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

YRLSP BUDGET—SUMMARY—21 December 2021

CONTRIBUTOR	AMOUNT Committed	% of TOTAL		AMOUNT Contributed or Invoiced to Date	% of Total Project Commitment
CASH					
YWG Basin WSRF Request	$ 89,000	54%	54%	$ 88,978	100%
Moffat County	15,000	9%	26%	$ 15,000	100%
Routt County	15,000	9%		$ 15,000	100%
University of Wyoming	12,572	8%		$ 12,572	100%
IN-KIND					
YRLSP volunteers	20,000	12%	20%	$ 29,260	146%
Other Partners (BLM, NPS, TNC, CDA, CPW, Moffat County, Routt County, CSU Extension and NRCS)	14,000	8%		$ 17,412	124%
TOTAL PROJECT COST	**$ 165,572**			$ 178,222	108%

ACKNOWLEDGEMENTS

The Yampa River Leafy Spurge Project is grateful for the support of the Yampa-White-Green Basin Roundtable, Moffat County, Routt County, Colorado Parks and Wildlife, Bureau of Land Management, Colorado Department of Agriculture, Natural Resources Conservation Service, CSU Extension, National Park Service, The Nature Conservancy, University of Wyoming and our many volunteers and supporters. This work is not possible without many willing hands.

Special thanks to Dr. Dan Tekiela (formerly with the University of Wyoming)—he provided brains, inspiration and mentorship for his students. And we are grateful for and proud of the work that Hannah Kuhns and Chloe Mattilio put into this project. We extend our congratulations and best wishes for their future careers.

EXECUTIVE SUMMARY

This executive summary is respectfully submitted to The Yampa-White-Green Basin Roundtable and the Colorado Water Conservation Board on December 22, 2021. It accompanies a final invoice for reimbursement of expenditures in the amount of $7,560.40. In addition, separate files contain final deliverables for each of the three tasks identified in the Statement of Work. As we dug into this project, it became obvious very quickly that we were developing large amounts of data and information. Our website has now become an integral instrument in the organization and dissemination of information related to our work on this problem. We continue to update it and intend to maintain it until a permanent home presents itself.

Task #1 [$40,900 allocated from CWCB/YWG Basin account—100% invoiced]

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—YRLSP

- YRLSP volunteers developed and maintain GIS products and systems to facilitate field mapping of leafy spurge, using electronic tablets.
- YRLSP volunteers developed a landowner permission/access form and tracked down landowners to seek permission for field mapping of approximately 120 miles of the Yampa River from the Hayden pump station downstream to the head of Cross Mountain Canyon. Field mapping was completed in 2021.
- The maps resulting from this work are available on the YRLSP web site: https://www.yampariverleafyspurgeproject.com/fieldmapping. 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. Shapefiles are available on request from interested stakeholders (where permission has been granted for data sharing).
- Leafy spurge field mapping data were provided to the University of Wyoming for use in their spatial analysis and predictive modelling work.

Geospatial Analysis, Remote Sensing and Predictive Modelling—University of Wyoming

- Initially, this component of task #1 was envisioned as a master's thesis project. After an unsuccessful effort to recruit a student suitable to the task, YRLSP worked with the University of Wyoming to allow for the addition of the project to a PhD candidate's work program. Thus, a PhD dissertation chapter (Chapter 3) has been substituted for a master's thesis as the deliverable accepted by YRLSP. (The full dissertation will not be completed until late in 2022.)
- University of Wyoming graduate student Chloe Mattilio has been working on two mapping applications that will make substantial contributions to the control of leafy spurge across the entire Yampa River Basin. Chloe's remote-sensing mapping application promises to accurately detect existing leafy spurge infestations using high spatial resolution, multispectral satellite imagery. Her invasive risk modelling application will also facilitate monitoring potential habitats for the arrival of new leafy spurge infestations.

