GRANTEE: **FRIENDS OF THE YAMPA**
PROJECT NAME: **YAMPA RIVER LEAFY SPURGE PROJECT**
ROUNDTABLE: **Yampa-White-Green**
[NTP Date: 19 November 2018]

General Project Status

- Most Tasks described in the Yampa River Leafy Spurge Project (YRLSP) are nearing completion, with minor exceptions due to Covid-19 impacts, specifically related to travel restrictions and postponement of outreach events.
- A request for extension of the period of performance from end date of 30 June 2021 to end date of 31 December 2021 was approved by CWCB on 23 November 2020. The extension will allow for completion of tasks impacted by Covid-19 restrictions.
- In-kind contributions continue to accrue, in excess of anticipated totals, from volunteers, partners and additional cooperators. Several volunteer activities are planned for June, 2021.
- In 2019 leafy spurge was mapped along approximately 60 miles of the Yampa River, from Hayden through Little Yampa Canyon. In 2020 an additional 50 miles were completed, from the mouth of Little Yampa Canyon downstream to the head of Cross Mountain Canyon. Related mapping was also completed on the Yampa River State Wildlife Area and on BLM land in Tepee Draw (tributary of the Yampa River, north of Dinosaur National Monument).
- View maps on our web site: https://www.yampariverleafyspurgeproject.com/mapping.
- A small YRLSP volunteer team traveled to the Front Range in mid-June 2020 to work with CDA to collect and process 13,000 biological control insects for transport back to our project area, in an effort to bolster local biocontrol insect populations. Insects were released on 13 sites in Routt and Moffat counties over a two-day period, with assistance from CPW & BLM.
- CPW purchased 10,000 additional insects, which were distributed on the State Wildlife Area.
- The 2020 youth engagement event is rescheduled to June 29-30, 2021, due to Covid-19.

<table>
<tr><th colspan="6">YRLSP BUDGET—SUMMARY—15 May 2021</th></tr>
<tr><th>CONTRIBUTOR</th><th>AMOUNT Committed</th><th colspan="2">% of TOTAL</th><th>AMOUNT Contributed or Invoiced To-Date</th><th>% of Total Project Commitment</th></tr>
<tr><td colspan="6">CASH</td></tr>
<tr><td>YWG Basin WSRF Request</td><td>$ 89,000</td><td>54%</td><td>54%</td><td>$ 87,431</td><td>98%</td></tr>
<tr><td>Moffat County</td><td>15,000</td><td>9%</td><td rowspan="3">26%</td><td>$ 15,000</td><td>100%</td></tr>
<tr><td>Routt County</td><td>15,000</td><td>9%</td><td>$ 15,000</td><td>100%</td></tr>
<tr><td>University of Wyoming</td><td>12,572</td><td>8%</td><td>$ 12,572</td><td>100%</td></tr>
<tr><td colspan="6">IN-KIND</td></tr>
<tr><td>YRLSP volunteers</td><td>20,000</td><td>12%</td><td rowspan="2">20%</td><td>$ 27,300</td><td>137%</td></tr>
<tr><td>Other Partners (BLM, NPS, TNC, CDA, CPW, Moffat County, Routt County, CSU Extension)</td><td>14,000</td><td>8%</td><td>$ 14,529</td><td>104%</td></tr>
<tr><td>TOTAL PROJECT COST</td><td>$ 165,572</td><td></td><td></td><td></td><td></td></tr>
</table>

Status of Tasks Identified in the Statement of Work

Task #1 [$40,900 allocated from CWCB/YWG Basin account—99.1% invoiced—estimated percent completion for Task #1 = 90%]

Develop a watershed scale management framework for leafy spurge in the Yampa Valley through mapping and predictive modelling.

This task involves two distinct components:

1. Field mapping of leafy spurge in riparian habitat along the Yampa River—conducted by YRLSP volunteers.
2. Geospatial analysis, remote sensing and predictive modelling—conducted by the University of Wyoming.

Field Mapping Report

- YRLSP volunteer Peter Williams developed and maintains GIS products and systems to facilitate field mapping of leafy spurge, using electronic tablets.
- YRLSP volunteers John Husband and Ben Beall developed a landowner permission/access form and tracked down busy landowners to seek permission for field mapping of more than 100 miles of the Yampa River from Hayden downstream to the head of Cross Mountain Canyon.
- In 2019 and 2020, Peter Williams and Ben Beall, with logistical assistance from additional volunteers, mapped leafy spurge along both banks (where permission allowed) of that same 100+-mile reach. The maps resulting from this work are available on the YRLSP web site: https://www.yampariverleafyspurgeproject.com/mapping.
- Many landowners and/or managers granted permission for accessing land along the river for mapping and data sharing. In cases where permission was not granted, leafy spurge was mapped from rafts only by visual inspection, but the resulting data is not visible on our public web site. If future permissions are obtained, this data can be unmasked.
- Leafy spurge mapping data were provided to the University of Wyoming for use in their spatial analysis and predictive modelling work.
- Plans for 2021 include completion of mapping in a 5-mile reach between Government Bridge and Juniper Mountain boat ramp. We also hope to map the reach from Cross Mountain Canyon to Dinosaur National Monument, if permission can be obtained. This work will complete all of the planned field mapping in the scope of work for this grant.
- The mapping team is also working on plans to assist UW graduate student Chloe Mattilio with a field-based accuracy assessment of her remote sensing project in June of 2021. The results of Chloe's modelling effort have exceeded our expectations and we are pleased to be able to provide logistical support to help her refine the model further.

