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Leafy spurge is a perennial invasive species that is well-established and difficult to control across North America. Leafy spurge management research has traditionally been performed in upland rangeland habitats, where long-term control requires multiple treatments. Leafy spurge control is more difficult in wet, seasonally flooded areas like riparian edges, since fewer treatment options exist and water provides an additional propagule dispersal vector. The objective of this research was to (1) quantify the impacts of sheep grazing and herbicide applications, alone or in combination, on leafy spurge density and seed production in riparian areas; (2) evaluate germination potential under different temperature and moisture conditions; and (3) quantify vegetative propagule viability in response to duration of submersion. There was no evidence of a synergistic effect of sheep grazing and herbicide applications; however, independent applications of quinclorac and aminopyralid + florpyrauxifen-benzyl caused a reduction in leafy spurge seed production. Leafy spurge seed was highly dormant; substantial germination was only observed with abundant water availability (0 Ψ) at the highest temperature (30 °C). Finally, heavier leafy spurge root fragments were able to produce the most shoots after a short exposure to wet conditions. Understanding best management practices for and physiological responses of leafy spurge in riparian systems is important for controlling this persistent species that has widespread negative ecological and economic impacts.

Leafy Spurge (*Euphorbia esula* L.) Seed Production, Germination, and Vegetative Propagule Potential in a Riparian Ecosystem

By

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Chapter 1: Background information and project introduction

Leafy spurge is an aggressive perennial invasive species that has become widely established in North America, beyond the point of eradication (Selleck *et al*. 1962, Dunn 1979). Within the intermountain west, specifically, it has invaded and established itself on millions of acres of rangeland, displacing native vegetation and reducing the quality of the land. Leafy spurge infestations across the northern great plains have accounted for millions of dollars in ecological damages and economic losses (Bangsund *et al*. 1993, Leistritz *et al*. 2004). Leafy spurge is difficult to control over the long term, with one-time applications of herbicides not providing sufficient damage to eradicate the plant (Alley and Messersmith 1985). Thus, leafy spurge management needs to be viewed as a long-term process, with thought and dedication placed on continued efforts, rather than quick fixes. Because leafy spurge infests swaths of rangeland and has such detrimental effects, it has been most studied in those upland, often arid, systems. However, leafy spurge can thrive in wet or seasonally flooded areas as well. In fact, irrigation ditches, drainage systems, and riverbanks are often the location for new leafy spurge infestations in an area (Messersmith *et al*. 1985), where the plant can readily establish itself and then spread out and away into surrounding areas. Larger bodies of water are no exception and can also be readily infested with leafy spurge populations. A particularly concerning example is in the Yampa River Valley in northwestern Colorado. Leafy spurge has existed in along the Yampa River for decades; however, after a major flood event in 2011, populations began establishing downstream as far as Dinosaur National Monument. Additionally, leafy spurge populations have spread out and away from the Yampa River through irrigation ditches. Despite its prevalence in such an ecologically and economically important ecosystem, there exists little information on how to manage leafy spurge in a wet, seasonally flooded area. Traditional methods, specifically use of the chemical picloram (Tordon® 22K, Corteva Agriscience), that have had success in dry, upland areas are not applicable and access to populations can be difficult.

In order to gain a better understanding of how to manage leafy spurge in a riparian area, a field study was developed to investigate the potential for integrated management of leafy spurge utilizing sheep grazing and herbicide applications, either in combination or applied separately. The field study aimed to answer the questions 1) do sheep grazing or herbicide applications individually reduce leafy spurge seed production in a riparian area and 2) is there a synergistic effect of integrated management when the two treatment types are combined. Treatments were applied in the summer of 2019 with an intensive sheep grazing event occurring in the end of May/early June of 2019, as an early season treatment. Herbicide treatments were applied at the end of July 2019 as a late season treatment with four different herbicides applied either on their own or in places that had already been grazed. Data were collected within the treatment season (2019) as well as one-year post-treatment season (2020). Chapter 2 explores the data collected from this field study and the figures and tables are formatted based on the guidelines for the Invasive Plant Science and Management journal for submission for publication.

Chapter 2 prompted thinking about other ways leafy spurge populations in riparian systems may differ from those in upland, dry areas. Specifically, in the spring of 2019, at a time when leafy spurge populations should be emerging in full force, it was difficult to find sufficient sites for the research plots in Chapter 2 because the plants simply had not emerged. That year was unseasonably wet in the Yampa River Valley, as well as uncommonly cool. Riparian areas often have fluctuations in moisture availability, especially during the beginning of the growing season, due to seasonal flooding. Additionally, river systems in the intermountain west are prone to late spring and early fall frosts, which can impact the length of the growing season. Combined, these factors could potentially impact the emergence of leafy spurge populations in the spring and subsequently affect the best timing for management efforts. Thus, a germination study was designed to answer the following question: Is there an optimum intersection between moisture availability and temperature that provides the ideal conditions for germination? A thermogradient table was utilized to investigate the impact of different moisture availabilities at a gradient of temperatures. Due to the large number of treatments and replications, the fully replicated experimental design was split into five separate trials, each running for 21 days. The table was checked daily for germination. The data collected from the germination trials are presented in Chapter 3 and the figures and tables are formatted based on the guidelines for the Invasive Plant Science and Management journal for submission for publication.

Historically, the main focus of the research on leafy spurge control and management has been the aboveground biomass and seed production. As the most accessible part of the plant, this makes sense. Further, in upland rangeland systems where leafy spurge is prevalent, the spread of the plant is most often attributed to dispersal of seeds, while the root system is cited for the plant's persistence (Messersmith *et al*. 1985). Although the root system is relatively inaccessible, it is known to prolifically reproduce if disturbed (Hanson and Rudd 1933, Messersmith *et al*. 1985). However, there is little known about how leafy spurge root fragments could be a potential source of natural population spread. In riparian systems water acts as a known vector for dispersal for leafy spurge seeds, and could also vector leafy spurge root fragments. Specifically, in seasonally flooded areas like the Yampa River Valley where leafy spurge populations grow right to the water's edge, it is feasible that when a flooding event occurs, parts of the root system may become exposed or break away into the river and be deposited downstream. A combined laboratory and greenhouse study was designed to examine if leafy spurge root fragments could establish new populations after prolonged exposure to wet conditions. The main questions for this project were, 1) can leafy spurge root fragments form viable root buds after prolonged exposure to wet conditions and 2) after prolonged exposure to wet conditions are leafy spurge root fragments able to produce new shoots and thus establish a new population? To do so, the laboratory experiment was established and leafy spurge root fragments were exposed to wet or dry conditions for varying amounts of time. The laboratory experiment culminated in a greenhouse planting to determine the viability of treated root fragments to produce new shoots. Chapter 4 reports the findings from this joint laboratory and greenhouse experiment and the figures and tables are formatted based on the guidelines for the Invasive Plant Science and Management journal for submission for publication.

This thesis explores the management of leafy spurge in a riparian ecosystem as well as the plant's physiological responses to environmental conditions in a wet, seasonally flooded area. As leafy spurge populations begin to spread beyond the upland, rangeland systems they are most associated with, it is important to understand the differences between such systems and how that can impact the spread and management of leafy spurge.

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Chapter 2: Integrated management of leafy spurge (*Euphorbia esula* L.) seed production in a riparian ecosystem

Introduction

Leafy spurge (*Euphorbia esula* L.) is an invasive perennial species that has become well established in the North America and is particularly widespread in the north and central plain states of the U.S. (Goodwin *et al*. 2003). Leafy spurge produces both from seed and from vegetative reproduction allowing the plant to spread rapidly and establish near monocultures, outcompeting native vegetation and reducing land quality (Messersmith *et al*. 1985). Further, because leafy spurge plants establish such extensive root systems, populations are very difficult to control, especially over the long term. Even if reductions in aboveground biomass and thus seed production can be achieved, the root system is very seldom damaged to the same extent and can reestablish the population in following seasons, if follow-up treatments are not applied (Lym and Messersmith 1994).

Leafy spurge has most severely impacted rangeland ecosystems, from both ecological and economic perspectives (Noble *et al*. 1979, Leitch *et al*. 1996, Leistritz *et al*. 2004). In addition to displacing native vegetation, it does not provide a replacement forage source for cattle, with small amounts of leafy spurge ingested causing mouth irritation and large amounts causing death (Selleck *et al*. 1962, Lym and Kirby 1987). Due to these issues, leafy spurge control and management has been most extensively studied in rangeland systems, where chemical control is often used as a way to manage leafy spurge populations (Lym and Messersmith 1983). In some cases, where populations are large and difficult to access, biocontrol agents have also provided a certain amount of control (Anderson *et al*. 1999, Kirby *et al*. 2000). It should be noted that biocontrol options are utilized once a population has well surpassed eradication and should be seen as a long-term control method, not a means of eradication.

The rangeland systems in which leafy spurge has historically become dominant and difficult to control are semiarid ecosystems, where interference from associated species is generally less intense (Selleck *et al*. 1962). Although leafy spurge does thrive in dry and disturbed systems, it is by no means confined to them. In fact, leafy spurge populations can establish just as well in areas with more moisture, such as flood plains and riverbanks (Goodwin *et al*. 2003). These riparian edges often have sensitive or unique plant communities, which can be especially harmed by the introduction of such an aggressive species like leafy spurge (Sheley *et al*. 1995). Moving water can also provide an additional vector for dispersal by which leafy spurge populations can further spread.

Despite the fact that leafy spurge populations can thrive in wet, riparian edges, there is much less understanding of how to control it in such ecosystems. Additionally, management efforts in seasonally wet or inundated areas are met with roadblocks that do not exist in dry, upland rangeland habitats. Foremost, perhaps, is the use of chemical control. While many herbicides can be sprayed near or up to water lines (Sheley *et al*. 1995), the main herbicide that has shown promise when it comes to any semblance of long-term control of leafy spurge is picloram (Alley and Messersmith 1985). Picloram can have lasting effects on soil biology and thus plant communities and cannot be sprayed near water, as there is risk of environmental contamination (Tordon® 22K, Corteva Agriscience). Of the chemical products that can be used near water lines, there is less known about their efficacy in controlling leafy spurge populations or they are non-selective formulations that could damage native vegetation (e.g., glyphosate). Beyond chemical control limitations, biocontrol agents can take a long time to establish and, even when they do, it has been suggested that the local environment plays a role in establishment, with very wet conditions impeding establishment (Rees 1994, Lym 1998, Nelson and Hirsch 1999). *Aphthona* spp., which feed on the root system, have had the most success in providing leafy spurge control (Lym 1998). Although they encompass a relatively wide range of habitats, from xeric to mesic, establishment and impact on leafy spurge is variable (Nowierski and Pemberton 2002). Thus, if riparian populations of leafy spurge have established beyond the means of eradication, biocontrol agents need time and proper conditions to establish.

In upland range systems, there are plenty of examples of small, seasonal streams, or irrigation ditches that are lined with leafy spurge. Such populations are likely on the fringe of larger populations that may or may not be receiving management. In some cases, the cost to manage leafy spurge populations that have been established for years or decades far exceeds the monetary value of the land, were it being used for traditional grazing purposes (Lym and Messersmith 1985, Bangsund *et al*. 1996). However, large and ecologically important riparian beltways in the Western United States are also being negatively affected by expanding leafy spurge populations. A prime example is the Yampa River Valley, which runs through northwestern Colorado and is home to an ecologically rare riparian forest habitat.

In the Yampa River Valley, Colorado, USA, leafy spurge is a main component of the plant community in the riparian edge. Leafy spurge has been spreading downstream along the Yampa River for decades from an inception point in Hayden, Colorado. The Yampa River Valley is an extensive riparian beltway that is both ecologically and economically important. The Yampa River is home to one of the largest remaining examples of a rare riparian habitat dominated by narrowleaf cottonwood, boxelder, and red-osier dogwood. It is also used as an irrigation source for the adjacent agricultural lands. Despite being aided by the river as an additional vector of seed dispersal, prior to 2011 the spread of leafy spurge was relatively slow. After an unprecedented flood year in 2011, more and more populations of leafy spurge have been detected downstream. These new populations established quickly and, in spreading, have contributed to the persistence of leafy spurge in the Yampa River Valley. Dinosaur National Monument, which is on the western side of the state and well downstream of leafy spurge's inception point in Hayden, CO, USA, has also seen an increase in leafy spurge populations. This only serves to underscore the importance of understanding how to control leafy spurge in riparian ecosystems, as the input of economic resources directed towards leafy spurge control increases.

For leafy spurge populations in the Yampa River Valley, we are interested in exploring ways to reduce the seed production as a first step in understanding how to potentially slow the spread of the plant in a riparian ecosystem. Leafy spurge seeds are dispersed by dehiscence of the seed pod which propels the seeds away from the plant (Hanson and Rudd 1933). In seasonally flooded areas along the Yampa River, water becomes an additional vector of dispersal, depositing seeds downstream of the source population; thus, understanding how to reduce leafy spurge seed production is an important goal. The main objective of this project is to utilize targeted grazing, herbicide applications, or a combination of the two to reduce the seed production of leafy spurge in the Yampa River Valley. We hypothesize that each of these treatments individually will reduce leafy spurge seed production and cover but together will work synergistically to reduce seed production and cover at a greater level than would have been achieved by utilization of the treatments individually.