Remote Sensing Mapping

- Chloe's work began in 2019 with searching out appropriate satellite photography (recent, little or no cloud cover, inclusive of our areas of interest in the Yampa River corridor . . . and reasonably priced!). She then applied a number of sophisticated processes to further refine the pixel resolution of the photography, before processing it into multiple spectral band combinations. These were further tweaked by applying different contrast, brightness and gamma values—all in an attempt to tease out the subtle spectral differences recorded in each photographic pixel.
- The end goal is to develop an algorithm that can effectively classify each enhanced photographic pixel as to whether it recorded light that was, or was not, reflected from a patch of leafy spurge on the ground.
- Chloe's development of the remote-sensing application includes correlation with the field mapping data collected by YRLSP, followed by groundtruthing of the initial model and further corrections. The final model classification identifies leafy spurge on the ground correctly with an overall accuracy rate of 91.3%—a remote sensing classification performance that is significantly better than random. The final step was to produce a comprehensive map of leafy spurge infestations (detected by remote sensing) in the Yampa River Valley.
- Two versions of the map appear on page 9 of the Chapter 3 document, which has recently been posted to the YRLSP website: https://www.yampariverleafyspurgeproject.com/research. We will be working with partners in the coming months to work out how best to make this information available to the public and interested partners and collaborators.

Invasion Risk Modelling

- The leafy spurge invasion in the Yampa River Basin is still in progress—what we see today does not define the potential extent of future infestations. Chloe Mattilio has also developed an invasion risk predictive model for the basin that will aid leafy spurge control efforts now and into the future.
- A primary resource is the extensive spatial dataset that has been developed in nearby Fremont County, Wyoming. Locations of leafy spurge populations have been recorded by Fremont County Weed & Pest for years, resulting in a robust dataset cataloging over 17,000 individual populations. By correlating environmental data for the Fremont County and Yampa River study area infestations (including soil type, texture, and pH; annual and monthly mean climate temperature and precipitation; location slope, elevation, and aspect; as well as infestation proximity to roads and developed areas), Chloe built a predictive model that can be applied to the Yampa River Basin to identify and map locations where new leafy spurge infestations are more likely to occur in the future.
- The best-fitting model, depicted on page 17 of the Chapter 3 document, classifies 359,680 acres in Routt and Moffat counties as having "high suitability" or risk of leafy spurge invasion, and another 2 million acres with "moderate suitability." The model helps us understand the need to continue efforts to thwart the progress of this pernicious weed.
- At the August 2021 YRLSP Working Group meeting, Chloe presented an update on her progress with remote-sensing mapping and invasive risk modelling applications. A downloadable PDF of her PowerPoint presentation is available on our website: https://www.yampariverleafyspurgeproject.com/chloemattilio.

Task #2 [$40,800 allocated from CWCB/YWG Basin account—100% invoiced]

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

- 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. During the study, one of the private parcels was withdrawn from the study due to changing management priorities of the landowner.

- UW graduate student Hannah Kuhns submitted her completed master's thesis as a final deliverable to YRLSP in July of 2021. Her thesis is available for download on the YRLSP website: https://www.yampariverleafyspurgeproject.com/research, and it is submitted as a separate file to the YWG Roundtable and CWCB. Hannah also presented a portion of her thesis work in a seminar on April 9, 2021. A video of her excellent presentation is also available on the YRLSP website: https://www.yampariverleafyspurgeproject.com/hannahkuhns.

- Hannah's work further supported our understanding of water as a vector for spreading leafy spurge downstream. Not only are seeds moving in the water, but Hannah showed convincingly that root fragments eroding out of banks and sandbars during runoff season are capable of floating downstream and re-establishing new plants that contribute to a burgeoning infestation.

- Her management treatment studies tell us that targeted sheep grazing is not practical in most riparian settings, and probably not something to pursue in other than very limited circumstances.

- Unfortunately, the herbicide studies did not point to a clear favorite chemical or strategy, but rather hinted that more study of Quinclorac and Duracor might eventually give us an appropriate substitute for the chemicals, such as Tordon, which are effective in upland settings, but not labeled for use in riparian areas. Quinclorac and Duracor appeared to cause a reduction in seed production, but more work on timing of application, rate of application, and duration of effect is warranted.

- Because the herbicide treatment results were inconclusive, one of the deliverables is not yet possible; the original grant application identified an Extension publication as one of the deliverables on this task. Instead, YRLSP will continue to consult with weed managers in Moffat and Routt counties as they experiment with various combinations of new chemistry and timing of application, and will ensure that every effort is made to continue to make new information available to local producers and land managers. Moffat County Weed Supervisor Jesse Schroeder has had some early promising results with a combination of QuinStar (Quinclorac) and Overdrive sprayed in late May–early June. Progress will take time, willingness to try new combinations, conversation and collaboration.

Task #3 [$ 3,000 allocated from CWCB/YWG Basin account—99.2% invoiced]

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.