University of Wyoming Report

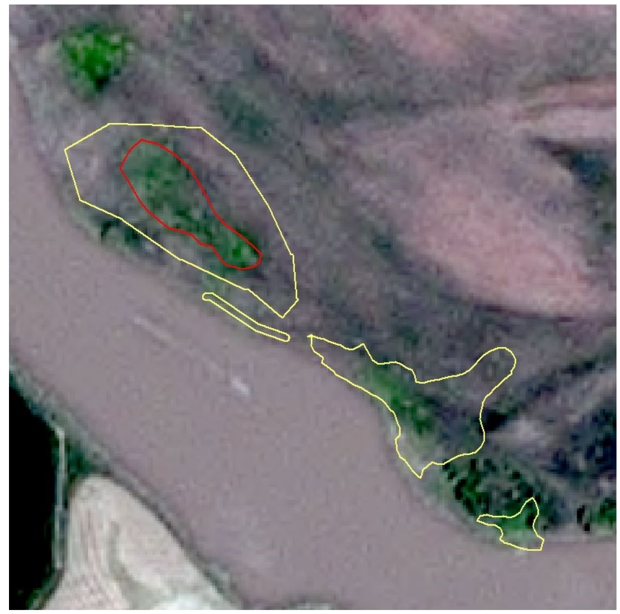
(Submitted by Chloe Mattilio – University of Wyoming, Department of Plant Sciences – 6 May 2021)

Update on Leafy Spurge Mapping with Remote Sensing and Invasion Susceptibility Modeling

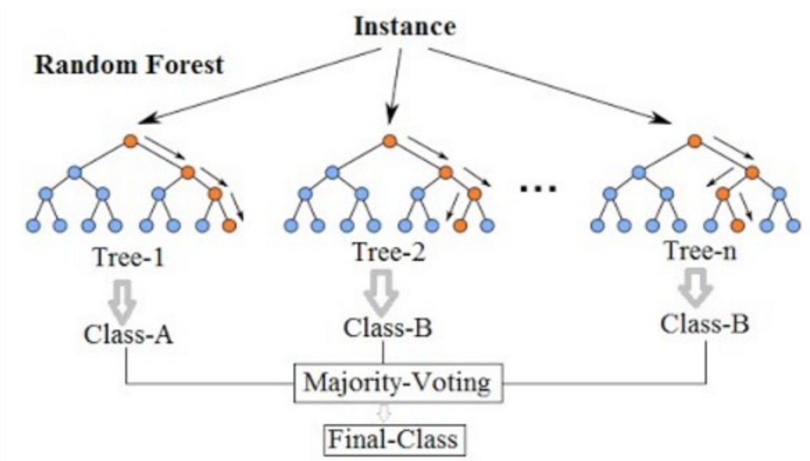
Results from Final Random Forest Classification

Using our training/validation set of

- Presence polygons for leafy spurge, based on extensive mapping on the Yampa River, knowledge of the area, and imagery interpretation(image show, right)
- Absence polygons, that capture the range of various landcover features



We classified all pixels in the imagery as “spurge” or “not spurge” using a Random Forest machine learning method. Random Forest grows a “forest” of decision trees, with branches splitting and sorting pixels into a set of classes (figure below)



In this figure, the “instance” is a pixel

The various decision trees classify the pixel, using all of the imagery bands

The results of the trees are pooled, and the pixel is classified according to the majority class voted for across all decision trees

In this classification, 351 trees were grown, to ensure all pixels were classified many times.

Resulting internal validation shows 8.24% overall classification error, with lower classification error for “spurge” than for the “not spurge” class.

Overall, this is an acceptable, and encouraging classification result!