Materials & Methods

Study sites and experimental design

Sites were scouted, chosen, and marked in May 2019. Sites were selected based on leafy spurge density (> 50% cover), ease of access and type of site i.e., riparian edge, hay meadow, etc. Four sites were selected and represent three unique riparian habitat types: riparian edge, hay meadow, and oxbow island. Three sites are in Craig, Colorado, USA along the Yampa River while one site is directly north of Craig, Colorado, USA along Fourmile Creek. In Craig, the hay meadow site is hayed annually, while a riparian edge is directly adjacent to an annually hayed area. A second riparian edge is adjacent to a property that is utilized for cattle grazing. Finally, the oxbow islands are along a tributary of the Little Snake River, which confluences with the Yampa River in western Colorado, USA and are utilized as rangeland and grazed by cows.

A fifth site was scouted and the grazing treatment was applied as specified below for the other four plots; however, due to miscommunication between the landowners and contract workers, the plots were hayed over prior to the herbicide treatment being applied. Initially, it was thought that the mowing could act as a different type of "grazing" treatment but since the windrows were still laying across the plots it was determined that it would not be practical to apply the herbicide treatments and still collect meaningful data. The site was therefore abandoned and excluded from further research.

Each site consisted of ten 3 m x 9 m plots, which were assigned treatments utilizing a randomized block design. Half of the plots at each site were grazed by sheep as an early season treatment. Sheep will readily graze leafy spurge, even though cattle will not (Landgraf *et al*. 1984). Grazing treatments occurred early in the growing season as an attempt to damage the plant and force it to utilize resources to regrow the aboveground vegetation before producing more seed, potentially reducing its total seed production and creating new vegetation for herbicide applications. Four different herbicide treatments were applied two months after the grazing treatment as a late-season application. Herbicides have been shown to be very effective when applied as a late-season treatment when carbohydrates are being transported to the roots for winter storage (Lym and Messersmith 1983). Each of the four herbicides were applied to areas that had either been grazed or not grazed. In the plots that had already received a grazing treatment, we hypothesized that the subsequent application of herbicide will place additional pressure on the plants and have a synergistic effect, more greatly reducing leafy spurge cover and seed production compared to plots that do not receive both treatments.

Sheep grazing

At each site, five of the ten 3 m x 9 m plots were fenced off together with portable electric fencing. Seven mature Hampshire blackface ewes (~200 lb./sheep) grazed the designated plots for a full day, for a stocking rate of 82 sheep/hectare. Due to travel restraints, multiple sites were grazed for two half days to equal a total grazing time of one full day. The hay meadow was grazed for two half days on May 28, 2019 and May 31, 2019 for a total of 12 hours of grazing. The hay meadow adjacent riparian edge was grazed for two half days on June 10, 2019 and June 12, 2019 for a total of 10 hours and 20 minutes of grazing. The grazing adjacent hay meadow was grazed for a full day on May 29, 2019 for a total of 10 hours of grazing. The oxbow island was grazed for a full day on June 11, 2019 for total of 10 hours of grazing.

Herbicide applications

Herbicide applications of quinclorac (Facet® L, BASF), aminopyralid (Milestone®, Corteva Agriscience), imazapic (Plateau®, BASF), and aminopyralid + florpyrauxifen-benzyl (DuraCor®, Corteva Agriscience) were made at the recommended rate, either on their own or in plots that had previously been grazed. Herbicide treatments were applied at the end of July 2019 to ensure that the herbicide was applied before the first fall frosts, which can occur as early as August in the Yampa River Valley. Quinclorac was applied at 67 g a.e./hectare. Aminopyralid was applied at 26 g a.e./hectare. Imazapic was applied at 140 g a.i./hectare and mixed with methylated seed oil (MSO) at 4.9 pints/hectare. Aminopyralid + florpyrauxifen-benzyl was applied at 7 g a.e./hectare and 9 g a.i./hectare, respectively, and mixed with MSO at 1.2 pints/hectare.

Data collection

Leafy spurge begins a dormant period after seed dispersal, usually at the end of August, with fall regrowth generally stimulated in early September by cooler weather and increased rainfall (Lym and Messersmith 1983). Within the treatment season, leafy spurge percent cover and seed quantification counts were done on September 12, 2019 for the hay meadow, the hay meadow adjacent riparian edge, and the grazing adjacent riparian edge and on September 14, 2019 for the oxbow islands. Due to timing, most plants were still in their dormant stage with most leaves fallen from the stems. Some plants did have new fall growth, which is characterized by a leafless main stem with two or more branches developing below the original flowering branches (Lym and Messersmith 1983). One-year post-treatment season, the same leafy spurge percent cover and seed quantification counts were done during peak growing season. Data was collected at the hay meadow on July 26, 2020. At the hay meadow adjacent riparian edge, the grazing adjacent riparian edge, and the oxbow islands data was collected on July 27, 2020.

Percent cover was quantified for all species within each treatment plot at every site. Quantification was broken down by individual percentages up to five percent and above five percent was quantified in increments of five percent.

A 0.25 m^2 quadrat was used to quantify stem counts and seed production and this was haphazardly subsampled five times within each treatment. Total stem counts were recorded for each quadrat and within the same quadrat a subset of 10 stems were randomly chosen to quantify seed production. Of the subset of 10 stems that were chosen, not all had quantifiable seed production. These stem counts, either first year growth or a stem that was too far senesced either due to treatment or seasonality, were recorded separately. Seed counts for all remaining viable stems of the subset were quantified in three separate stages to ensure an accurate representation of seed production: burst (post-capsule), capsule, and bract (pre-capsule). These three metrics encompass seeds that have been dispersed, seeds that have not been dispersed, and seeds that have not yet formed but have the potential to do so within the current season, respectively. In this way we can also gain insight in the differentiation between viable seed production (burst and capsule) and non-viable seed production (bract) although there is some uncertainty of the viability of the seed when it comes to the capsule stage.

Statistical analysis

Data was analyzed in Program R (version 3.6.1). Each model contained fixed effects of grazing and herbicide. The grazing factor has two levels – grazed or not grazed – and the herbicide factor has five levels – quinclorac, aminopyralid, imazapic, aminopyralid + florpyrauxifen-benzyl, or no herbicide.

Seed counts were related back to the total mature stem count in a given quadrat. In this way, a seed per m^2 metric was obtained and most concisely represents any changes in the system. Seed counts were analyzed at the total seed level, rather than the individual burst/capsule/bract stage, as had been recorded during data collection. Initially, seed counts were analyzed for each separate stage (Appendix A); however, there were no discernable trends in the data, based on either of the treatments. It was decided to move forward with reporting on the combined total seed counts for each year since all seeds have potential to become dispersed propagules.

Total cover was further split into resident and non-resident vegetation in order to analyze the impact of the grazing and herbicide treatments on the native plant community. Native vegetation was considered resident while exotic, non-native, or invasive species were considered non-resident. Species like smooth brome (*Bromus inermis* Leyss.) and timothy (*Phleum pratense* L.) that are not native, yet considered desirable from a grazing and haying standpoint, were classified as non-resident vegetation.

2019 total seed counts at the quadrat level were log transformed and analyzed using a two-way ANOVA. The model included an interaction term between the grazing and herbicide factors as well as a random effect of plot within location. 2020 total seed counts at the quadrat level were analyzed using a zero inflated approach with a binomial logistic regression due to the large number of zeroes in the dataset. Of the seed that was produced, the values were log transformed and analyzed using a two-way ANOVA with an interaction term between the grazing and herbicide factors.

2019 and 2020 total vegetation cover, leafy spurge cover, and non-resident vegetation cover at the plot level were analyzed using individual two-way ANOVAs. The models included an interaction term between the grazing and herbicide factors as well as a random effect of location. 2019 and 2020 resident vegetation cover at the plot level was analyzed using a zero inflated approach with a binomial logistic regression. Both models contained an interaction term between the grazing and herbicide factors. Of the resident vegetation present, the data were analyzed using a two-way ANOVA with an interaction term between the grazing and herbicide factors.

Results

Within treatment season (2019)

Total seed counts were not impacted by an interaction between the grazing and herbicide factors ($p = 0.2815$). Plots that were grazed reduced total seed production by 40% when compared with plots that where not grazed (p = 0.0203, Figure 1). Herbicide treatments did not have a significant effect on total seed counts (p = 0.5743).

Total cover and leafy spurge cover were not significantly affected by an interaction between the grazing and herbicide factors (p = 0.8510, p = 0.9560, respectively). Individually, grazing and herbicide treatments did not significantly impact total cover (p = 0.1395, p = 0.0538, respectively) or leafy spurge cover (p = 0.4730, p = 0.1210, respectively).

There was no significant impact of an interaction between the grazing and herbicide factors on the presence or absence of resident vegetation (p = 0.1206). Individually, the grazing and herbicide treatments did not have an effect on presence or absence of resident vegetation cover (p = 0.1072, p = 0.9026, respectively). Of the resident vegetation cover present, there was no effect of an interaction between the two factors (p = 0.4860). Individually, the grazing and herbicide treatments did not have an effect on the resident cover that was present (p = 0.6700, p = 0.8790, respectively).

Non-resident vegetation was not significantly impacted by an interaction between the grazing and herbicide factors (p = 0.2140). Plots that were grazed reduced non-resident vegetation cover by 11% when compared with plots that were not grazed (p = 0.0009, Figure 2). Herbicide treatments did not have a significant effect on non-resident vegetation cover (p = 0.6086).

One-year post-treatment season (2020)

The presence or absence of total seed production was not impacted by an interaction between the grazing and herbicide factors (p = 0.7536). The grazing and herbicide treatments did not have an effect on presence or absence of total seed production (p = 0.3267, p = 0.4751, respectively). Of the seed produced, there was no significant impact of an interaction between the grazing and herbicide factors (p = 0.6053). Plots that were grazed had increased total seed production by 48% when compared to plots that were not grazed (the opposite of the previous year) (p = 0.004, Figure 1). Herbicide treatments also had a significant effect on total seed production with plots that received herbicide applications of quinclorac or aminopyralid + florpyrauxifen-benzyl reducing total seed production when compared to no herbicide being applied (73% and 66%, respectively) (p = 0.0013, Figure 3).

Total cover was significantly impacted by an interaction between the grazing and herbicide factors (p = 0.0459). The treatment combination of grazing and aminopyralid + florpyrauxifen-benzyl reduced total cover more greatly than the combinations of no grazing and quinclorac, no grazing and aminopyralid + florpyrauxifen-benzyl, and no grazing and aminopyralid (Figure 4). After accounting for the interaction, plots that were grazed reduced total cover by 10% when compared with plots that were not grazed (p = 0.0016). Individually, herbicide treatments did not have an effect on total cover (p = 0.3988).

Leafy spurge cover was not significantly impacted by an interaction between the grazing and herbicide factors (p = 0.6273). Plots that were grazed reduced leafy spurge cover by 11% when compared with plots that were not grazed (p = 0.0044, Figure 5). Herbicide treatments did not have a significant effect on leafy spurge cover (p = 0.5227).

There was no impact of an interaction between the grazing and herbicide factors on the presence or absence of resident vegetation cover (p = 0.4551). The grazing and herbicide treatments did not have an effect on presence or absence of resident vegetation cover (p = 0.2524, p = 0.9568, respectively). Of the resident vegetation cover present, there was no impact from the interaction between the two factors (p = 0.6960) as well as no effect of either the grazing or herbicide treatments (p = 0.9540, p = 0.7920, respectively).

Non-resident vegetaion was not significantly impacted by an interaction between the grazing and herbicide factors (p = 0.9810). There was no significant effect of either the grazing or herbicide treatments on non-resident vegetation cover (p = 0.1200, p = 0.1080, respectively).

Discussion

An intensive grazing treatment in the spring places stress on the plant during a critical growing period, which decreases plant vigor (Sedivec *et al*. 1995). A reduction in plant vigor during the early growing season can be highlighted by the within treatment season (2019) leafy spurge seed production, which was reduced in plots that were grazed compared with plots that were not grazed (Figure 1). There was no effect of herbicide on seed production within the treatment season, as late season applications will control seedling leafy spurge plants, but viable seed has already been produced (Lym and Messersmith 1983).

Although there was a reduction in seed production due to grazing, there was no effect of either grazing or herbicide treatments on total vegetation cover, leafy spurge cover, or resident vegetation cover. This is counterintuitive, specifically for leafy spurge cover, given that a reduction in leafy spurge seed production would seem to point to a similar reduction in leafy spurge cover. However, it is possible that by the time the data collection occurred in September of the treatment season, the vegetation had ample time to recover from the early season grazing treatment. Sedivec and colleagues note that one type of grazing management plan for controlling leafy spurge is to remove the bracts and flowering parts of the plant in the spring; however, this type of grazing does not reduce the root system (1995), which could still readily produce aboveground biomass. Indeed, aboveground disturbances can actually increase stem densities by removing apical dominance and stimulating growth of root buds (Selleck *et al*. 1962). Additionally, it is possible that the lack of impact on leafy spurge percent cover can be attributed to the regrowth that happens in the fall. New fall regrowth is characterized by a leafless main stem with two or more branches from the original flower branches (Lym and Messersmith 1983) and could account for lack of impact on the leafy spurge percent cover as well as on the total vegetation cover.

Although leafy spurge cover was not reduced, it is positive that there was also no detrimental effect on the resident vegetation cover within the treatment season. Indeed, a metric of successful targeted grazing is that the resident vegetation was not negatively impacted (Frost and Launchbaugh 2003). Non-resident vegetation cover was reduced in plots that were grazed when compared to plots that were not grazed, despite total vegetation cover and leafy spurge cover not being impacted. Likely, this reduction is a reflection of the large amounts of smooth brome present, which was readily grazed by the sheep.