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

A full record of all YRLSP activities related to biological control and education/outreach are available on our website: https://www.yampariverleafyspurgeproject.com/biological-control.

YOUTH ENGAGEMENT

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. The success of the 2019 youth engagement event encouraged YRLSP partners to plan for a similar event in 2020. Covid-19 intervened, however, and the event was rescheduled for June 29–30, 2021. Despite efforts to recruit kids for participation in 2021, it seemed that Covid-19 was still keeping kids from participating in group activities to a significant degree. When we failed to sign up a minimum number of kids, the event was reluctantly cancelled a week before it was scheduled. We do have all of the t-shirts and materials and supplies to try again in 2022, which we intend to do, even though the CWCB-WSRF grant will be closed out on 31 December 2021.

BIOLOGICAL CONTROL

In 2018–2019, 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 of 2019 and 2020, YRLSP volunteers and partners visited 24 legacy sites representing 26 documented release records. All of the identified legacy sites proximate to the mainstem Yampa River and where access was granted were visited.

Preliminary results from our assessment of legacy sites were surprising because many people believed that local efforts to establish persistent populations of biological control agents had failed. It is notable that all but one of the visited sites that still support leafy spurge also support small numbers of biological control insects. The apparent persistence of biocontrol insects over several years to several decades was encouraging.

It is also notable that the leafy spurge mapping crew (Task 1) detected biocontrol insects in areas along the Yampa River that are significantly distant from known legacy biocontrol release sites. Dinosaur National Monument staff also detected insects in 2020. This suggests that biocontrol agents have been present and active throughout the Yampa Valley for some time, possibly for nearly three decades. If biocontrol agents have been active in the Yampa Valley for +/-30 years, it is possible that the leafy spurge infestation has been thwarted to some degree over this same period of time.

In cooperation with our agency partners and multiple private-landowning stakeholders, in 2019 the YRLSP began an accelerated leafy spurge biological control release program. The goal is to distribute large numbers of biological control insects in appropriate locations throughout the full extent of the riparian habitat along the mainstem Yampa River. Each new release site will be subject to periodic monitoring in the future. We will continue to make a dedicated effort on this project at least through 2023, and possibly longer, if it seems to be having a measurable effect.

Ultimately the YRLSP hopes to establish a number of viable local nursery sites to support a Yampa-Basin-sourced biocontrol insect collection and redistribution effort.

Future Plans

YRLSP will continue to work with interested partners and private landowners in the coming years to identify appropriate sites for future releases of leafy spurge biocontrol agents. The objective will 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 section as new information becomes available.

In collaboration with NRCS and Routt and Moffat county weed managers, YRLSP is planning to host a field tour in July 2022 to help local producers learn about biocontrol options for leafy spurge management. We have tentatively scheduled a meeting with Patrick Stanko in January to discuss ideas for working with the YWG Roundtable Public Education, Participation and Outreach (PEPO) committee to share new information with a broad audience.

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. The biological control aspect of the original project has grown in importance to the overall effort as new information has come to light.

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.

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

A full record of all YRLSP activities related to biological control and education/outreach are available on our website: https://www.yampariverleafyspurgeproject.com/biological-control.