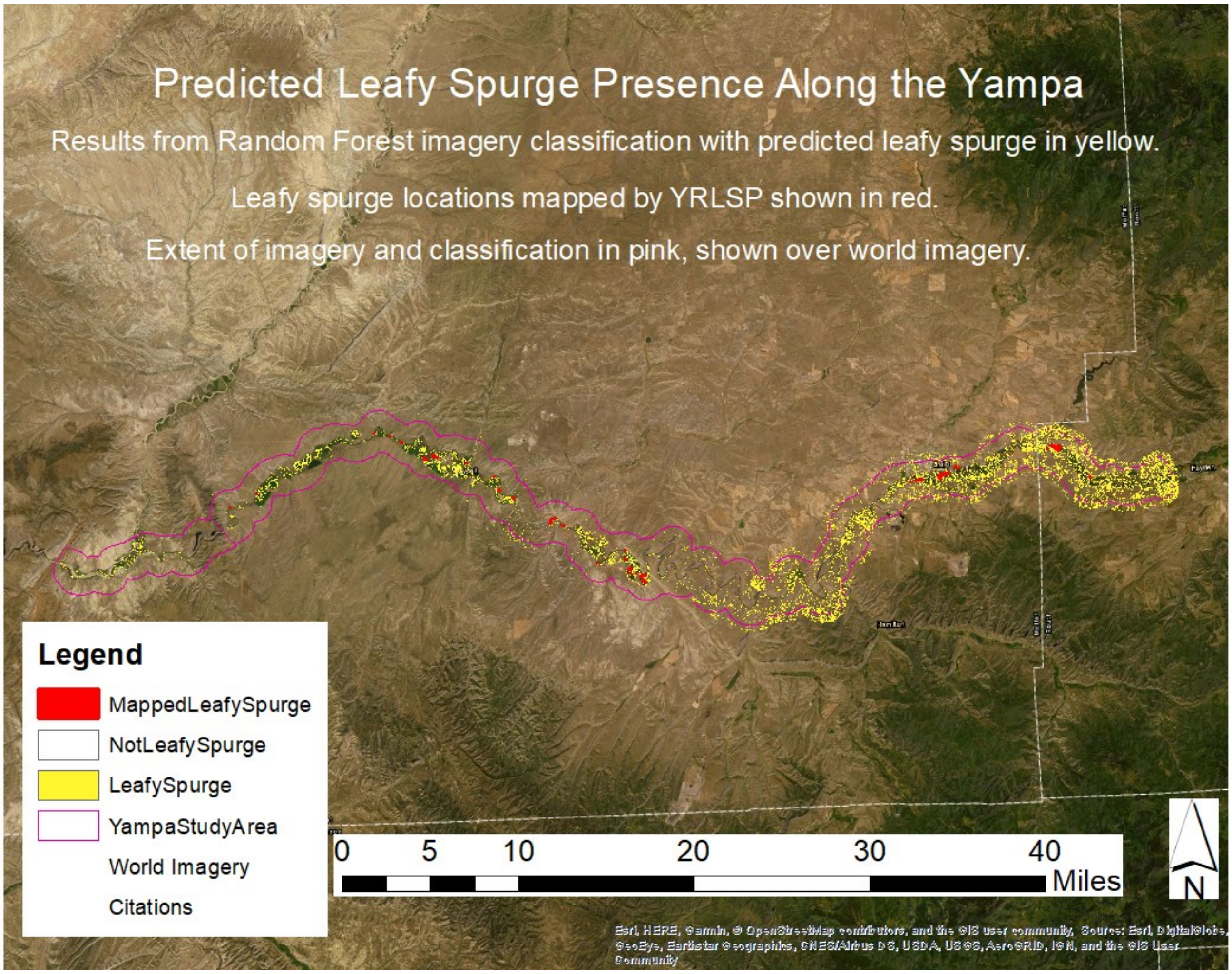
Producer’s Accuracy for leafy spurge is 89.58%, meaning that 89.58% of ground leafy spurge was correctly shown in the classified map.

User’s Accuracy for leafy spurge is 95.55%, meaning that 95.55% of classified leafy spurge on the map will align with what is on the ground.

By comparing these multiple accuracy assessments, we estimate that 95.55% of our classified leafy spurge pixels match the leafy spurge training and validation data set provided.

<table>
<tr><td colspan="2" rowspan="2"></td><td colspan="4">Data from validation mapping</td></tr>
<tr><td></td><td></td><td>Totals</td><td>User’s Accuracy</td></tr>
<tr><td rowspan="4">Class assigned from imagery classification</td><td></td><td>86
Correctly classed leafy spurge</td><td>4
Not-spurge incorrectly classed as spurge</td><td>90
(86 + 4)</td><td>95.55
(86 / 90) *100</td></tr>
<tr><td></td><td>10
Spurge, incorrectly classed as not-spurge</td><td>70
Not-spurge correctly classed as not-spurge</td><td>80
(10 + 70)</td><td>87.5
(70 / 80) * 100</td></tr>
<tr><td>Totals</td><td>96
(86 + 10)</td><td>74
(4 + 70)</td><td>170</td><td></td></tr>
<tr><td>Producer’s Accuracy</td><td>89.58
(86 / 96) *100</td><td>94.59
(70 / 74) *100</td><td></td><td>Total Accuracy 91.76%</td></tr>
</table>

A map of predicted leafy spurge by this Random Forest Classification is shown below.



Within this leafy spurge identification workflow, we have:

1. Mapped leafy spurge locations that were marked with GPS by the Yampa River Leafy Spurge Project, in points, lines, and polygon form (shown in red in map above).

2. Multispectral remote sensing imagery from summer of 2019 with red, green, blue, and near infrared (NIR) bands, covering study extent, in raster form.

3. Spectral training samples that were digitized from imagery interpretation and ground mapping reference data for leafy spurge presence and absence, in polygon form.

4. Classified imagery results with a binary prediction of leafy spurge presence and absence from Random Forest classification, in raster form then converted to polygons for further comparison (shown in yellow and transparent in map above).

Comparing Spectral Properties of Classified and Missed Leafy Spurge

I am interested in the differences in reflectance for correctly classified and incorrectly classified leafy spurge. To investigate differences in reflectance for red, green, blue, and near infrared (NIR) light, I selected correctly and incorrectly classified leafy spurge from my Random Forest classification map, extracted reflectance values for all four bands for each class, and combined them into a single data frame. To test differences in reflectance, I performed a two-way analysis of variance (ANOVA) testing for correctly classified and missed leafy spurge polygons. Correctly classified leafy spurge mean reflectance was not significantly different from missed leafy spurge mean reflectance for the red, green, and blue bands of light, but did have significantly higher reflectance for the NIR band of light (p-value 0.0305). Though differences were not tested, correctly classified leafy spurge polygons had smaller standard deviations than missed leafy spurge in each band of light (See table below).

	Band of Light of Multispectral Imagery											
	Red			Green			Blue			NIR		
Class	MRef	SDRef	p-value	MRef	SDRef	p-value	MRef	SDRef	p-value	MRef	SDRef	p-value
Spurge	307.67	102.38	0.801	439.93	79.5	0.149	368.44	51.54	0.626	1359.4	261.06	0.0305*
Missed Spurge	309.31	123.89		432.68	95.27		366.86	61.6		1324.98	285.12	

Table 1. Results table from two-way ANOVA testing for differences between reflectance in each band of light (red, green, blue, and NIR) for correctly classified (Spurge) and incorrectly classified (MissedSpurge) leafy spurge polygons. Values shown are mean reflectance (MRef), standard deviation of reflectance (SDRef), and p-values (p-value) testing the differences between the class means for each band of light. P-values marked with * are significantly different.