Despite a decrease in leafy spurge seed production within treatment season in plots that were grazed, the one-year post-treatment season (2020) saw the opposite effect in plots that were grazed, with an increase in leafy spurge seed production. Since there was no interaction between the grazing and herbicide treatments, the increase cannot be contributed to an antagonistic effect of combining treatments. It is possible that due to the stress placed on the plants in the previous season, while treatments were being applied, the plants in the plots that were grazed responded to that stress in an often-documented way: increased production of aboveground biomass (Detling *et al*. 1979, Hilbert *et al*. 1981) and subsequently, seed production (Paige and Whitham 1987).

On their own, though, herbicide treatments did reduce leafy spurge populations when compared to no herbicide being applied, specifically in plots treated with quinclorac or aminopyralid + florpyrauxifen-benzyl. Fall applications of herbicides have been shown to have generally consistent control of leafy spurge the season following application (Alley and Messersmith 1985).

As mentioned previously, stress response to grazing can sometimes manifest as an increase in aboveground biomass and seed production; yet, while one-year post-treatment season leafy spurge plants produced more seeds in plots that were grazed, the same plots that were grazed also had a decrease in leafy spurge percent cover. Although the reduction in cover is positive for controlling the leafy spurge population, it is counteracted by the increase in seed production, which will ultimately release more propagules into the system.

Again, resident vegetation cover was not impacted by either of the treatments, which is positive for the small native plant community that exists amongst vegetation that is heavily comprised of leafy spurge or grasses specifically utilized for grazing (cattle) or haying purposes.

Overall, there were some desired outcomes from the sheep grazing i.e., a reduction of seed production within the treatment season and a reduction of leafy spurge cover one-year post-treatment season. However, there were also confusing signals like the increase in seed production one-year post-treatment in plots that had been grazed. There is no clear story based solely on grazing, in fact, much of the literature suggests that rotations or continued seasons of grazing in the same location has much more success in providing control of leafy spurge than a single grazing event alone (Sedivec and Maine 1993, Olson and Lacey 1994, Sedivec *et al*. 1995).

From a herbicide perspective, there was a clear reduction of leafy spurge seed production one-year post-treatment season, which is what was expected. However, the herbicides did not significantly impact any other aspects of the leafy spurge plants/aboveground biomass. As with sheep grazing, though, herbicides have had the most efficacy in controlling leafy spurge through reapplications over multiple seasons (Alley and Messersmith 1985, Lym and Messersmith 1994).

Since there were no significant interactions between the grazing and herbicide factors that more greatly reduced leafy spurge seed production or cover than either treatment alone, focusing on herbicide applications over multiple seasons makes the most sense moving forward. The logistics of transporting and overseeing targeted grazing events, especially in such small, albeit dense, populations of leafy spurge right along the Yampa River, is not economically effective. Many of the very dense populations that line the edge of the river are difficult to access, if not completely inaccessible from a herding perspective. Additionally, successful control of leafy spurge has only been achieved through continuous grazing of sheep over four growing seasons (Helgeson 1942, Johnston and Peake 1960). Further research on herbicides that are safe to spray near water, specifically quinclorac and aminopyralid + florpyrauxifen-benzyl, should be pursued to better understand how to manage leafy spurge populations in riparian ecosystems and reduce propagule load to the river.

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Chapter 2 figures and tables

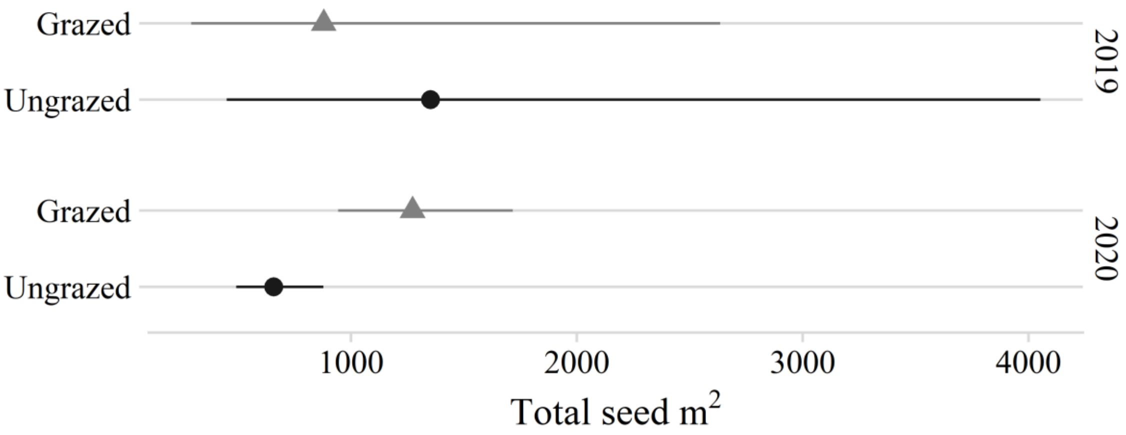

Figure 1. Within treatment season (2019) and one-year post-treatment season (2020) effect of grazing on leafy spurge total seed production (error bars represent 95% confidence intervals). Grazed plots had a reduction in leafy spurge seed production in 2019 ($p = 0.0203$) and an increase in leafy spurge seed production in 2020 ($p = 0.004$).

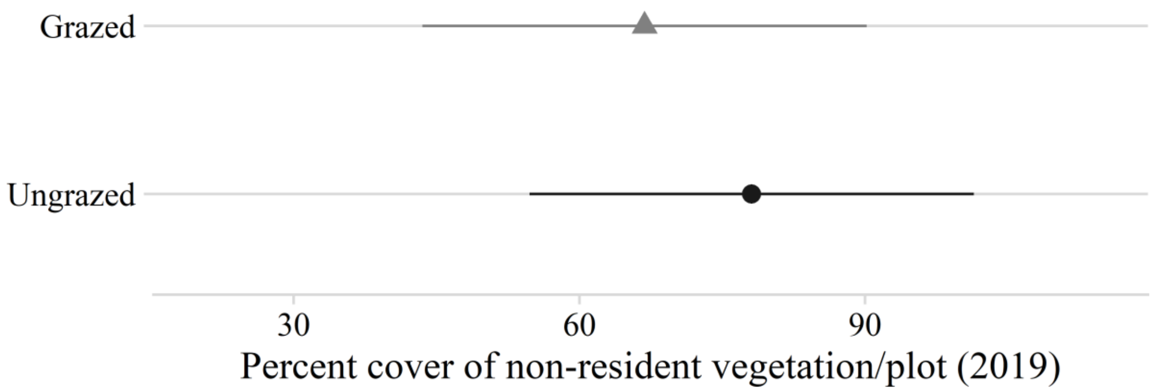

Figure 2. Within treatment season (2019) effect of grazing on non-resident vegetation cover (error bars represent 95% confidence intervals). Non-resident vegetation cover was reduced in the plots that were grazed (p = 0.0009).

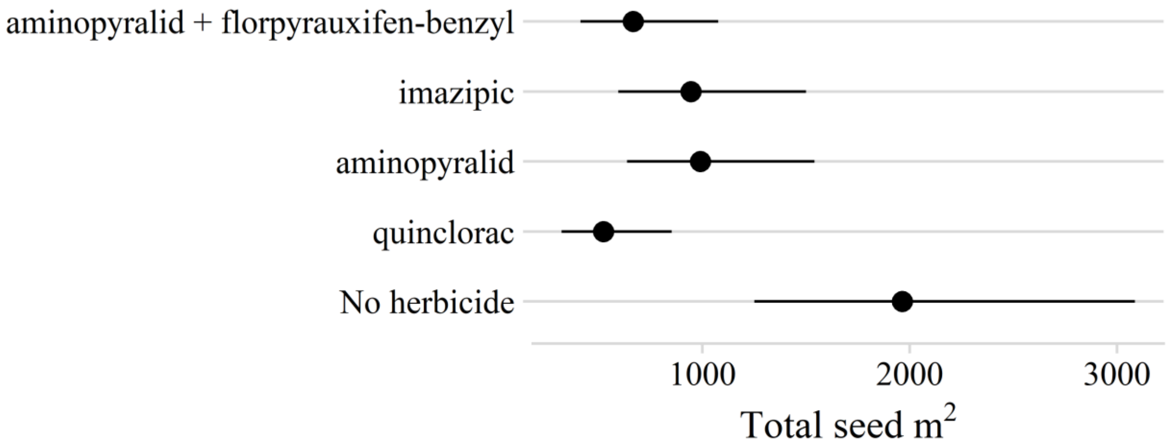

Figure 3. One-year post-treatment season (2020) effect of herbicide on leafy spurge total seed production (error bars represent 95% confidence intervals). Herbicide treatments significantly reduced the total leafy spurge seed production compared to plots that did not receive applications ($p = 0.0013$). Specifically, aminopyralid + florpyrauxifen-benzyl and quinclorac applications most significantly reduced the total seed production.

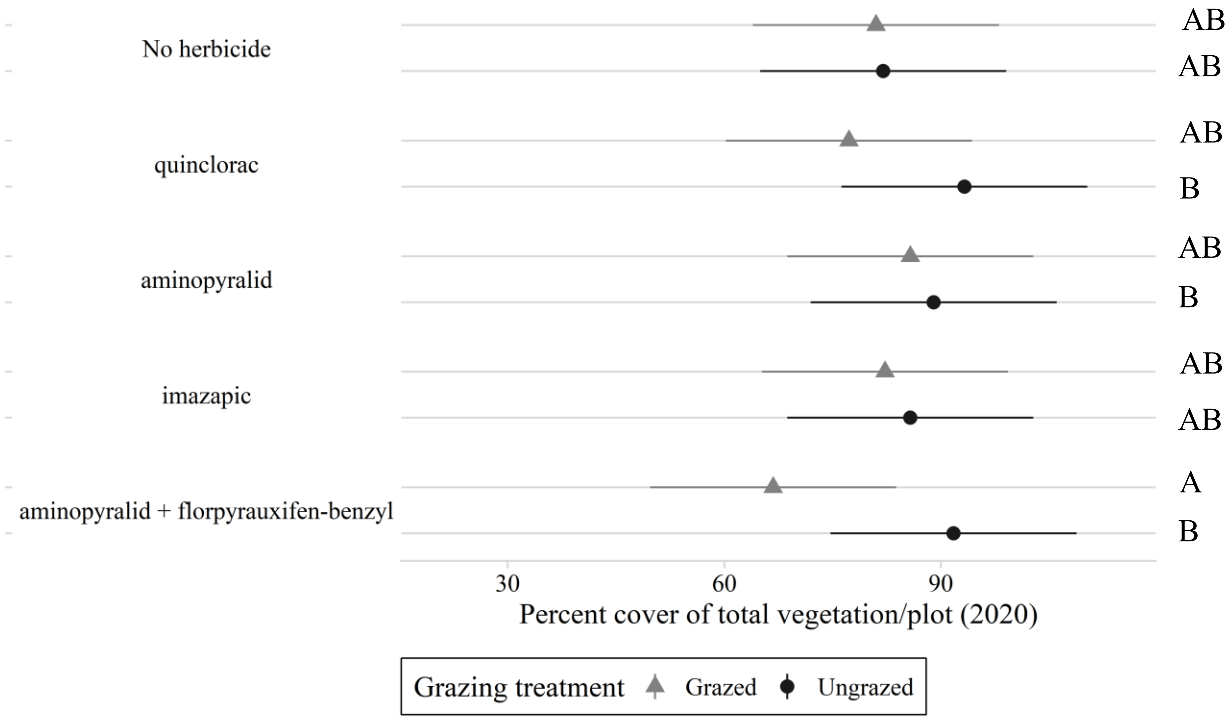

Figure 4. One-year post-treatment season (2020) effect of grazing and herbicide interaction on total vegetation cover (p = 0.0459), percentages with the same letter are not significantly different (error bars represent 95% confidence intervals). The combination of grazing and aminopyralid + florpyrauxifen-benzyl provided a greater reduction in total vegetation cover compared to aminopyralid + florpyrauxifen-benzyl being applied independently.

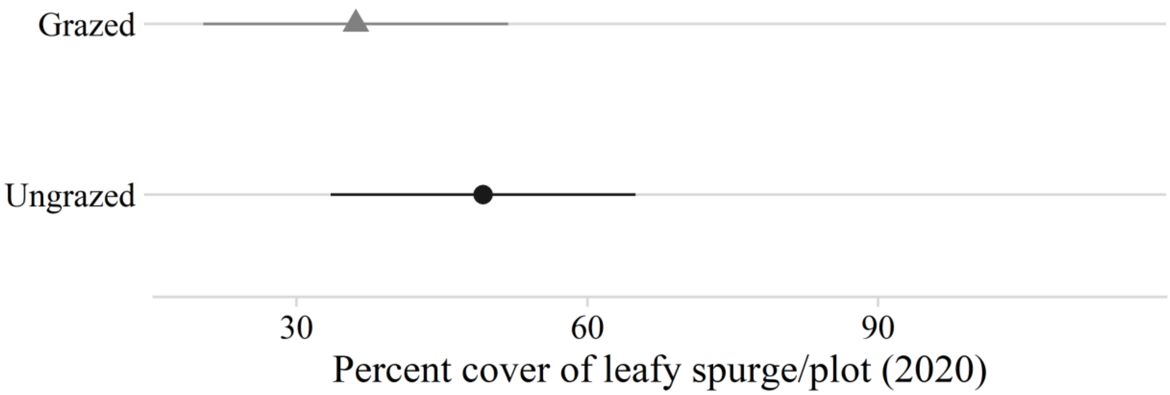

Figure 5. One-year post-treatment (2020) effect of grazing on leafy spurge cover (error bars represent 95% confidence intervals). Grazed plots had a greater reduction in percent cover of leafy spurge compared to plots that were not grazed (p = 0.0044).