YOUTH ENGAGEMENT

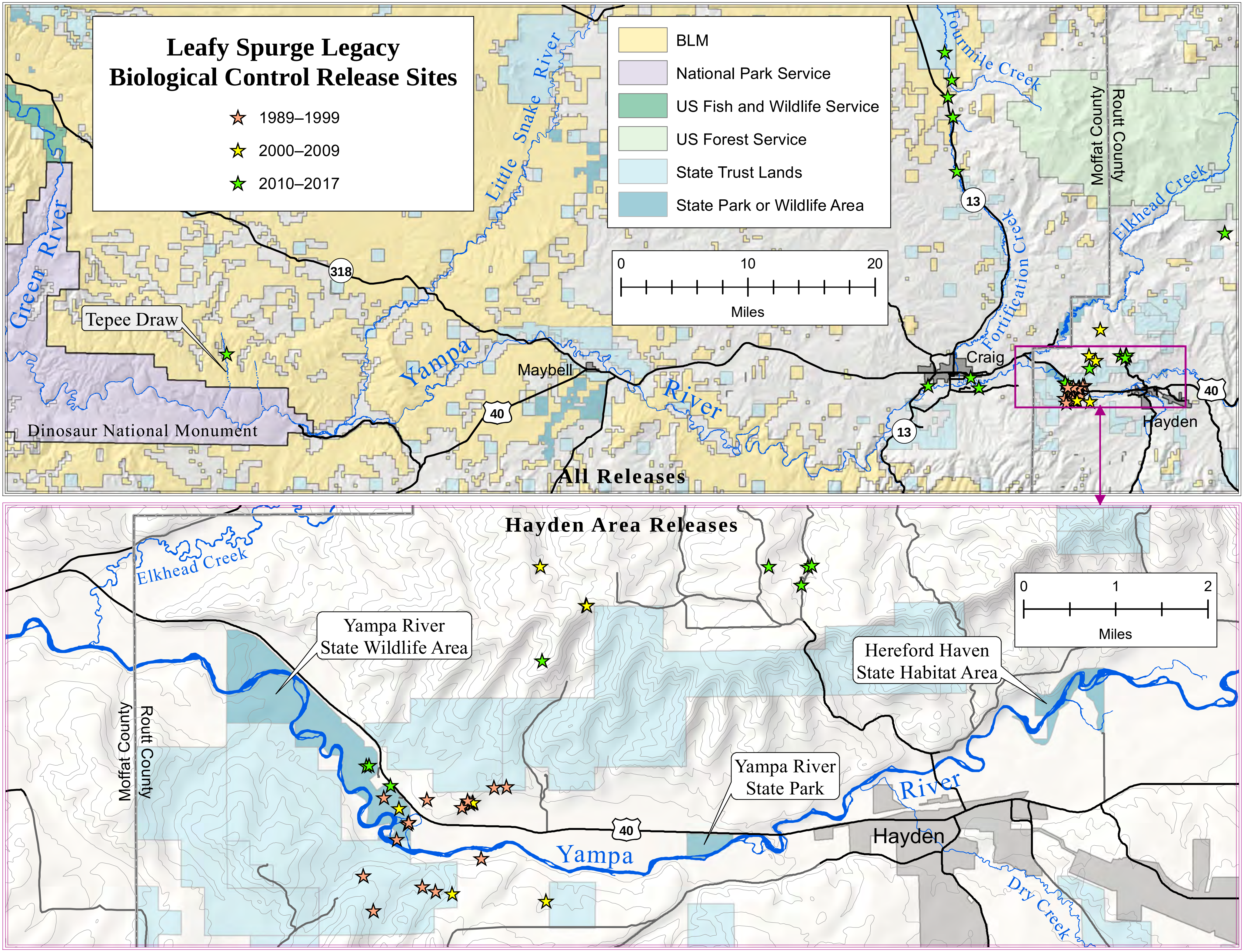
In July 2019, the YRLSP sponsored a two-day kids workshop on invasive weeds and biological control. Partner agencies contributed time and expertise to ensure the Boys and Girls Club kids had a quality educational and fun experience. Kids spent a half day of invasive weed orientation at Loudy Simpson 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 for a similar event in 2020. Covid-19 intervened, however, and the event was rescheduled for June 29–30, 2021. Despite efforts to recruit kids for participation in 2021, it seemed that Covid-19 was still keeping kids from participating in group activities to a significant degree. When we failed to sign up a minimum number of kids, the event was reluctantly cancelled a week before it was scheduled. We do have all of the t-shirts and materials and supplies to try again in 2022, which we intend to do, even though the CWCB-WSRF grant will be closed out on 31 December 2021. The YRLSP has set aside enough funding from other sources to pay for lunch and other costs associated with holding the event in late June 2022.

Biological Control

YRLSP volunteer Peter Williams and Colorado Department of Agriculture Bio-Control Specialist 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 (*YRLSP Biological Control Species ID Guide*) is available for download from the YRLSP website: https://www.yampariverleafyspurgeproject.com/resources.

In 2018–2019, 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 of 2019 and 2020, YRLSP volunteer Tamara Naumann visited 24 legacy sites representing 26 documented release records, 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). All of the identified legacy sites proximate to the mainstem Yampa River were visited.