Summer Validation Mapping of Leafy Spurge

Based on our classification and subsequent testing, we have a good idea of where classification performance varies, but not necessarily why classification performance varies there. To understand factors that influence classification accuracy on the ground, we have planned for summer validation mapping by foot and by raft along the Yampa. We have identified locations of interest based on their cover type, accessibility, and range of spurge populations present (according to 2020 cumulative YRLSP mapping efforts). These locations cover a broad range of habitat types, including riparian forest, riparian meadows, irrigated agricultural lands, upland vegetation, and rangeland riparian tributaries. All these locations have been compiled into a spatial data frame with coordinates, ground mapping status (and accompanying data from YRLSP mapping efforts) and classification status. Additional parameters that will be collected in the field while validation mapping are presence/absence of leafy spurge, leafy spurge population size, leafy spurge abundance, vegetation type, geomorphology, proportional bare ground, and canopy coverage. We will use this validation dataset to improve presence mapping, inform presence map interpretation, and predict leafy spurge invasion susceptibility.

Invasion Susceptibility Modeling of Leafy Spurge on the Yampa River

Ecological niche modeling (ENM) has been completed for Fremont County, WY, using the following environmental predictors: Soil type, texture, pH, and flooding frequency, temperature and precipitation annual and monthly means, temperature and precipitation annual and monthly variation, annual and monthly solar radiation, distance to nearest population of leafy spurge, and slope, elevation, and aspect. ENM was completed using the ENTML package in Program R. This model will be shifted to the Yampa River basin using the same predictors, but for our study area, and leafy spurge invasion susceptibility will be predicted continuously. This work will be completed by early June, 2021.

Task #2 [$40,800 allocated from CWCB/YWG Basin account—99.4% invoiced—estimated percent completion for Task #2 = 95%]

Identify best integrated management practices for reducing leafy spurge seed production in riparian habitat in the Yampa Valley.

YRLSP received permission to access many private parcels for research purposes. The University of Wyoming team found suitable conditions on two private parcels, one Moffat County parcel, and one Colorado Trust Land parcel. We are grateful for the amount of community support received from landowners and public agencies. One of the private parcels was withdrawn from the study due to changing management priorities of the landowner.

UW graduate student Hannah Kuhns has submitted the first chapter of her Master's thesis as her progress report for inclusion in this document. She will be submitting her completed Master's thesis in the next several months as a final deliverable. She also presented her thesis seminar on April 9, 2021. A video of her excellent presentation is available on the Yampa River Leafy Spurge Project website: https://www.yampariverleafyspurgeproject.com/hannahdefense

University of Wyoming Report
(Submitted by Hannah Kuhns – Master's student – University of Wyoming, Department of Plant Sciences – 26 April 2021)

In the past six months I have solidified the statistical analyses for the data collected in 2019 and 2020. The report included below is a nearly final draft of my first thesis chapter and will also be the template for publication later this year.

Otherwise, the data collection for the project exploring the relationship between root fragment size, moisture exposure time, and root bud formation/shoot emergence was completed in November 2020. The data was worked up and presented in conjunction with the dataset from the Yampa River Valley on April 9, 2021 during my public presentation, which can now be accessed

on the Yampa River Leafy Spurge Project website:
https://www.yampariverleafyspurgeproject.com/.

Data collection for the project exploring the relationship between a temperature and moisture gradient on leafy spurge seed germination was completed in March 2021. I am currently working with the data now.

Chapter 1: Integrated management of leafy spurge (*Euphorbia esula* L.) seed production in a riparian ecosystem

Introduction

Leafy spurge 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 K, Dow AgroSciences). 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 the ecologically rare riparian forest habitat.

In the Yampa River Valley, Colorado, 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, 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 achieve long-term control 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 flagged in May 2019. Sites were selected based on leafy spurge density, ease of accessibility 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 (Location 1, Location 2, Location 3) are in Craig, Colorado along the Yampa River while one site is directly north of Craig, Colorado along Fourmile Creek (Location 4). In Craig, Location 1 is a hay meadow site that is hayed annually, while Location 2 is a riparian edge that is directly adjacent to an annually hayed area. Location 3 is not utilized for grazing or haying but is adjacent to a property that is utilized for cattle grazing. Location 4 is along a tributary of the Little Snake River, which confluences with the Yampa River in western Colorado. Location 4 is utilized as rangeland and is 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 did not receive herbicide treatments and was not visited again.

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 was applied to areas that had either been grazed or not grazed. In the plots that had already received a grazing treatment, the hope is 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 and seven Hampshire blackface ewes grazed for a full day, for a stocking rate of 203 sheep/acre. Due to travel restraints, multiple sites were grazed for two half days to equal a total grazing time of one full day. Location 1 was grazed for two half days on May 28, 2019 and May 31, 2019 for a total of 12 hours of grazing. Location 2 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. Location 3 was grazed for a full day on May 29, 2019 for a total of 10 hours of grazing. Location 4 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 Location 1, Location 2, and Location 3 and on September 14, 2019 for Location 4. 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 Location 1 on July 26, 2020. At Location 2, Location 3, and Location 4 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 were 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, Rinskor active, 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; 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*) and timothy (*Phleum pratense*) 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 an error term of plot within location. 2020 total seed counts at the quadrat level were analyzed using a zero inflated approach with a binomial logistic regression. 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 an error term 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 as well as a fixed effect of location. 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. Plots that were grazed reduced total seed production by 40% when compared with plots that were not grazed (Figure 1). Herbicide treatments did not have a significant effect on total seed counts.