Chapter 3: Leafy spurge (*Euphorbia esula*) seed germination across a temperature and moisture gradient

Introduction

Leafy spurge is a deep-rooted perennial invasive species that has become widespread in North America (Selleck *et al*. 1962, Dunn 1979). The underground root system allows leafy spurge populations to establish beyond eradication, outcompeting other species and forming near monocultures. These root systems are considered the main reason for the persistence of the species (Messersmith *et al*. 1985). Spread of leafy spurge populations, however, is attributed to the plant's seed production and subsequent dispersal, both naturally and by aid of animals and humans (Watson 1985). Leafy spurge seeds are formed in capsules that, over time, dry out and break open, exploding the seeds out and away from the parent plant, up to 5 meters (Bowes and Thomas 1978a). Not only do leafy spurge populations spread quickly, but they are also difficult to control. Plants with reduced seed production capabilities due to management practices can still produce viable seed. Additionally, in areas of heavy competition, leafy spurge plants are able to produce high seed quantities (Selleck *et al.* 1962). Further, leafy spurge seeds are able to remain dormant in seedbank for up to eight years (Bowes and Thomas 1978b), although dormancy does seem to be site specific and potentially influenced by environmental factors or genetic differences (Selleck *et al*. 1962). Because seed dispersal is the main mechanism by which leafy spurge populations spread, understanding the conditions under which leafy spurge seeds germinate is key to management efforts.

Germination of leafy spurge seeds has been well studied. Optimum germination has been recorded at steady temperatures of 20 °C and 30 °C (Hanson and Rudd 1933) and fluctuating temperatures that mimic the natural world also providing high levels of germination (Hanson and Rudd 1933, Selleck *et al*. 1962). Previous research on germination of leafy spurge seeds has focused specifically on temperature, as leafy spurge has become widespread in mainly arid rangeland systems. However, it is also important to consider areas in the intermountain west that are wet or seasonally flooded. Leafy spurge populations are often found along irrigation streams and ditches; in fact, such areas are often inception points for populations in new areas (Messersmith *et al*. 1985), due to water as a vector for seed dispersal. In the intermountain west, there are also large riparian corridors that leafy spurge has begun to overtake. A prime example is the Yampa River Valley in northwestern Colorado, USA. Like many systems in the intermountain west, the Yampa River Valley is subject to late spring frosts as well as early growing season flooding. This combination of fluctuating temperatures and a range of moisture availability provide a unique environment for leafy spurge seeds to germinate in.

While many leafy spurge populations that are well established return each year due to their extensive root system, seeds are considered the main contributor to the dispersal of leafy spurge populations. Germination of leafy spurge seeds cannot be ignored in a riparian system where water is naturally dispersing propagules more quickly than would have been possible in an upland, dry system. Moisture availability is important for leafy spurge germination (Bakke 1936), with available moisture in the early growing season allowing the most seedlings to emerge (Best *et al*. 1980). Thus, considering the impact of moisture availability on leafy spurge seed germination is important to better understand systems in which water is not a limiting factor. This research examines the intersection of temperature and moisture availability and if there is an impact on leafy spurge seed germination.

Methods

Leafy spurge seed capsules were collected from two locations, Martin Luther King Jr. Park and Dry Creek Disc Golf Course, in Cheyenne, Wyoming, USA weekly during peak seed production in June and July of 2020. The seed capsules were kept in a refrigerator at 1 °C and were only removed from cold storage separate seeds from capsules. Seeds were sorted based on collection location (Selleck *et al* 1962) and color, which is an indication of maturity and germination potential (Wicks and Derscheid 1964). Leafy spurge seeds that are brown, gray-brown, gray, and mottled are considered mature and were selected for experimentation while all other seeds were discarded. As seed color is an indication of maturity, the four color classes (brown, gray-brown, gray, and mottled) of mature seeds were kept separate from one another and were an imposed fixed effect for this experiment. Once seeds were fully sorted, they remained in cold storage (1°C) for a minimum of four months (and up to eight months) after their collection date. This process was designed to mimic an overwinter period as a type of afterripening and intended to prompt the seeds to germinate to their full potential during the trial.

Six temperature treatments were set up on a thermogradient table – 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, and 30 °C – with an initial set of five moisture treatments – 0 Ψ, -3.75 Ψ, -7.5 Ψ, -11.25 Ψ, and -15 Ψ – per temperature. Within each moisture and temperature combination each seed color class was replicated three times for each collection location. Due to the holding capacity of the thermogradient table used in this experiment (72 petri dishes) it was not possible to fit all moisture treatment and seed color class treatment combinations within each temperature treatment in a single trial. Thus, a total of five runs were prepared with moisture treatment, seed color class, and replicate randomized within each imposed temperature. Each replicate was represented by a split petri dish that contained 40 leafy spurge seeds on top of four layers of seed germination filter paper on either half. The two halves of each petri dish received the same unique treatment combination with only collection location differing across the split. Each petri dish half was moisturized with 7 mL of the appropriate moisture solutions at the beginning of the experiment. Petri dishes were wrapped with M4 parafilm to reduce evaporation.

Moisture solutions were made by the appropriate amount of polyethylene glycol (PEG) 8000 to distilled water based on the equation (Michel 1983):

$$[PEG] = (4-(5.16(\Psi T-560(\Psi + 16){}^\wedge 05)/(2.58T-280) \tag{1}$$

Each of the five runs was observed for 21 days with germination status recorded daily. Upon germination, the successful seed was removed from the petri dish. Run 1 began on November 17, 2020 and ended on December 8, 2020. Run 2 began on January 4, 2021 and ended on January 25, 2021. Run 3 began on January 25, 2021 and ended on February 15, 2021. Run 4 began on February 15, 2021 and ended on March 8, 2021. Run 5 began on March 8, 2021 and ended on March 29, 2021.

Data was analyzed in Program R (version 3.6.1) using dose-response analysis models. Models accounted for temperature treatments and water potential treatments only, as anything more complicated would not allow the models to converge.

Results

The 0 Ψ and 30 °C treatment was the only combination that produced significant amounts of germination over the duration of the runs (Figure 1), with the seeds projected to reach 5% overall germination at extended time intervals (well past the 21-day run length). At the 0 Ψ and 30 °C treatment combination, it took 11 days for the seeds to reach 50% germination of an overall total of 5%. No other treatment combinations allowed for model convergence and thus did not produced meaningful germination results. In total, all other treatment combinations had an overall germination rate of 5%.

Discussion

The treatment combination of the most available water and the warmest available temperature, 0 Ψ and 30 °C, was the only treatment to produce meaningful germination results. A total of 5% germination potential over an extended period of time, while significant compared to other treatment combinations, is still small. This, in concert with no other treatment combinations producing meaningful germination results, signals that there is likely an overall underlying reason for why minimal germination occurred.

Leafy spurge seeds can stay dormant in the seedbank for years (Selleck *et al*. 1962, Bowes and Thomas 1978b) and it can be difficult to produce germination results if afterripening efforts are not taken into account (Foley 2004). Unfortunately, afterripening options for leafy spurge are not well understood. Periods of cold to induce overwintering and chemical options have been explored (Selleck *et al*. 1962, Foley 2004, Foley 2008, Foley and Chao 2008). This research attempted a period of induced overwintering afterripening; however, this was performed on seeds collected during the growing season before the experiment was run. There are discrepancies and unknowns in the literature about leafy spurge seed dormancy that could also play a factor in germination potential (Brown and Porter 1942, Bowes and Thomas 1978b). Additionally, it is known that seeds from different sites can have different viabilities or expressions of dormancy (Selleck *et al*. 1962). If this experiment were to be replicated, it may be prudent to have an older and potentially more reliably viable seed source, as well as consider more disparate populations. No seed viability tests were run prior to the germination trails, which may also be an option to consider in the future.

Despite minimal germination across the board, the treatment combination that produced significant results suggests that leafy spurge seeds need ideal conditions to germinate. It is noted in the literature that moisture availability does play a role in when seedlings emerge, with Bakke (1936) observing that germination can occur whenever sufficient moisture is available and Best *et al*. (1980) documenting that maximum seedling emergence occurred in the early spring of a growing season, with any emergence later in the growing season following heavy rains. It is important to understand how leafy spurge seeds respond in riparian ecosystems where water is amply available. In upland, arid systems leafy spurge has become a dominant species in the areas it has invaded. It is well established and difficult to control. There are further limitations to controlling leafy spurge populations in riparian systems where leafy spurge is beginning to readily establish itself with the aid of water as an additional vector for dispersal. As this research supports, seeds need plenty of moisture available in the system for them to germinate. A seasonally wet area, especially in the early growing season, could be providing more than sufficient moisture availability to leafy spurge seeds that are swept downstream and allow them to more readily establish new populations.

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Chapter 3 figures and tables

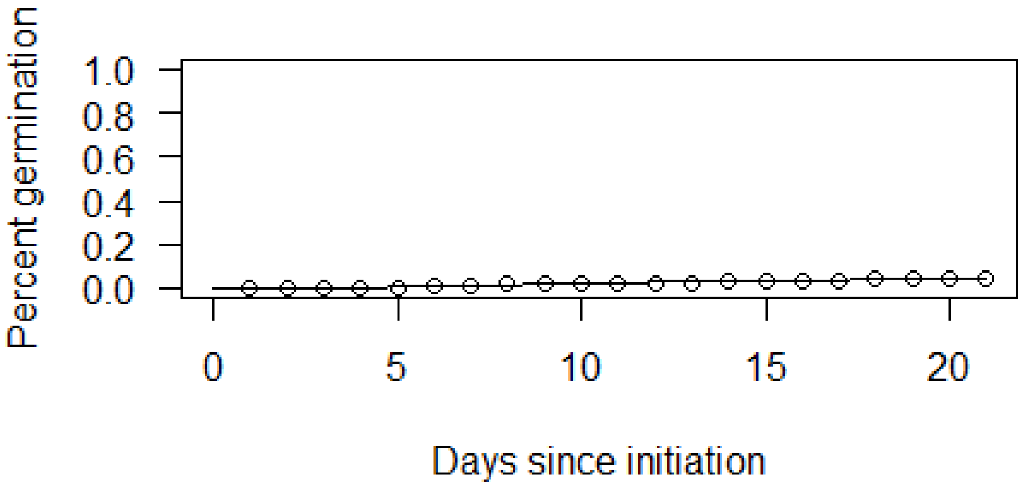

Figure 1. Percent germination of leafy spurge seeds over 21 days based on dose-response analysis model output for 0 Ψ and 30 °C treatment combination. The d parameter represents the upper asymptote, or the proportion of seeds that can germinate at the longest interval (d = 0.05, p-value < 0.001). The e parameter is the inflection point of the curve, or the time at which 50% of the maximum potential germination has been reached (e = 11.00, p-value < 0.001). The b parameter is the slope of the curve at the e parameter (b = -2.67, p-value < 0.001).

Chapter 4: Leafy spurge (*Euphorbia esula* L.) root bud formation and shoot emergence after prolonged moisture exposure

Introduction

Leafy spurge is an aggressive perennial invasive species that has become widespread and established beyond the point of eradication across North America (Selleck *et al*. 1962, Dunn 1979). Known to have prolific seed production throughout the growing season, the dispersal of seeds is considered a main factor in the spread of leafy spurge populations. Additionally, the leafy spurge root system produces asexual vegetative buds that overwinter under the soil surface and allow for vegetative spread. Although the root system of leafy spurge is not considered as prolific in the spreading of leafy spurge populations as the spread of seed, it is the most important factor for the persistence of the plant (Messersmith *et al*. 1985). Leafy spurge has a deep and extensive root system that is difficult to control, thus contributing to the survival of the plant (Messersmith et al. 1985). A high proportion of the plant's biomass is in the root system (Bakke 1936), which is relatively inaccessible, especially in natural areas (Heidel 1982). Furthermore, leafy spurge is generally resistant to stress, with moderate drought resistance and extensive carbohydrate reserves in the roots, allowing it to regrow even if the aboveground biomass is removed (Selleck *et al*., 1962).

Leafy spurge has a heterorhizic root system that is composed of both "long" and "short" roots (Raju *et al*. 1963). Long roots have cambial activity and can produce root and shoot buds while short roots, which arise from long roots, lack cambial activity and consequently cannot produce shoot buds (Raju *et al*. 1963). Thus, the long roots of leafy spurge are the primary contributing factor to the permanent framework of the root system, with the ability to grow rapidly horizontally, eventually turning downward to become vertical roots (Raju 1985). The deeply penetrating root system allows individual plants to produce patches where no other plants can establish themselves (Bakke 1936), aiding in its difficulty to control.

The leafy spurge root system can produce buds along the long roots at almost any segment (Messersmith *et al*. 1985). Vegetative buds that are produced by the root system can be classified as either crown buds or shoot buds. The leafy spurge crown develops at the base of the stem and consists of buds that produce new stems at the same location annually (Messersmith *et al*. 1985). Crowns can live for several years, producing roots that contribute to the spread of leafy spurge, but the number of years is unknown (Bowes and Thomas 1978). Alternatively, the adventitious shoot buds that are produced on the underground parts of leafy spurge can be produced after an injury to the plant (reparative buds) and can also arise spontaneously without any apparent injury (additional buds) (Raju *et al*. 1966). Adventitious buds are pinkish and are more abundant in shallower depths than in deeper levels, a trend which also corresponds to root density (Coupland and Alex 1954, Coupland and Alex 1955). The maximum depth at which buds can develop has been found to vary between 35 and 174 cm (Coupland and Alex 1955); however, buds can occasionally occur at greater depths with Raju *et al*. (1964) finding buds down to 2.29 meters.