Leafy Spurge Legacy
Biological Control Release Sites
1989–1999
2000–2009
2010–2017
BLM
National Park Service
US Fish and Wildlife Service
US Forest Service
State Trust Lands
State Park or Wildlife Area
0
10
20
Miles
Green River
Little Snake River
Fourmile Creek
Fortification Creek
Elkhead Creek
Moffat County
Routt County
Tepee Draw
Dinosaur National Monument
Yampa River
Maybell
Craig
Hayden
318
13
40
All Releases
Hayden Area Releases
Elkhead Creek
Yampa River
State Wildlife Area
Hereford Haven
State Habitat Area
Yampa River
State Park
Yampa
River
Hayden
Dry Creek
0
1
2
Miles

Each of the visited legacy sites was evaluated, using a field protocol developed with assistance from John Kaltenbach (CDA). The protocol is available on the YRLSP website: https://www.yampariverleafyspurgeproject.com/resources. A companion digital version of the data sheet facilitated field data collection on tablets and subsequent data management tasks.

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 (e.g., downy brome and/or annual mustards)
- 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 48	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 efforts to establish persistent populations of biological control agents 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. The apparent persistence of biocontrol insects over several years to several decades was encouraging.

As observers visited an increasing number of legacy sites, a possible pattern emerged 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 legacy 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.

It is notable that the leafy spurge mapping crew (Task 1) detected biocontrol insects in areas along the Yampa River that are significantly distant from known legacy biocontrol release sites. Dinosaur National Monument staff also detected insects in 2020. This suggests that biocontrol agents have been present and active throughout the Yampa Valley for some time, possibly for nearly three decades. If biocontrol agents have been active in the Yampa Valley for +/-30 years, it is possible that the leafy spurge infestation has been thwarted to some degree over this same period of time.

While available scientific literature suggests that riparian habitat is not ideal for proliferation of leafy spurge biological control agents (Lym 2013), at least one Idaho study showed that heavy inundation of riparian habitat with large numbers of flea beetles (*Aphthona* spp.) can be successful (Progar 2010 and PNWRS 2012). Very few studies have been conducted to determine efficacy of the stem-boring beetle (*Oberea erythrocephala*), which seems to prefer riparian habitat. One researcher went so far as to suggest that *Oberea* must not be effective, since no one studies it (Progar 2011)! Over three years of observation in riparian habitat in the Yampa Valley, we have found *Oberea* almost universally present in riparian environments, albeit in small numbers in most locations; we have also observed them in upland areas that are many miles from documented biocontrol release sites. They are clearly extremely mobile creatures, and perhaps underestimated in terms of their effect on leafy spurge populations over time. We suggest that more work is needed over longer time frames than previous studies have managed.

We are now convinced that the "inundation" strategy is worth a solid try. To this end, in cooperation with our agency partners and multiple private-landowning stakeholders, in 2019 the YRLSP began an accelerated leafy spurge biological control release program. The goal is to distribute large numbers of *Aphthona* (flea beetles) and *Oberea* (stem-boring beetles) in appropriate locations throughout the full extent of the riparian habitat along the mainstem Yampa River. Each new release site will be subject to periodic monitoring in the future. We will continue to make a dedicated effort on this project at least through 2023, and possibly longer, if it seems to be having a measurable effect.

Currently leafy spurge biological control insects are available to us (in seasonally variable quantities) from collections made by the Colorado Department of Agriculture (CDA) at various Colorado Front Range locations, or from a commercial vendor collecting in Montana. However, ultimately the YRLSP hopes to establish a number of viable local nursery sites for its own Yampa-Basin-sourced beetle collection and redistribution.

CHRONOLOGY OF YRLSP BIOLOGICAL CONTROL ACTIVITIES

2019

In the first year of the YRLSP biological control release program, approximately 7,300 flea beetles collected by the CDA on the Front Range (and donated to the YRLSP) were released on or in the vicinity of the Yampa River State Wildlife Area (YRSWA) in Routt County.

2020

In June 2020, four YRLSP volunteers upped the ante by traveling to the Front Range to aid the CDA in their annual collection of leafy spurge biological control beetles. Under the tutelage of the CDA's John Kaltenbach, on the first day approximately 27,000 flea beetles were collected from the former Lowry Bombing and Gunnery Range east of Denver. Day two was then spent at the CDA facility in Broomfield, sorting the flea beetles from stray plant parts and the rest of the insect "bi-catch," before packaging them into 1000-insect lots for distribution by the CDA across Colorado. In return for our contributions, the YRLSP's share of the take was thirteen 1000-insect lots of *Aphthona*, and one lot of *Oberea* (*Oberea* is typically released in lots of only 100 insects).