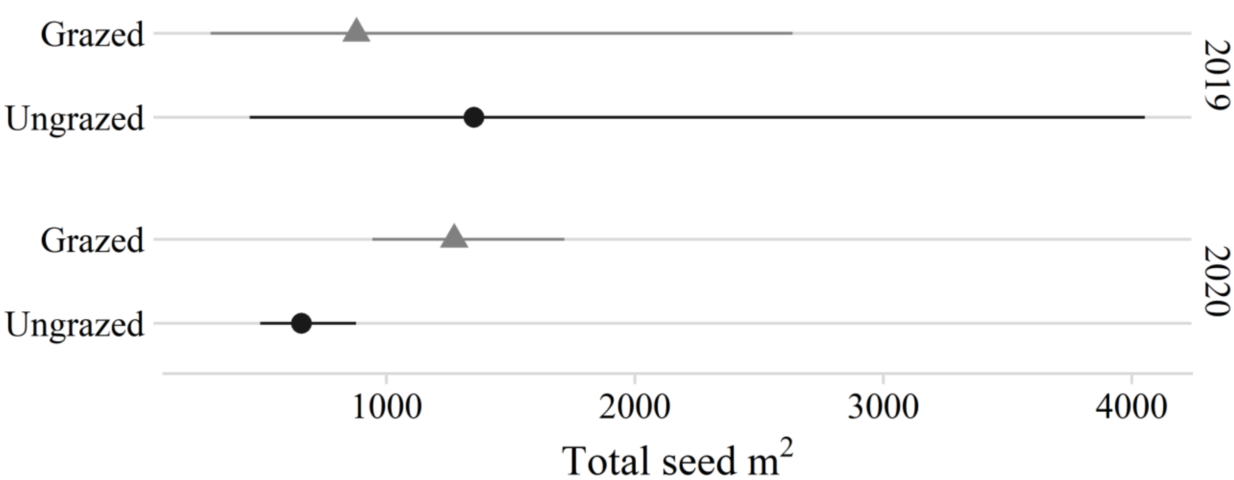


Figure 1. Within treatment season (2019) and one-year post-treatment season effect of grazing on leafy spurge total seed production (error bars represent 95% confidence intervals)

Total cover and leafy spurge cover were not significantly affected by an interaction between the grazing and herbicide factors. Individually, neither treatment significantly affected total cover or leafy spurge cover.

There was no significant impact of an interaction between the grazing and herbicide factors on the presence or absence of resident vegetation. Individually, the grazing and herbicide treatments did not have an effect on presence or absence of resident vegetation cover, neither did location. Of the resident vegetation cover present, there was no effect of an interaction between the two factors. Individually, the grazing and herbicide treatments did not have an effect on the resident cover that was present.

None-resident vegetation was not significantly impacted by an interaction between the grazing and herbicide factors. Plots that were grazed reduced non-resident vegetation cover by 11% when compared with plots that were not grazed (Figure 2). Herbicide treatments did not have a significant effect on non-resident vegetation cover.

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. The grazing and herbicide treatments did not have an effect on presence or absence of total seed production. Of the seed produced, there was no significant impact of an interaction between the grazing and herbicide factors. Plots that were grazed had increased total seed production by 48% when compared to plots that were not grazed (the

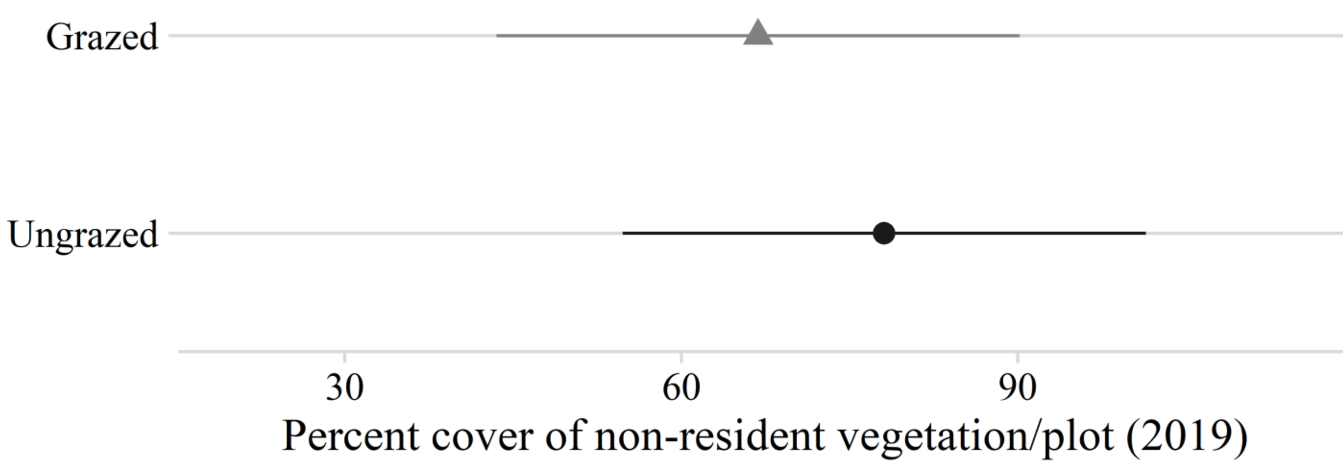


Figure 2. Within treatment season (2019) effect of grazing on non-resident vegetation cover (error bars represent 95% confidence intervals)

opposite of the previous year) (Figure 1). Herbicide treatments also had a significant effect on total seed production with plots that received herbicide applications of quiclorac or Rinskor active reducing total seed production when compared to no herbicide being applied (73% and 66%, respectively) (Figure 3).