These adventitious vegetative buds can be further classified as active, inactive, and dead. Active buds are pink to white in color, inactive buds are yellow to light-brown in color and are composed of living tissue that is essentially dormant, from a development viewpoint, and dead buds are composed of dead or lignified tissue (Coupland and Alex 1955). The underground distribution of these vegetative buds and the ability of new shoots to be readily produced by small pieces of root (Hanson and Rudd 1933) are major reasons for the plant's persistence.

Despite the extensive distribution and persistence of the leafy spurge root system, dispersal of roots is considered a minor factor compared with the dispersal of seeds in the spread of leafy spurge (Messersmith *et al*. 1985). A handful of researchers are responsible for the bulk of knowledge concerning the leafy spurge root system. As early as the 1930s, scientists were describing the root system (Hanson and Rudd 1933, Bakke 1936) while in later decades Canadian scientists performed experiments to understand specific morphology and characteristics of the root system (Coupland and Alex 1954, Coupland and Alex 1955, Raju *et al*. 1963, Raju et al. 1964a, Raju et al. 1964b). Only one study has begun to scratch the surface of the potential of leafy spurge root fragments as a means of population dispersal (Raju et al. 1964a), despite leafy spurge populations continuing to spread outside of the more classical rangeland systems where the plant is often studied. In upland populations it is understandable that the root system is much more a factor for persistence of a population rather than the spread over greater distance. However, leafy spurge populations are not limited to these upland, typically rangeland, areas; in fact, the plant thrives in wet conditions.

Specifically, in riparian areas, the additional vector of water which aids leafy spurge seed dispersal could also serve to aid root dispersal. Leafy spurge populations often grow right up to the water’s edge. In this sense, the root system could be providing some amount of stabilization to the riverbanks; however, erosion of the banks can be extensive, especially in areas that are seasonally flooded. Since leafy spurge populations grow so close to the water, any erosion that occurs has the potential to break off root fragments, which can then be deposited downstream. It has been postulated that a primary means of leafy spurge dispersal in a riparian area would occur from root segments carried downstream, especially during high water events (Progar et al. 2010). These root fragments, through pre-formed root buds or the formation of new buds, have the potential to establish new populations of leafy spurge. Thus, the dispersal of roots cannot be ignored, especially when seeking to reduce the spread of leafy spurge populations.

With this in mind, the following questions concerning leafy spurge root fragments and water exposure were posed: 1) can leafy spurge root pieces still produce root buds after prolonged water exposure, 2) does duration of exposure to water affect the ability to produce root buds, and 3) does size of root fragment in combination with duration of exposure to water affect the ability to produce root buds? These questions have not been explored in the literature and will be useful information for a more complete understanding of population dispersal dynamics of leafy spurge in riparian systems.

Materials & Methods

Root collection

Leafy spurge root material was collected at Martin Luther King Jr. Park in Cheyenne, Wyoming, USA over a four-day period at the end of July in 2020. Leafy spurge root fragments were dug up with small hand trowels, digging into and up a small hillside to best access the root system. Once extracted, roots were cleaned of excess soil, wrapped in damp paper towels, and stored in coolers until return to the laboratory. In the laboratory, the roots were kept wrapped in damp paper towels and stored in refrigerators at 1 °C until preparation for treatments and pre-treatment measurements.

Experimental design

Root fragments were measured into three different classes: 21, 14, or 7 cm. Once measured, roots were placed in a wet block or a dry block. A wet block consisted of two 0.61 m x 1.22 m plastic bins that were joined together by corrugated plastic pipe at either end. One end was connected with a small pond pump to ensure that the water was continuously circulated. A dry block consisted of two 0.61 m x 1.22 m plastic bins with bottoms lined with a stable, porous clay gravel. The gravel was meant to act as a neutral substrate to lay the roots on, rather than a man-made substrate, like plastic. There were three wet blocks and three dry blocks, for a total of six blocks.

This was a full-factorial complete randomized block design. Within each block, roots were left in either a wet or dry treatment for six different time intervals: 0-time, 1 day, 1 week, 2 weeks, 1 month, and 2 months. Each time interval had five replicates of each root length within each block for a total of 540 root fragments. Measurements of initial weight (g), diameter (mm), and number of root buds were taken before roots were placed in water or on the gravel. Not all roots had uniform diameter along the entire length of fragment, in which case two measurements were made at the thinnest and widest parts of the root and averaged to obtain a representative diameter. Root buds for each root fragment were further classified as active buds, inactive buds, or dead buds (Coupland and Alex 1955). For the 0-time interval, the wet block roots were briefly submerged in water prior to measurements and dry block roots were immediately measured. The 0-time interval roots were planted in the greenhouse directly following the data collection.

Despite the pond pumps circulating water through each wet block, there was still concern that water conditions could stray from the baseline of the lab water being used and influence the roots. Thus, measurements of pH, nitrate, nitrite, and ammonia were made on a weekly basis to ensure that there were no major nutrient or water quality fluctuations. Additionally, fresh water was added, when necessary, if evaporation was decreasing the water line below the pump intake/output level. The water in the wet blocks fluctuated between 12.7 °C and 15.5 °C and the room temperature between 18.3 °C and 21.1 °C.

Greenhouse planting

After being in wet or dry conditions for the allotted period of time, roots were reweighed and buds were recounted. Roots were planted in shallow trays that contained a 50/50 mix of mortar sand and potting mix (bark mix), just below the surface. The 50/50 mix was used to best represent the sandy soils along riparian beltways like the Yampa River. The trays were watered twice a day. Any new shoots that arose during the planted time were accounted for as soon as they were observed. Throughout the planting period, some of the arisen shoots died, which was also accounted for. The asymptote of a cumulative distribution function was used to determine when the roots should be dug up. Based on the cumulative distribution function, root fragments were planted for a minimum of 35 days before being dug up (Figure 1). Once dug up, the roots were reweighed and the buds were recounted.

Statistical analysis

Data was analyzed in Program R (version 3.6.1). Active and inactive bud counts were combined to form a viable bud counts parameter, which was used for analyses. After creating a viable bud count parameter, four other parameters were derived from the root bud data: post-treatment viable buds, post-treatment dead buds, post-plant viable buds, post-treatment dead buds. This was done in order to express the overall change in the two different bud parameters – viable and dead – for the root fragments after they had been exposed to a moisture treatment and after they had been planted in the greenhouse.

Change in root buds analysis

The four parameters derived from the root bud data were analyzed with generalized linear mixed-effect models with Poisson distributions. Each model had fixed effects of root length, initial root weight, exposure time, and moisture treatment. There was an interaction term included between exposure time and moisture treatment as well as a random effect of block.

Shoot emergence analysis

Emerged shoot data (whether or not shoots emerged from root fragments) were analyzed with a generalized linear mixed-effect model with a binomial distribution. The model contained fixed effects of root length, initial root weight, exposure time, and moisture treatment and included an interaction term between exposure time and moisture treatment as well as a random effect of block.

Root fragments with emerged shoots analysis

Of the shoots that did emerge, the data on those root fragments were further analyzed with a generalized linear model. This was to better understand the driving factors of quantity of shoots that can be produced by a root fragment. The model included fixed effects of root length, initial root weight, exposure time, and moisture treatment, and included an interaction term between exposure time and moisture treatment.

The threshold of significance was set at an alpha of 0.1 for all analyses, in order to best understand any trends or relationships in the dataset.

Results

Change in root buds

Post-treatment viable buds were not significantly affected by an interaction between exposure time and moisture treatment ($p > 0.1$). Individually, root length, initial weight, and exposure time did not significantly affect post-treatment viable buds ($p > 0.1$). Moisture treatment did have a significant impact on post-treatment viable buds (Figure 2), with the wet treatment causing a 1.6% greater increase of viable buds than the dry treatment ($p = 0.07$).

Post-treatment dead buds were not significantly affected by an interaction between exposure time and moisture treatment ($p > 0.1$). Individually, root length, initial weight, exposure time, and moisture treatment did not significantly affect post-treatment dead buds ($p > 0.1$).

Post-plant viable buds were not significantly affected by an interaction between exposure time and moisture treatment ($p > 0.1$). Individually, root length, initial weight, exposure time, and moisture treatment all had significant effects on post-plant viable buds ($p = 0.053$, $p < 0.001$, $p = 0.087$, $p = 0.064$, respectively).

Post-plant viable buds were reduced by 0.1% for every centimeter increase in root length (Figure 3a). Post-plant viable buds were reduced by 0.7% for every gram increase of initial root weight (Figure 3b). Post-plant viable buds were reduced by 0.04% for every added day of exposure to treatment (Figure 3c). Post-plant viable buds were reduced by 2% more in the wet treatment than in the dry treatment (Figure 4).

Post-plant dead buds were not significantly affected by an interaction between exposure time and moisture treatment ($p > 0.1$). Individually, root length, initial weight, and moisture treatment did not significantly affect post-plant dead buds ($p > 0.1$). Exposure time did have a significant effect on post-plant dead buds (Figure 5), with post-plant dead buds increasing by 0.1% for every added day of exposure to treatment ($p < 0.001$).

Shoot emergence

There was no significant impact of the interaction between exposure time and moisture treatment on whether or not root fragments would produce shoots. Root length also did not have a significant impact on shoot emergence. Root fragments with heavier initial root weights were more likely to produce shoots when planted in the greenhouse ($p = 0.007$, Figure 6).

Individually, exposure time significantly impacted whether or not shoots would emerge with root fragments that had a shorter duration of exposure being more likely to produce shoots ($p < 0.001$, Figure 7). Moisture treatment on its own also significantly impacted whether or not shoots would emerge. Root fragments exposed to the wet treatment were more likely to produce shoots than root fragments that were exposed to the dry treatment ($p = 0.001$).

Root fragments with shoot emergence

Of the shoots that did emerge, there was a significant impact of the interaction between exposure time and moisture treatment (Figure 8). Root fragments that were exposed to the wet treatment for shorter amounts of time produced more shoots than root fragments exposed to the dry treatment for longer periods of time ($p = 0.015$).

After taking into account the interaction term, duration of exposure and moisture treatment did not individually affect the number of shoots a root fragment could produce ($p = 0.852$, $p = 0.667$, respectively). Root length also did not significantly affect how many shoots a root fragment could produce ($p = 0.422$). Initial root weight did have a significant impact on the number of shoots produced (Figure 9), with root fragments that had heavier initial root weights being able to produce more shoots than root fragments with lighter initial root weights ($p < 0.001$).

Discussion

The root system of leafy spurge is not often considered a means of population spread, but rather, persistence of established populations (Messersmith *et al*. 1985). Riparian areas represent a unique situation in which water acts as an additional vector to move propagules downstream – and not just seeds but also root fragments, which are able to reproduce asexually through the formation of root buds. The results of this research confirm that leafy spurge root fragments are an additional way for leafy spurge populations to spread in riparian areas: being moved by water and deposited downstream.

Not all results that were statistically significant had ecological relevance. For example, although there was a trend of a 0.04% reduction in post-plant viable buds for every added day of exposure to treatment, this is such a small amount of change that it isn’t actually speaking to a meaningful physiological process for the root fragments and whether or not they can produce viable buds. Similarly, with the change in post-plant viable buds based on root length and initial root weight, we see such small trends that we cannot draw meaningful, ecologically relevant conclusions.

Change in root buds

There are still statistically significant and ecologically relevant results based on this research. A prime example is the greater increase in post-treatment viable buds after exposure to the wet moisture treatment compared to the dry moisture treatment. This can be attributed to the fact that competition for water is a factor in the mechanism of root bud inhibition for leafy spurge (McIntyre 1979). When water is removed as a limiting factor, the root fragments exposed to the wet treatment were able to produce more viable root buds than the fragments exposed to the dry treatment, where access to water was still a limiting factor.

Further, we see that the longer the root fragments were exposed to a moisture treatment, the more post-plant dead buds they had. The longer the root fragments were exposed to either the wet or dry treatment, the less viable the roots are likely to be as they either begin to decompose in the water or dry out on the porous gravel substrate. In both cases they were not getting the resources they needed and upon planting, could not be revitalized; thus, any viable buds that did exist began to die off, increasing the number of post-plant dead buds. Leafy spurge plants can survive several months of submergence; however, prolonged exposure can also kill the plants, with the root system also unable to recover (Selleck *et al*. 1962).

Shoot emergence

Although this research set out explicitly to explore the viability of root fragments as dispersal agents based on root bud formation, the main takeaway from this research surrounds shoot emergence. Leafy spurge root fragments are more likely to produce shoots if they have heavier weights, which speaks to the idea of carbohydrate storage and resource availability. Another perennial invasive species, Canada thistle (*Cirsium arvense* L.), that can reproduce vegetatively is also able to produce more and larger shoots from larger pieces of roots that, again, have greater carbohydrate storages than smaller root pieces (Hayden 1934). Even after going through extended exposure to moisture treatments, a root fragment that is heavier, and, by association, larger, can still have the potential to produce new shoots.

Leafy spurge root fragments that were exposed to the wet treatment were more likely to produce shoots than root fragments exposed to the dry treatment, which ties in with the fact that leafy spurge root fragments exposed to the wet treatment had an increase in post-treatment viable buds when compared to those exposed to the dry treatment. Removing water as a limiting factor is a main takeaway from this research – root fragments that have exposure to water and are no longer inhibited by competition (McIntyre 1979) are able to produce more viable buds and this, in turn, allows them to be more likely to produce shoots.