Upon our return to the Yampa Valley, two additional days were spent releasing the biological control insects at thirteen separate locations in Routt and Moffat counties. Then, in July 2020, YRLSP volunteers also helped release an additional 10,000 flea beetles on the Yampa River State Wildlife Area, purchased by the YRSWA from the Montana vendor.

This brought the total for just the 2020 biological control releases alone in the Yampa Valley to approximately 23,100 insects—roughly a third as many again as recorded during the entire "legacy release" period of 1989–2017.

2021

The YRLSP was looking forward to another successful volunteer collection trip to the former Lowry Bombing and Gunnery Range in 2021, but (despite the extreme drought conditions prevailing on the Western Slope) continuously cool, wet spring conditions on the Front Range resulted in poor collection numbers during test runs by CDA crews. Ultimately it became clear that a collection trip to the Front Range by YRLSP volunteers would have produced only limited returns. We look forward to better collecting conditions in 2022.

Nevertheless, John Kaltenbach of the CDA was able to supply us with 3,000 *Aphthona* and 250 *Oberea*, while Tyler Jacox of the YRSWA was able to acquire an additional 6000 *Aphthona* and 200 *Oberea* from the commercial vendor in Montana. All of these insects were released at new locations in the YRSWA and in Moffat County.

In addition to the new biocontrol releases in 2021, YRLSP volunteers and partners conducted monitoring on nine sites where insects were released in 2019 and 2020. Biocontrol insects were still present on all nine sites. Detectable changes in leafy spurge cover were only recorded on two of the nine sites, but visible signs of insect activity were present on all nine sites.

YRLSP biocontrol releases are summarized in the table and maps on the following pages.

FUTURE PLANS

YRLSP will continue to work with interested partners and private landowners in the coming years to identify appropriate sites for future releases of leafy spurge biocontrol agents. The objective will be to provide a rapid and significant boost to the biocontrol insect population in the Yampa Valley. Plans are to collect (or purchase) and release at least another 10K–20K insects in June 2022, as conditions permit, primarily in Moffat County. Our primary target area in 2022 is Little Yampa Canyon, which has experienced significant increases in leafy spurge in recent years.

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 section as new information becomes available. In collaboration with NRCS and Routt and Moffat county weed managers, we are planning to host a field tour in July 2022 to help local producers learn about biocontrol options for leafy spurge management. We have tentatively scheduled a meeting with Patrick Stanko in January to discuss ideas for working with the YWG Roundtable Public Education, Participation and Outreach (PEPO) committee to share new information with a broad audience.