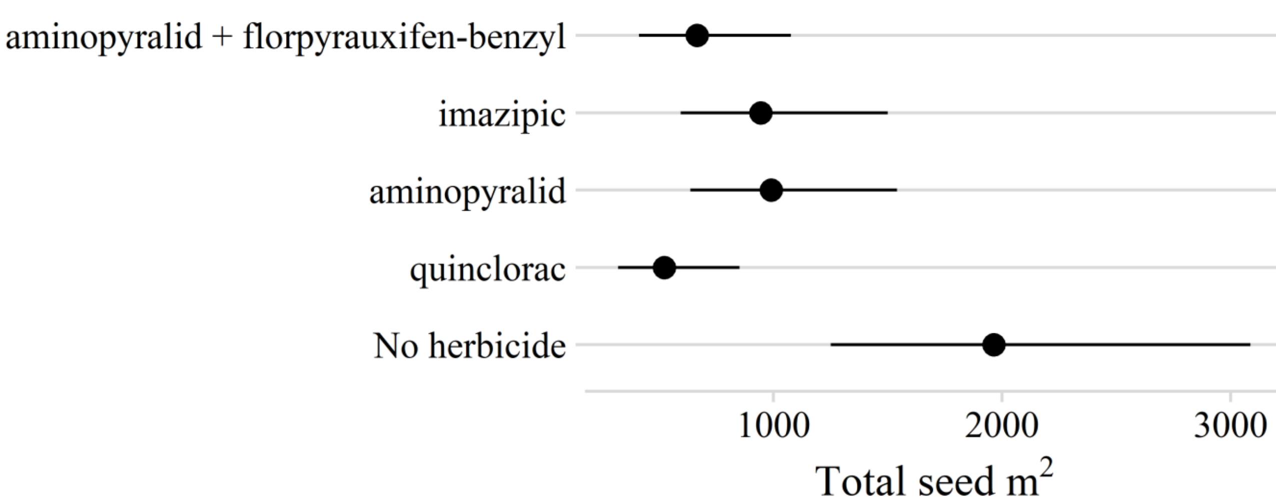


Figure 3. One-year post-treatment season (2020) effect of herbicide on leafy spurge total seed production (error bars represent 95% confidence intervals)

Total cover was significantly impacted by an interaction between the grazing and herbicide factors. The treatment combination of grazing and Rinskor active reduced total cover more greatly than the combinations of no grazing and quinclorac, no grazing and Rinskor active, 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. Individually,