Whether or not shoots emerged from root fragments was also influenced by the duration of exposure to the moisture treatment. Regardless of treatment, root fragments with a shorter duration of exposure were more likely to produce shoots. This ties back to the idea that the longer the root fragments were exposed to either moisture treatment, the more dead buds they accumulated, as the root fragments began to use up their carbohydrate and resource reserves. It is important to note that short amounts of exposure to either moisture treatment were found to be ideal for shoots being produced. Thus, areas that are seasonally flooded could be providing an ideal condition for short exposure times before fragments are washed ashore.

Root fragments with shoot emergence

Of the root fragments that had shoots emerge, two important factors stand out. First, heavier root fragments are able to produce more shoots. Thus, not only are heavier root fragments more likely to produce shoots, but they also produce more shoots than root fragments with lighter weights. Again, this speaks to the nutrient reserves available to heavier root fragments, such that even after exposure to wet or dry conditions, heavier root fragments still have ample resources to reproduce asexually.

Secondly, and perhaps most importantly, the specific combination of short durations of exposure and exposure to wet conditions allow leafy spurge root fragments to produce more shoots than longer durations of exposure to the dry treatment. Even root fragments exposed to the wet moisture treatment for longer amounts of time could still produce shoots, but very few in comparison to fragments exposed for short periods of time. This combination, again, highlights the fact that competition for water is a factor in the mechanism of root bud inhibition (McIntyre 1979). The leafy spurge root fragments seem to be able to take the most advantage of the removal of that competition for short periods of time – perhaps because after extended periods of exposure, despite competition for water being removed, the wet conditions begin to become unfavorable for other reasons, like increase in rate of decay. Thus, in seasonally flooded areas, not only are root fragments a viable way for leafy spurge populations to spread downstream, but conditions are also potentially ideal for fragments to readily establish new populations, with short exposure to wet moisture conditions producing more new shoots than other moisture and duration of exposure combinations.

Conclusion

Previously, it had only been speculated that leafy spurge root fragments might be able to contribute to the spread of populations in riparian systems. Now, we know that these root fragments do have the potential to establish new populations downstream of source populations; in fact, seasonally flooded riparian ecosystems provide seemingly ideal conditions for those root fragments to readily establish new populations if swept downstream. Documenting the establishment of these populations will be critical in understanding their spread and to reduce the spread and impact of leafy spurge in riparian ecosystems.

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Chapter 4 figures and tables

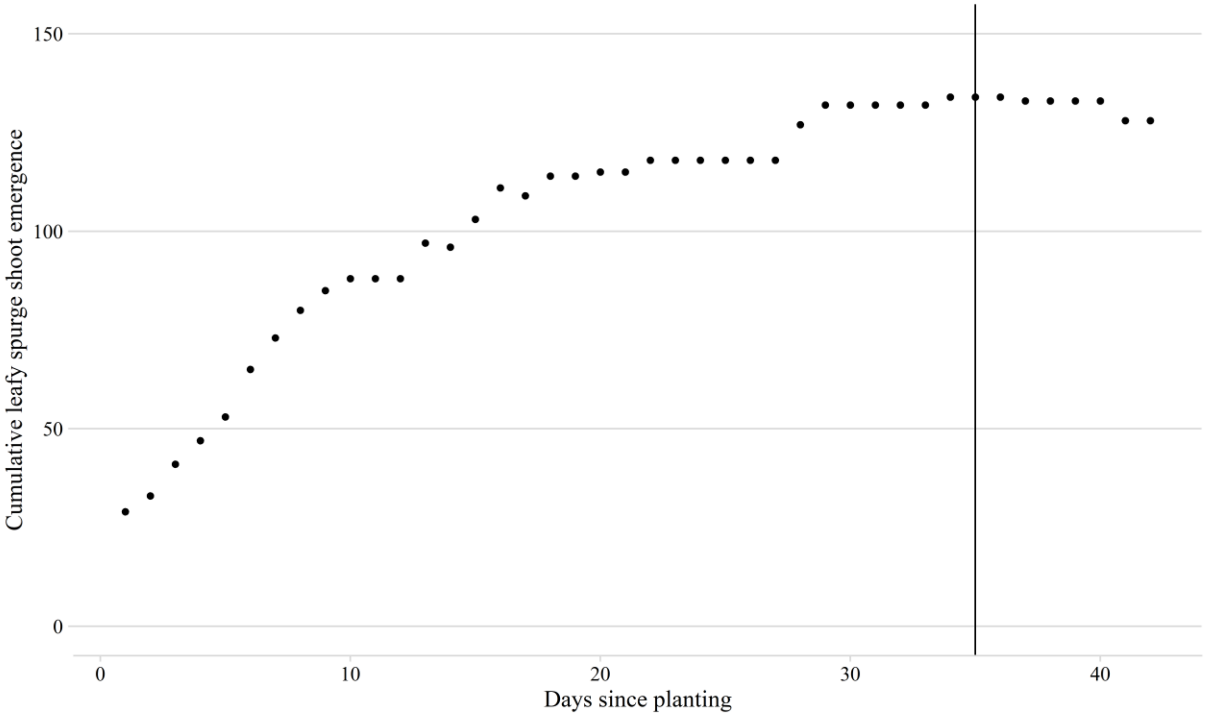

Figure 1. Cumulative distribution function for leafy spurge shoot emergence from root fragments after planting in the greenhouse. Based on the curve, root fragments were left planted for a minimum of 35 days (vertical line) before being dug up and quantified for buds and shoot emergence.

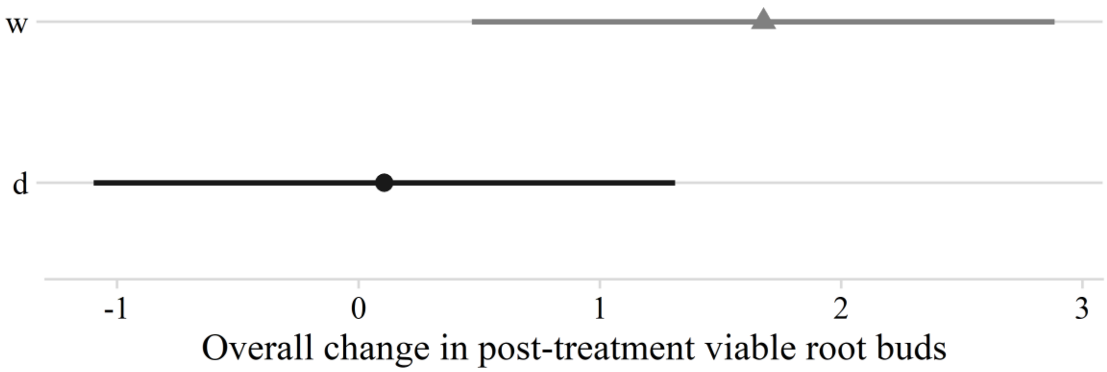

Figure 2. Overall change in post-treatment viable root buds for wet (w) and dry (d) treatments. Root fragments exposed to the wet treatment had a 1.6% increase in viable buds compared to the root fragments exposed to the dry moisture treatment (p = 0.07).

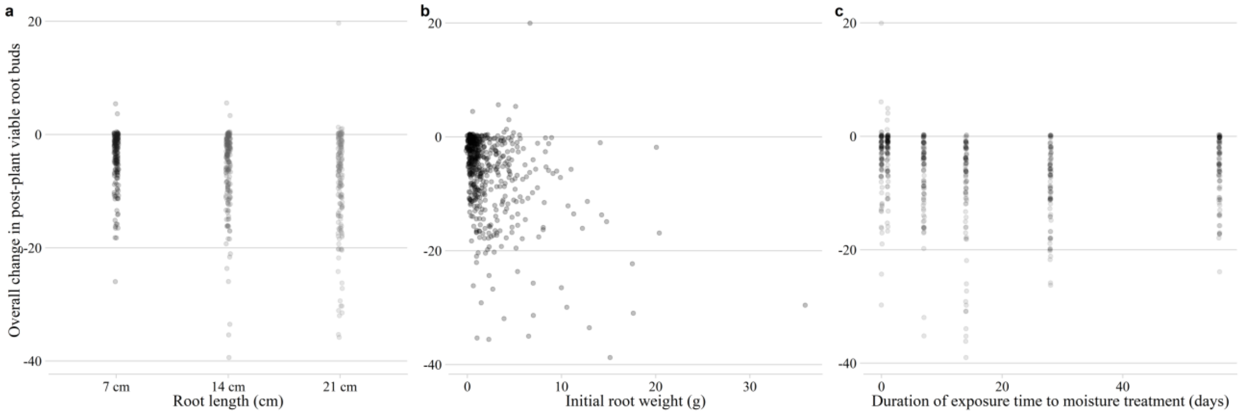

Figure 3. Overall change in post-plant viable root buds for root length (a), initial root weight (b), and duration of exposure time to moisture treatment (c). Longer root fragments had a 0.1% reduction in viable buds for every centimeter of increased root length ($p = 0.053$). Root fragments with heavier initial root weights reduced viable buds by 0.7% for every gram increase of initial weight ($p < 0.001$). Root fragments had a 0.04% reduction in viable buds for every additional day of exposure to treatment ($p = 0.087$).

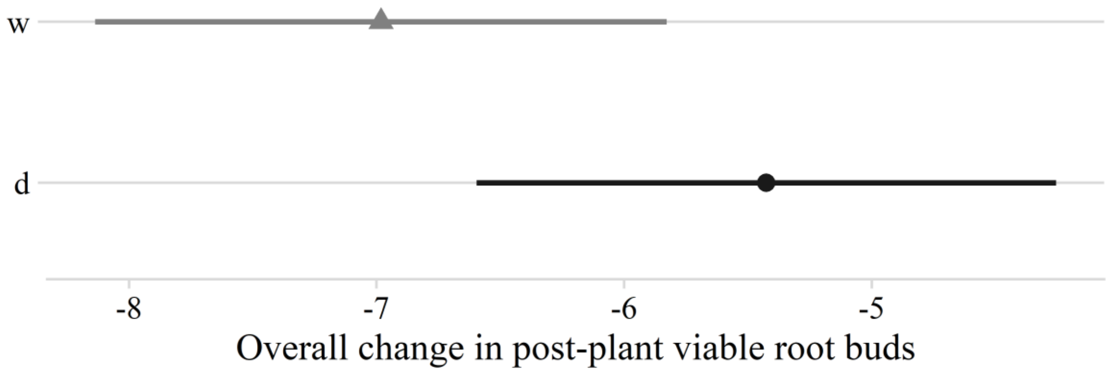

Figure 4. Overall change in post-plant viable root buds for wet (w) and dry (d) treatments. Root fragments exposed to the wet treatment had a 2% decrease inviable buds compared to the root fragments exposed to the dry treatment (p = 0.064).

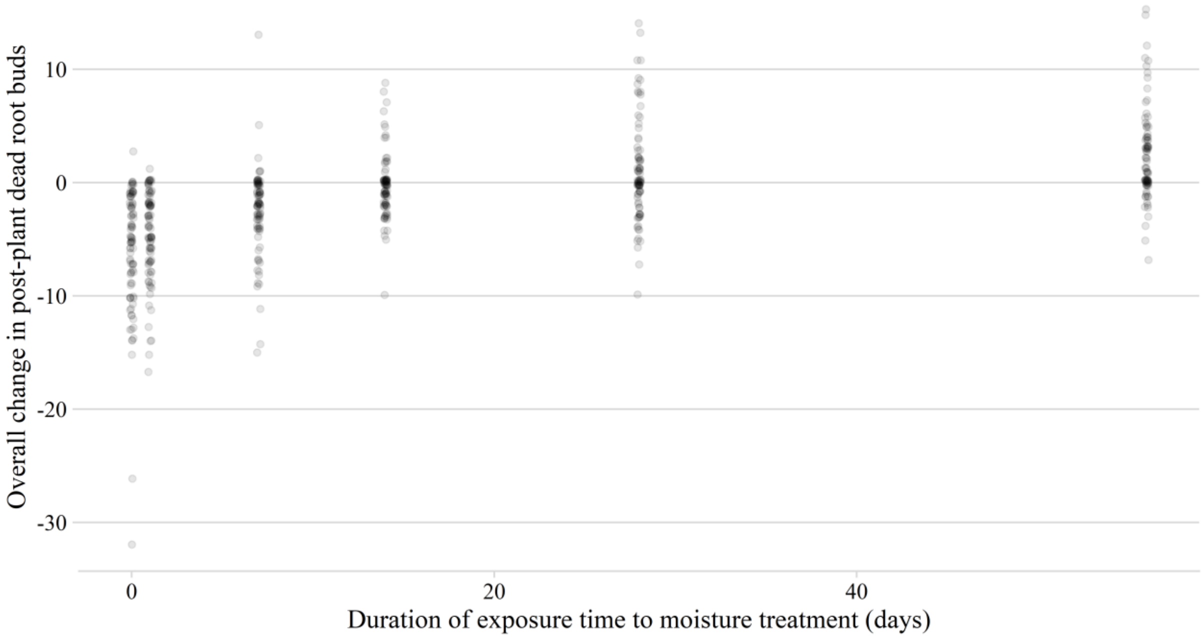

Figure 5. Overall change in post-plant dead root buds for the duration of exposure to the moisture treatments. Root fragments exposed to the moisture treatments for a longer duration of time had a 0.1% increase in dead buds for every additional day of exposure ($p < 0.001$).

