and No Name; therefore, it is crucial to consider stakeholders downstream who may use the water that passes through the property. For these stakeholders, concerns of herbicide use might include pollution of drinking water for people or for animals. To minimize this concern, managers should create a plan for herbicide use near water that is appropriate for the conditions of the area. Some herbicides are considered safe for use up to "water's edge" and some herbicides are considered safe for use on aquatic plant species such as the List A Species, purple loosestrife. Training on proper herbicide usage near water is provided to JCOS and OSMP applicators and will reduce the risk to water contamination. Elimination of all risks to aquatic systems is not entirely possible, but maintaining desired native vegetation through herbicide use will benefit downstream users with improved water quality, increased quantity, and enhanced stream flow (Sheley et al., 2011). When using herbicides near water, there is always a potential for some pollution, however, under appropriate application protocols, the risk is small and is worth the benefits of invasive species control near the waterways.

Similar to waterways, railways are another concern for managers. Union Pacific Railroad which operates a railroad section that divides the east and west ends of the property. Currently, managers have not been granted access by Union Pacific to the east end of the property. This makes it challenging to treat for invasive weeds on the eastern side. Managers should work with Union Pacific to develop a relationship that allows applicators access. Like with homeowners adjacent to Lippincott, it would be beneficial for Union Pacific to allow for this access because it will reduce the amount of vegetation control the railroad is required to perform in order to keep the railroad safely running (Torstensson, 2001).

Perhaps the most at-risk stakeholders in this situation are the herbicide applicators themselves. Since these employees are directly handling the herbicides, they are most threatened by health risks posed by herbicides. These risks can be mitigated by informing the employees of the risks associated with herbicide application and providing proper training for applicators. This training includes knowing how to read herbicide labels and knowing which personal protection equipment (PPE) is required for certain chemicals. It is the responsibility of the employer to provide this PPE and it is the responsibility of the applicator to wear it in accordance with the herbicide label. In addition to protecting themselves from exposure to the herbicide, applicators are responsible for applying the herbicide at the appropriate time, recognizing targeted species, and reducing risks to the public or the environment. For both JCOS and OSMP, employed applicators either work under a CDA-certified Qualified Supervisor or in many cases, obtain their own certification through the CDA. If applicators do not have their own certification, the Qualified Supervisor is responsible for providing the necessary education of herbicide application and associated risks to inform any employees. If applicators are trained properly, then the risk to themselves and the risk to the public and the environment can be greatly reduced.

There are many costs and benefits to invasive vegetation management through the use of herbicide on open space properties. JCOS and OSMP managers should create a management plan for Lippincott Ranch that includes a wide-range of appropriate herbicides for vegetation management. Although OSMP prefers to limit herbicide use, exclusively using weed control alternatives is not practical at this property and is not suitable for maintaining control of invasive vegetation. There is some risk to public and ecological health, but it is minimal under proper herbicide application. The consequences of limited control through alternative methods for listed invasive species is greatly outweighed by the benefits when managing with a large natural area such as Lippincott Ranch.

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