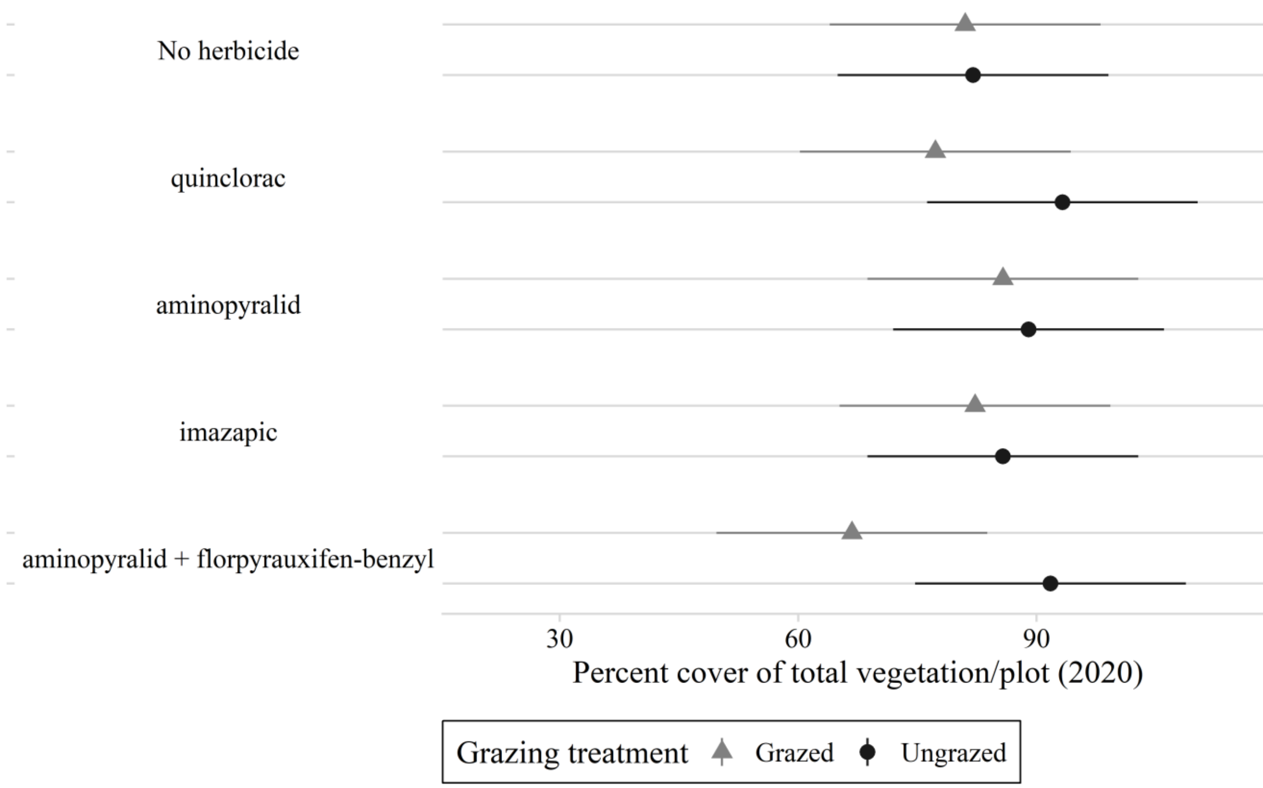


Figure 4. One-year post-treatment season (2020) effect of grazing and herbicide interaction on total vegetation cover, percentages with the same letter are not significantly ($p > 0.05$) different

herbicide treatments did not have an effect on total cover.

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

There was no impact of an interaction between the grazing and herbicide factors on the presence or absence of resident vegetation cover. The grazing and herbicide treatments did not have an effect on presence or absence of resident vegetation cover, neither did location. Of the resident vegetation cover present, there was no impact from the interaction between the two factors as well as no effect of either the grazing or herbicide treatments.

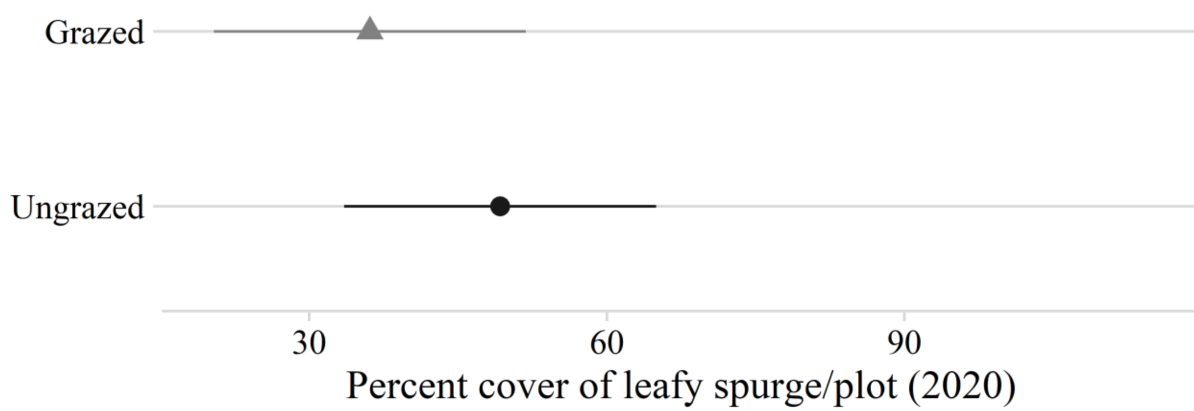


Figure 5. One-year post-treatment season (2020) effect of grazing on leafy spurge cover (error bars represent 95% confidence intervals)

Non-resident vegetaion was not significantly impacted by an interaction between the grazing and herbicide factors. There was no significant effect of either treatment on non-resident vegetation cover.

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 Rinskor active. 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, although 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 an 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 Rinskor active, 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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Task #3 [$ 3,000 allocated from CWCB/YWG Basin account—87.5% invoiced—estimated percent completion for Task #3 = 85%]

Education and Outreach—Engage youth in the Yampa River Leafy Spurge Project, using biological control as a means to encourage learning, participation and productive involvement.

Responsibility for completing Task #3 lies with YRLSP volunteers and partner agencies.

- CSU Extension—Moffat and Routt Counties
- Colorado Parks and Wildlife
- Colorado Department of Agriculture
- BLM—Little Snake Field Office
- NRCS—Routt and Moffat Counties

In July, 2019, the YRLSP sponsored a two-day kids' workshop on invasive weeds and biological control. Partner agencies contributed time and expertise to ensure the Boys and Girls Club kids had a quality educational and fun experience. Kids spent a half day of invasive weed orientation at Loudy Simpson Park in Craig. They were joined by Routt County Master Gardeners for a second day of leafy spurge biocontrol field science at the Highway 40 Rest Area between Hayden and Craig. The event wrapped up with a picnic lunch and good reviews from the young field scientists. More photos are available on the YRLSP web site: https://www.yampariverleafyspurgeproject.com/youth-outreach.

The success of the 2019 youth engagement event encouraged YRLSP partners to plan and host a similar event in 2020. Covid-19 intervened, however, so the event has been rescheduled for June 29-30, 2021.

YRLSP volunteer Peter Williams and Colorado Department of Agriculture (John Kaltenbach) worked together to develop an educational information sheet on leafy spurge biological control insects presently available for use in managing leafy spurge. This document is available for download from the YRLSP website:
https://www.yampariverleafyspurgeproject.com/resources

YRLSP volunteers collected information from a variety of sources to document historical releases of biological control insects in Moffat and Routt Counties. This effort yielded 44 records on 42 sites, dating back as far as 1989 (30 years). In July, 2019 and 2020, YRLSP volunteer Tamara Naumann tracked down 26 records on 24 sites in the field, with help from Tyler Jacox (CPW), Chris Rhyne (BLM), John Husband (YRLSP), Jesse Schroeder (Moffat County), Hannah Kuhns (UW), Todd Hagenbuch (CSU Extension) and Peter Williams (YRLSP). Each site was evaluated, using a field protocol developed with assistance from John Kaltenbach (CDA). Results are summarized below.