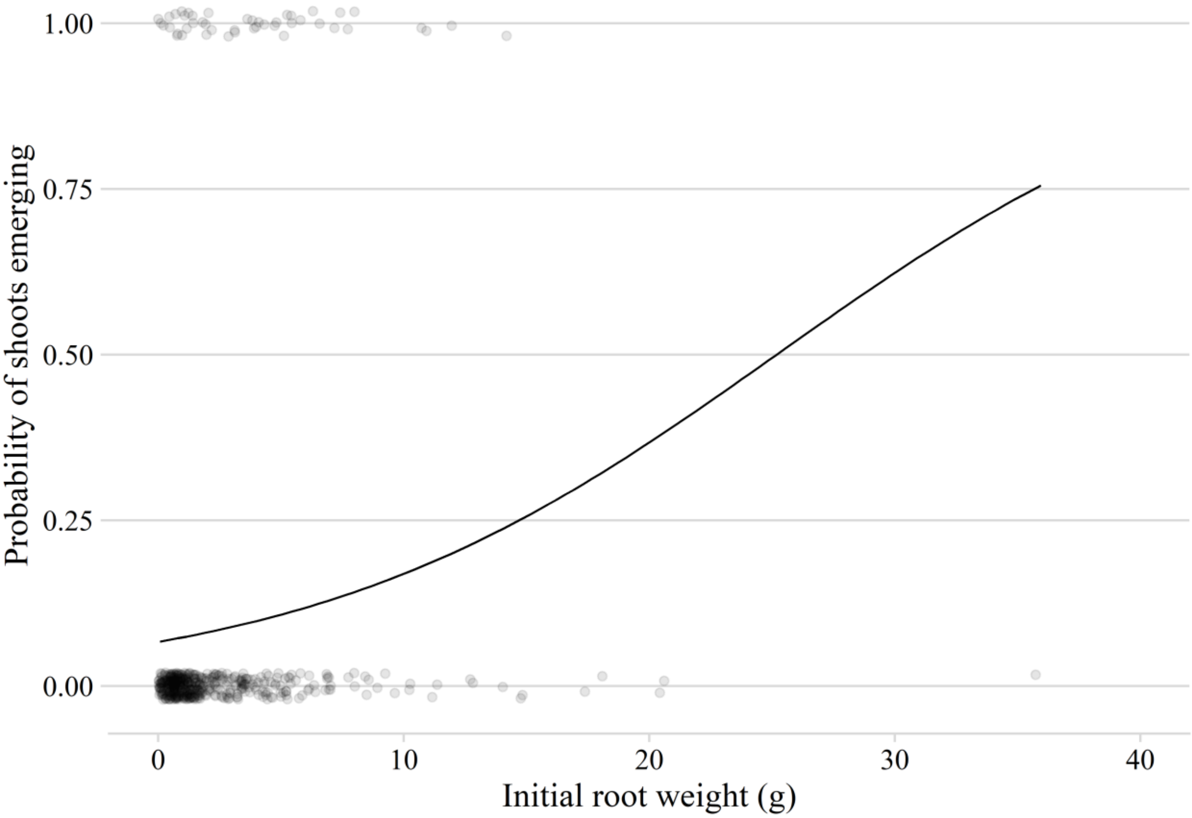

Figure 6. Probability of shoots emerging based on initial root weight (g). Root fragments with heavier initial root weights were more likely to produce shoots (p = 0.007).

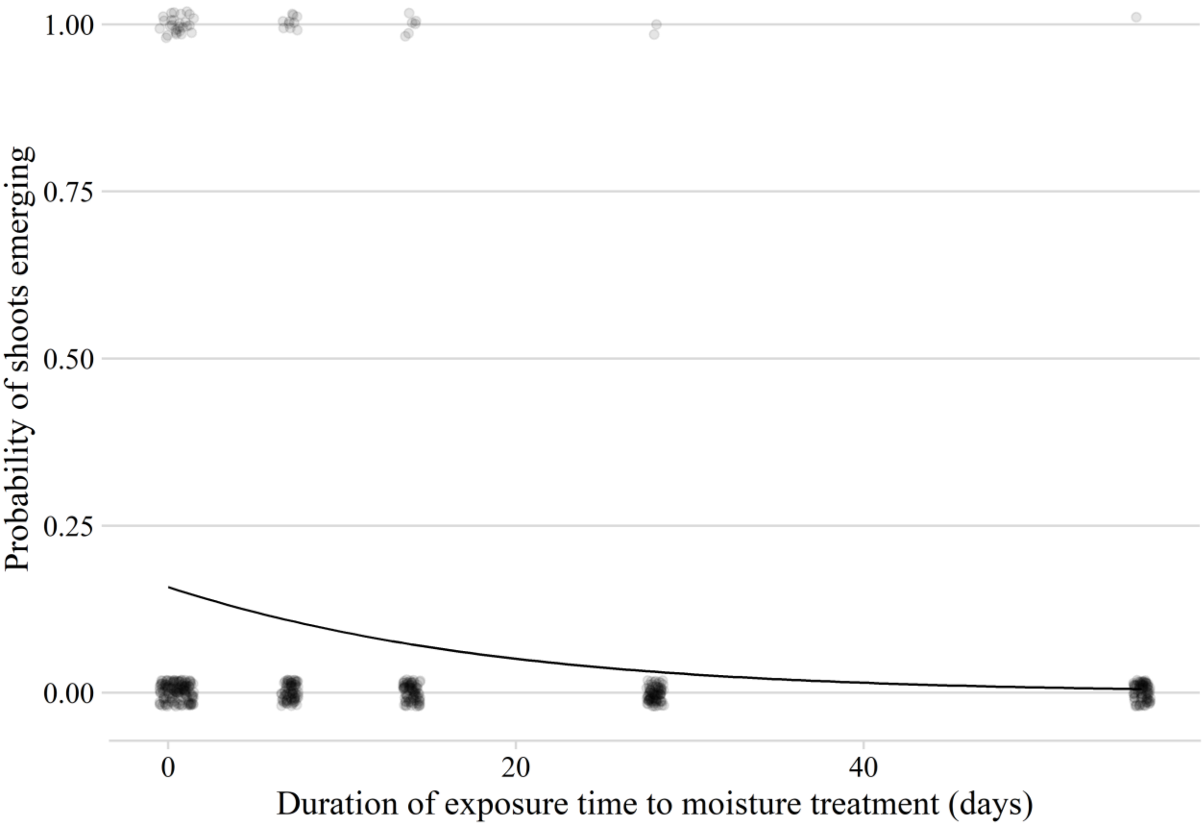

Figure 7. Probability of shoots emerging based on duration of exposure time to moisture treatment (days). Root fragments exposed to a moisture treatment for a shorter period of time were more likely to produce shoots ($p < 0.001$).

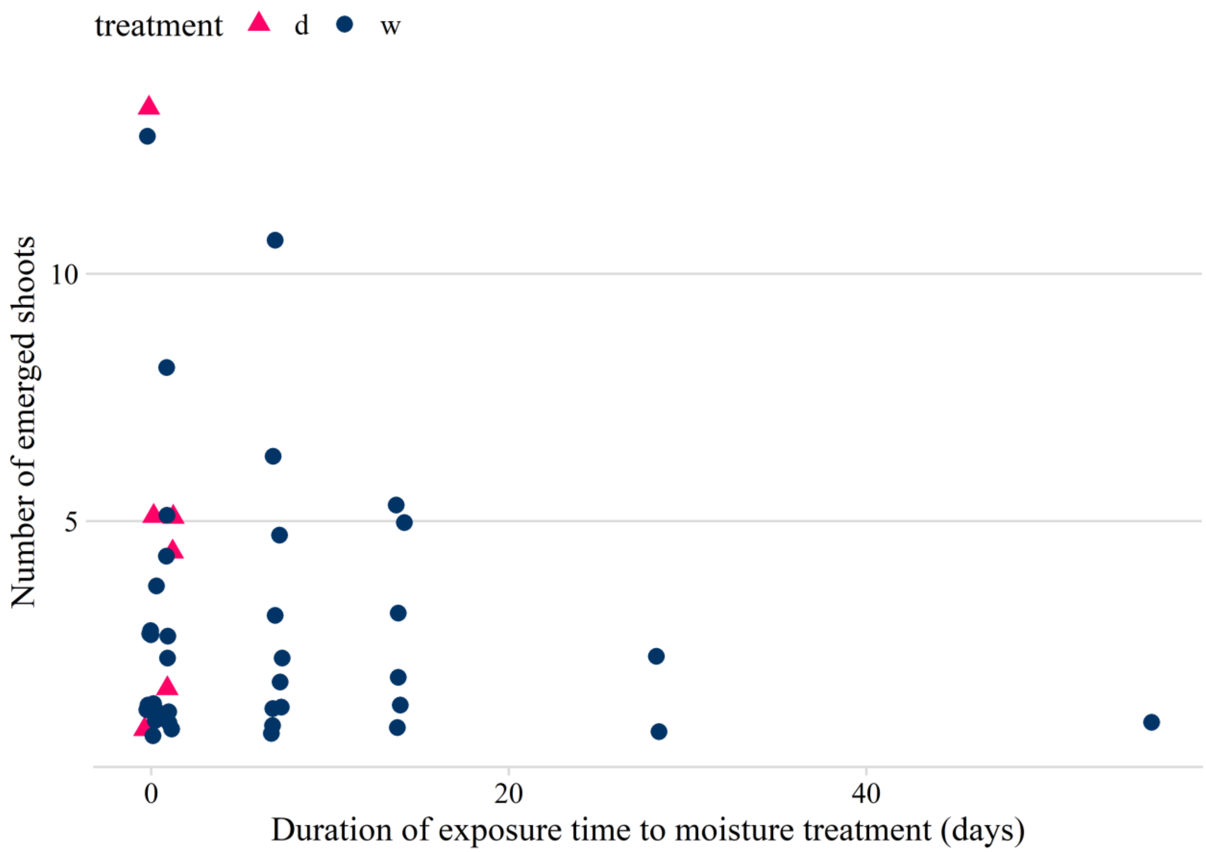

Figure 8. Number of emerged shoots based on an interaction between duration of exposure time and moisture treatment, either wet (w) or dry (d). Results based on the subset of root fragments that had shoots emerge. Root fragments produced more shoots when exposed to the wet moisture treatment for a short amount of time (p = 0.015).

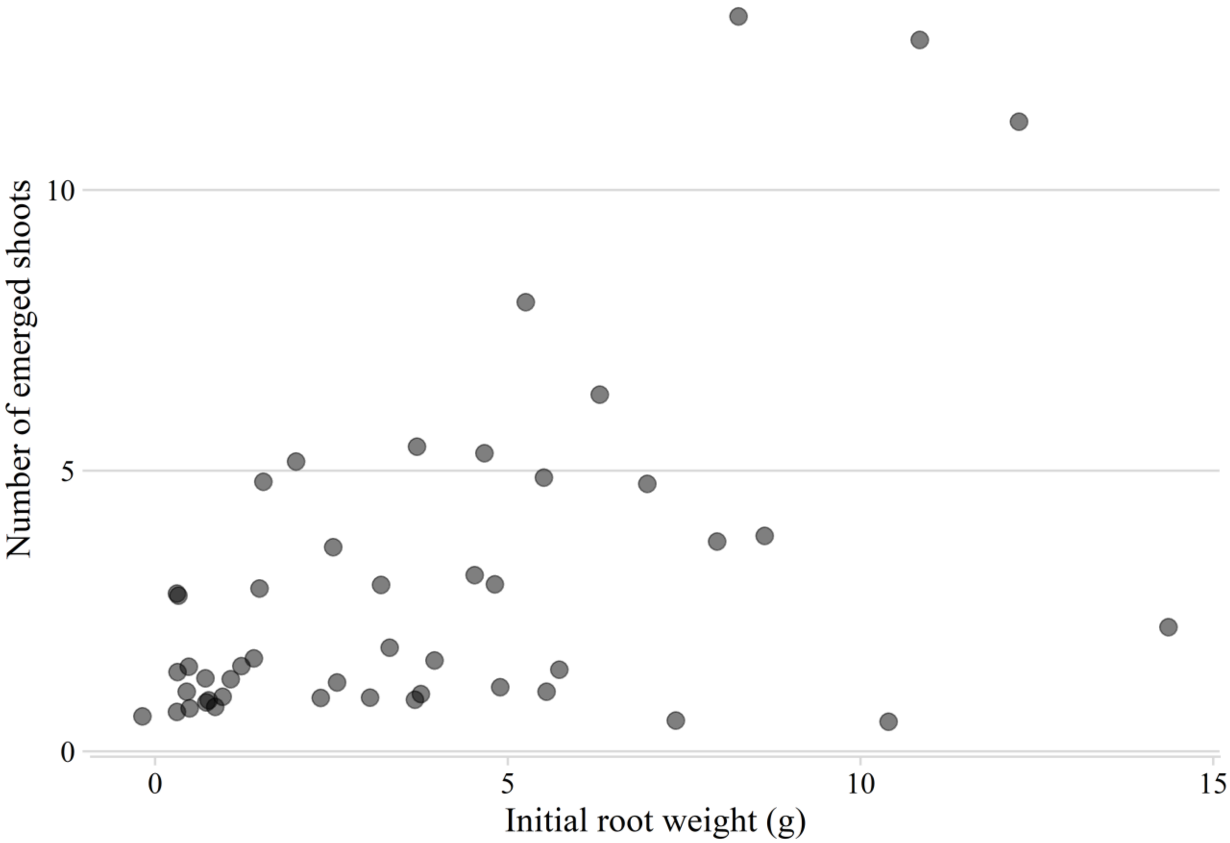

Figure 9. Number of emerged shoots based on initial root weight (g). Root fragments with heavier initial root weights were able to produce more shoots ($p < 0.001$).