- 15 sites still had spurge *and* leafy spurge biocontrol beetles (see table below)
- 1 site, with possibly questionable coordinates, had spurge, but biocontrol beetles were not found on site, although they were found nearby (Mack 39).
- 6 sites had clearly been sprayed with herbicide and now support little or no leafy spurge—most of these are now occupied primarily by annual weeds
- 1 site was an older record with obviously incorrect coordinates, so its history could not be reliably assessed
- 1 site was inaccessible (island in a pond), so could not be assessed (although leafy spurge was visible on the island)

Site Name	Release Year	Spurge Density	Years Since Release	Year Monitored by YRLSP
ROUTT COUNTY				
YRSWA 19	1991	Moderate	28	2019
YRSWA 6	1994	Low	25	2019
YRSTL 9	1997	Moderate	22	2019
J Quarter ◯ 4	1998	Low	21	2019
YRSWA 20	1999	Low	20	2019
YRSTL 22	2008	Moderate	11	2019
YRSWA 34	2016	Low	3	2019
YRSWA 37	2016	Low	3	2019
MOFFAT COUNTY				
BLM TEPEE 47	2010	Various*	10	2020
BLM CR38 43	2016	High	3	2019
CAMILLETTI 38	2016	Moderate	4	2020
FOURMILE 42 & 44	2016 & 2017	Moderate	3 & 2	2019
MACK 39	2016	High	4	2020
PEROULIS N 33	2016	High	3	2019
PEROULIS S 41	2016	Moderate	3	2019
WAGNER	2016	High	3	2019

* The BLM Tepee 47 site has had multiple integrated treatments (biocontrol, fire and herbicide) over the past decade, so it is not possible to determine the effect of a single biocontrol release. This site is suitable for future biocontrol releases, as much progress has been made in reducing the overall extent and density of the original infestation. A summary of treatments and results is available on our web site: https://www.yampariverleafyspurgeproject.com/tepee.

The preliminary results from our assessment of legacy sites were surprising because many people believed that local biological control efforts had failed. Although a sample size of 16 sites is small, it is notable that all but one of the visited sites that still support leafy spurge also support small numbers of biological control insects. These results are encouraging.

As observers have visited an increasing number of legacy sites, a possible pattern is emerging with respect to the appearance of sites occupied by biological control insects. While it is not possible to know with certainty how each of the sites looked at the time of release (because no photos or quantitative data were recorded), standard procedure for biological control involves using this management tool in areas where large, dense weed populations are present. It is reasonable to assume that historical release sites supported large, dense leafy spurge populations in most, if not all cases. Currently, a majority of the legacy sites support low or moderate spurge densities, especially on sites where biocontrol insects were released more than three years prior. A significant proportion of these sites present with stunted, non-flowering individual spurge plants distributed throughout a matrix of more desirable vegetation. Scattered small patches of dense, flowering leafy spurge also occur in many of these sites. The small sample size precludes definitive conclusions regarding efficacy of biocontrol in local riparian environments, but this pattern is consistent enough to suggest it may be beneficial to work toward enhancing local biological control efforts, including a more robust program of monitoring for efficacy.

All of the identified legacy sites proximate to the mainstem Yampa River were visited in 2019 or 2020. Data has been collected using the protocol developed in collaboration with the Colorado Department of Agriculture. The protocol is available on the YRLSP website: https://www.yampariverleafyspurgeproject.com/resources. A companion digital version of the data sheet facilitates field data collection on tablets and subsequent data management.

It is notable that the leafy spurge mapping crew detected biocontrol insects in areas along the Yampa River that are significantly distant from known biocontrol release sites. Dinosaur National Monument also detected insects in 2020. This suggests that biocontrol agents have been present and active in the Yampa Valley for some time, possibly for nearly three decades. If biocontrol agents have been active in the Yampa Valley for +/-30 years, as it now appears, it is possible that the leafy spurge infestation has been thwarted to some degree over this same period of time.

The Colorado Department of Agriculture (John Kaltenbach) has made additional leafy spurge biological control insects available to the YRLSP, free of charge, in exchange for the data we are collecting on historical and current release sites. As a result, five new biocontrol releases occurred in 2019, augmented by 13 new releases in 2020. In addition, CPW purchased 10,000 flea beetles in 2020, which added another six sites on the Yampa River State Wildlife Area. Data and photographs were collected at the time of each release. In the past two years, we have increased the biocontrol effort by adding 24 release sites—that is more than 30,000 insects. This 2-year effort represents a 38% increase over the total number of insects released in the Yampa Valley over the past three decades combined!

YRLSP will work with interested partners and private landowners in the coming years to identify appropriate sites for release of additional biological control insects in the future. The overarching goal would be to provide a rapid and significant boost to the biocontrol insect population in the Yampa Valley. As this effort is proving potentially more important than we anticipated, we have enhanced the biocontrol information and reporting section on our web site:

https://www.yampariverleafyspurgeproject.com/biological-control

and we will continue to update this as new information becomes available. Plans are to release at least another 10K-20K insects in June, 2021, as conditions permit, primarily in Moffat County. We are also planning to host a local field tour in July to help producers learn about biocontrol options for leafy spurge management. And finally, we are beginning to consider next steps for biocontrol in the Yampa Valley—a research needs assessment, several more years of aggressive insect augmentation, monitoring to determine whether insect populations are growing large enough to allow for hosting a local catch-and-take biocontrol insect event, and other ideas. This aspect of the original project has grown in importance to the overall effort as new information has come to light.