Chapter 5: Overall concluding thoughts on leafy spurge seed production, germination, and root bud formation in a riparian ecosystem

The pervasiveness of leafy spurge in North America has caused problems, both ecologically and economically, for decades (Noble *et al*. 1979, Leitch *et al*. 1996, Leistritz *et al*. 2004). As a perennial species, it is able to readily establish itself and, through sexual and asexual reproduction, it is able to persist and spread quickly once introduced (Hanson and Rudd 1933). Leafy spurge has formed near-monocultures across the rangeland systems of the plains and mountain states and, because of this, has been extensively studied in these upland, arid systems (Selleck *et al*. 1962, Messersmith *et al*. 1985, Leitch *et al*. 1996). It is difficult to control, with few options providing any semblance of long-term control (Watson 1985, Lym 1998). Long-term management efforts are still being explored and as the years have passed, leafy spurge has begun to establish itself in other ecosystems as well. Irrigated ditches and streams have often been inception points of leafy spurge populations in new areas (Messersmith *et al*. 1985); however, recent decades have seen leafy spurge begin to take hold of larger waterways and riparian corridors.

Control of leafy spurge in wet or seasonally flooded areas is not well understood and has been studied far less than control in upland, range systems. Chapter 2 explored different management options, and the potential of an integrated management option, to control leafy spurge in the Yampa River Valley, an ecologically and economically important riparian beltway in northwestern Colorado. It will be important to conduct longer-term projects in the future, but for now, we know that in the short-term chemical control is the best option for reducing leafy spurge populations and its subsequent seed production. This agrees with of many of the research conclusions in upland, range systems, where chemicals like picloram (Tordon 22K, Corteva Agriscience) have provided some control in the short-term (Lym and Messersmith 1983, Lym and Messersmith 1994). Picloram cannot be sprayed near water due to environmental contamination concerns and this research focused on four other chemicals that are safe to spray near water lines. In this study, a late-season herbicide application of either aminopyralid + florpyrauxifen-benzyl or quinclorac caused a greater reduction in leafy spurge seed production compared to no herbicide being applied. Further, the combination of aminopyralid + florpyrauxifen-benzyl (DuraCor, Corteva Agriscience) is a newly-labeled herbicide and more research should be done over longer timelines to determine the efficacy of one-time applications compared to reapplying at various intervals over three- or five-year periods.

Water acts as an additional vector for seed dispersal in riparian areas, which is why the focus of management options to control leafy spurge populations was on seed production. Leafy spurge seeds are impacted in other unique ways in seasonally flooded areas aside from additional dispersal aids. In a riparian area, leafy spurge seeds are exposed to an increased water availability in the early growing season, which could impact when seeds are able to germinate at the beginning of a growing season. Chapter 3 aimed to determine if there was an intersection between moisture availability and temperature that would produce optimum conditions for leafy spurge seed germination. Although there was minimal overall germination, the combination of the most available water and the warmest temperature, 0 Ψ and 30 °C, was the only treatment combination that produced meaningful germination results. There were likely underlying expressions of dormancy in the seed source used for this experiment; however, the results support established literature – leafy spurge seeds need moisture to be available in order for them to germinate (Bakke 1936, Best *et al*. 1980). In a riparian ecosystem, water is disseminating leafy spurge seeds throughout the system more quickly than would be possible in an upland, arid system. In combination with leafy spurge seeds requiring optimum amounts of moisture available to germinate, riparian corridors are in huge danger of being overrun with leafy spurge populations, once the plant becomes introduced.

Finally, the main reason for leafy spurge's persistence is often attributed to the root system. In upland, arid areas, this is a problem on its own; however, there is not much worry about root fragments widely dispersing the population, even though they can reproduce asexually (Messersmith *et al*. 1985). In a riparian ecosystem, the root system does have the potential to disperse and establish new populations, aided by the vector of water. It is known that leafy spurge can survive periods of submergence (Selleck *et al*. 1962), and in a wet, seasonally flooded area it stands to reason that leafy spurge root fragments could be a source of population dispersal. Chapter 4 explored the possibility that leafy spurge root fragments could withstand exposure to water over differing periods of time and still produce viable root buds and, subsequently, new shoots. The main takeaway from this research is that short periods of exposure to water provide the optimum conditions for leafy spurge root fragments to produce new shoots. This is important because riparian ecosystems like the Yampa River Valley could be providing these exact conditions in the early growing season when seasonal flooding sweeps root fragments downstream.

Since water can be a vector of dispersal for both seeds and root fragments in riparian ecosystems, which then are subject to seemingly ideal conditions for germination and shoot emergence, it is all the more important to understand how to control leafy spurge populations along waterways. The research in this thesis highlights the persistence and tenacity of leafy spurge populations in riparian ecosystems and underscores the need for more research in such areas. We cannot rely on knowledge of management practices in upland, range systems, when leafy spurge plants are differently influenced in riparian ecosystems and are potentially harder to control, with quicker dispersal and more ideal conditions for establishment.

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Appendix A: Statistical analysis progression of analyzing leafy spurge seed production in the Yampa River Valley, Colorado (Chapter 2)

Seed count data were analyzed in Program R (version 3.6.1). Initially, seed counts were analyzed separately with burst seed counts, capsule seed counts, and bract seed counts all separate response variables. This was done because the distinction was made during the field data collection. It was also an attempt to parse out any differences between treatments on the different stages of seed set for leafy spurge, which could have implications for management timing (e.g., if there are a lot of bract seed counts after a treatment, rather than burst, during peak growing season, perhaps the treatment delayed the formation of capsules/seeds, which is valuable information from a control standpoint).

Burst seed counts within treatment season were analyzed with a generalized linear mixed model with a negative binomial distribution. Capsule seed counts and bract counts within treatment season were analyzed with a generalized linear model with a zero-inflated negative binomial distribution. Two outliers were removed from the bract counts due to extreme values, which were from seemingly random locations and plots. Zero-inflated models were utilized due to a large portion of the count data being zero. Burst seed counts, capsule seed counts, and bract counts one-year post-treatment were analyzed with generalized linear mixed models with a negative binomial distribution.

Within the treatment season, burst seed counts, capsule seed counts, and bract counts were all impacted by the grazing treatment, with the grazed plots producing fewer seeds compared to the plots that were not grazed. There were no clear trends in the herbicide treatments having an effect on the seed production, in any of the three seed production categories.

One-year post-treatment burst seed counts and capsule seed counts were not significantly affected by the grazing treatment but were significantly reduced by the application of aminopyralid + florpyrauxifen-benzyl. No other herbicides affected burst and capsule seed counts one-year post-treatment and there were no interactions between the grazing and herbicide treatments. This is not aligned with the hypothesized synergistic effect of treatment combinations one-year post-treatment. It does make sense, though, that there was an effect of an herbicide application, while the effect of grazing seems to have worn off. Bract counts one-year post-treatment were significantly reduced by all treatments and treatment combinations when compared to no applied treatments (grazing or herbicide) which could speak to a possible synergistic effect of treatment combinations for reducing the quantity of seeds produced by leafy spurge plants in the later parts of the growing season. However, there are no clear trends to fit this in with burst or capsule seed production one-year post-treatment and may ultimately be of no ecological significance.

Overall, analyzing the data at each separate seed count level did not provide a clear picture and the seed counts for within treatment season and one-year post-treatment season were combined to form a single total seed count response variable for each year. This analysis is what is described in the main body of Chapter 2.

Appendix A figures and tables

Table 1. Generalized linear mixed model (negative binomial) output for burst seed counts within treatment season (2019) – bold values represent significance (α = 0.05)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**7.087**	**0.341**	**20.75**	**< 2e^{-16}**
Grazed	**-0.37**	**0.128**	**-2.89**	**0.0039**
quinclorac	0.353	0.199	1.78	0.0754
aminopyralid	**0.407**	**0.198**	**2.06**	**0.0393**
imazapic	**0.517**	**0.199**	**2.59**	**0.0095**
aminopyralid + florpyrauxifen-benzyl	0.384	0.198	1.94	0.0527

Table 2. Generalized linear model (zero-inflated negative binomial – zero-inflated half) output for capsule seed counts within treatment season (2019) – bold values represent significance (α = 0.05)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**-1.21553**	**0.56745**	**-2.142**	**0.03219**
Grazed	0.99039	0.72696	1.362	0.17308
quinclorac	0.52164	0.75018	0.695	0.48684
aminopyralid	0.53885	0.74656	0.722	0.47043
imazapic	**2.59047**	**0.79710**	**3.250**	**0.00115**
aminopyralid + florpyrauxifen-benzyl	0.28968	0.76568	0.378	0.70519
Grazed:quinclorac	-0.33735	0.98954	-0.341	0.73317
Grazed:aminopyralid	-0.74129	0.98941	-0.749	0.45372
Grazed:imazapic	**-2.19619**	**1.02524**	**-2.142**	**0.03218**
Grazed:aminopyralid + florpyrauxifen--benzyl	0.09974	1.0047	0.1	0.92066

Table 3. Generalized linear model (zero-inflated negative binomial – conditional half) output for capsule seed counts within treatment season (2019) – bold values represent significance (α = 0.05)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**3.82781**	**0.27308**	**14.017**	**< 2e^{-16}**
Grazed	**1.03437**	**0.41816**	**2.474**	**0.0134**
quinclorac	0.09937	0.40025	0.248	0.8039
aminopyralid	0.38088	0.39984	0.953	0.3408
imazapic	**1.23979**	**0.59177**	**2.095**	**0.0362**
aminopyralid + florpyrauxifen-benzyl	0.21951	0.39243	0.559	0.5758
Grazed:quinclorac	-0.77558	0.60965	-1.272	0.2033
Grazed:aminopyralid	-0.14136	0.59341	-0.238	0.8117
Grazed:imazapic	**-1.73855**	**0.75749**	**-2.295**	**0.0217**
Grazed:aminopyralid + florpyrauxifen-benzyl	-0.87552	0.61459	-1.425	0.1543

Table 4. Generalized linear model (zero-inflated negative binomial – zero-inflated half) output for bract counts within treatment season (2019) – bold values represent significance (α = 0.05)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	0.16505	0.35511	0.465	0.6421
Grazed	0.03169	0.29997	0.106	0.9159
quinclorac	0.6197	0.47339	1.309	0.1905
aminopyralid	**-1.06438**	**0.47771**	**-2.228**	**0.0259**
imazapic	0.06581	0.45634	0.144	0.8853
aminopyralid + florpyrauxifen-benzyl	0.43272	0.46177	0.937	0.3487

Table 5. Generalized linear model (zero-inflated negative binomial – conditional half) output for bract counts within treatment season (2019) – bold values represent significance ($\alpha = 0.05$)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**4.28462**	**0.30315**	**14.134**	**< $2e^{-16}$**
Grazed	**1.46656**	**0.23699**	**6.188**	**$6.09e^{-10}$**
quinclorac	0.40994	0.38483	1.065	0.2868
aminopyralid	-0.05169	0.31861	-0.162	0.8711
imazapic	0.54254	0.35796	1.516	0.1296
aminopyralid + florpyrauxifen-benzyl	**0.96585**	**0.39471**	**2.447**	**0.0144**

Table 6. Generalized linear mixed model (negative binomial) output for burst seed counts one-year post-treatment season (2020) – bold values represent significance (α = 0.05)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**5.966**	**0.893**	**6.68**	**$2.4e^{-11}$**
Grazed	0.428	0.401	1.07	0.2857
quinclorac	-0.986	0.702	-1.41	0.16
aminopyralid	-0.19	0.566	-0.34	0.7371
imazapic	0.72	0.815	0.88	0.3769
aminopyralid + florpyrauxifen-benzyl	**-1.763**	**0.565**	**-3.12**	**0.0018**

Table 7. Generalized linear mixed model (negative binomial) output for capsule seed counts one-year post-treatment season (2020) – bold values represent significance ($\alpha = 0.05$)

Treatment	Beta Estimate	Standard Error	Z-value	P-value
(Intercept)	**6.355**	**0.619**	**10.26**	**< 2e^{-16}**
Grazed	0.208	0.299	0.7	0.487
quinclorac	-0.64	0.477	-1.34	0.18
aminopyralid	-0.175	0.452	-0.39	0.699
imazapic	-0.351	0.492	-0.71	0.476
aminopyralid + florpyrauxifen-benzyl	**-0.99**	**0.451**	**-2.20**	**0.028**

Table 8. Generalized linear mixed model (negative binomial) output for bract counts one-year post-treatment season (2020) – bold values represent significance (α = 0.05)

Treatment	**Beta Estimate**	**Standard Error**	**Z-value**	**P-value**
(Intercept)	**5.883**	**0.948**	**6.21**	**$5.4e^{-10}$**
Grazed	**-3.506**	**0.817**	**-4.29**	**$1.8e^{-05}$**
quinclorac	**-3.052**	**0.806**	**-3.78**	**0.00015**
aminopyralid	**-1.716**	**0.778**	**-2.21**	**0.02733**
imazapic	**-1.962**	**0.769**	**-2.55**	**0.01077**
aminopyralid + florpyrauxifen-benzyl	**-1.786**	**0.78**	**-2.29**	**0.02199**
Grazed:quinclorac	**3.7**	**1.104**	**3.35**	**0.00081**
Grazed:aminopyralid	**3.411**	**1.067**	**3.2**	**0.00138**
Grazed:imazapic	**3.559**	**1.129**	**3.15**	**0.00161**
Grazed:aminopyralid + florpyrauxifen-benzyl	**2.729**	**1.148**	**2.38**	**0.01745**