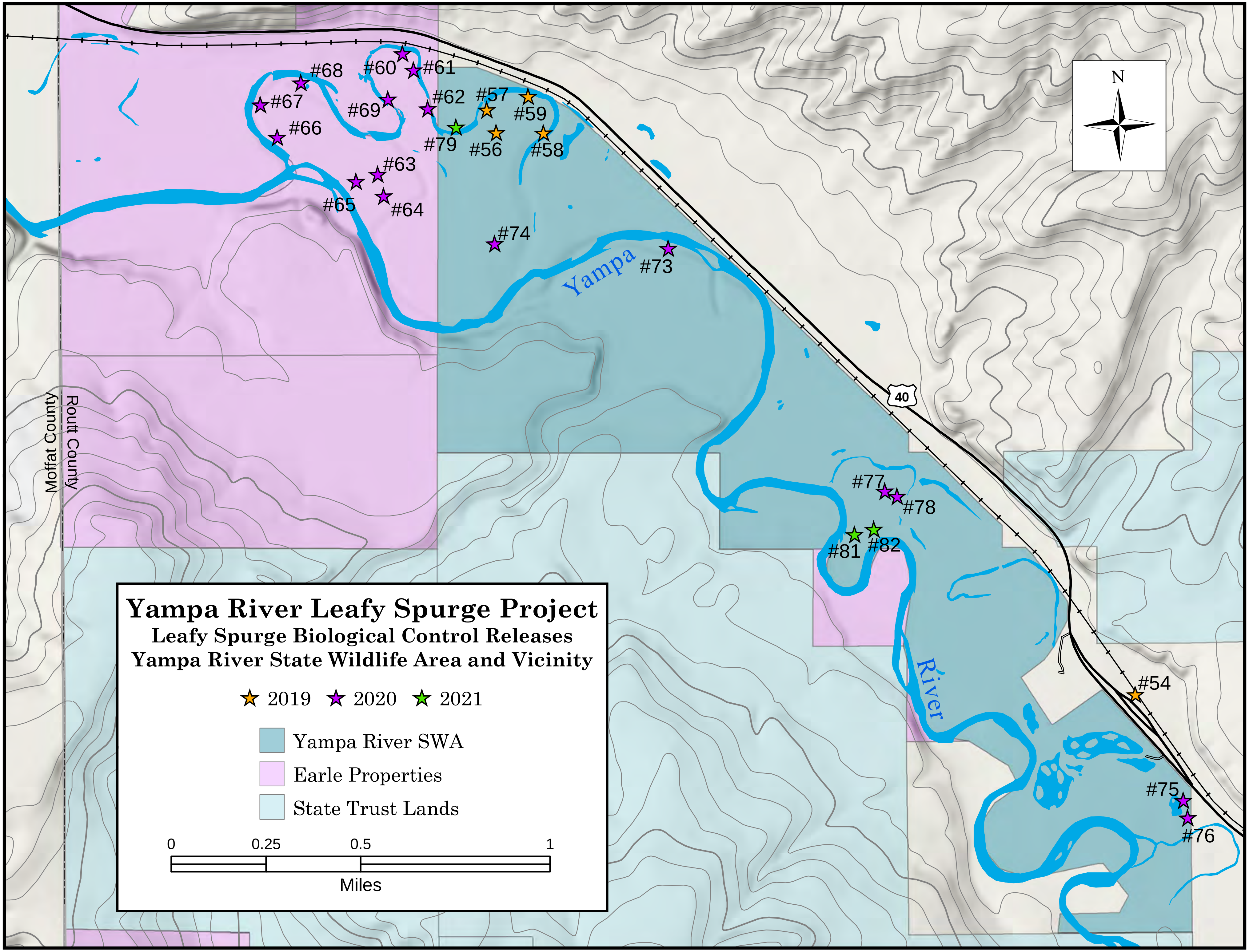
And finally, we are beginning to consider next steps for biocontrol in the Yampa Valley—a research needs assessment, several more years of aggressive insect augmentation, monitoring to determine whether insect populations are growing large enough to allow for hosting a local catch-and-take biocontrol insect event, and other ideas. The biological control aspect of the original project has grown in importance to the overall effort as new information has come to light.

YRLSP Biocontrol Release Summary 2019-2021						
Recent YRLSP Releases:	2019		2020		2021	
Species:	Aphthona	Oberea	Aphthona	Oberea	Aphthona	Oberea
Yampa River State Wildlife area						
Yampa River SWA #56	1,000	several				
Yampa River SWA #57	1,000	several				
Yampa River SWA #58	1,000	0				
Yampa River SWA #59	1,000	0				
Yampa River SWA #73			2,500	0		
Yampa River SWA #74			2,500	0		
Yampa River SWA #75			1,250	0		
Yampa River SWA #76			1,250	0		
Yampa River SWA #77			1,250	0		
Yampa River SWA #78			1,250	0		
Yampa River SWA #79					2000	150
Yampa River SWA #81					1200	100
Yampa River SWA #82					1200	100
Subtotal:	4,000		10,000		4,400	
3-year SWA Total:	18,400					
Other Public Lands						
Hwy 40 Rest Area #54	3,300	several				
BLM Fortification #70			1,000	0		
Loudy Simpson Island #83					1,200	0
South Beach #84					1,200	0
Private Lands						
Earle Oxbow #60			1,000	0		
Earle Oxbow #61			1,000	0		
Earle Oxbow #62			1,000	0		
Earle Oxbow #63			1,000	0		
Earle Oxbow #64			1,000	0		
Earle Oxbow #65			1,000	0		
Earle Oxbow #66			1,000	120		
Earle Oxbow #67			1,000	0		
Earle Oxbow #68			1,000	0		
Earle Oxbow #69			1,000	0		
McIntyre (upland) #71			1,000	0		
McIntyre (upland) #72			1,000	0		
McIntyre (river) #80					1,000	100
K-Diamond Ranch #85					1,200	0
Subtotal:	3,300	several	13,000	120	4,600	450
3-year (other) Total:	21,470					
3-YEAR GRAND TOTAL:	39,870					

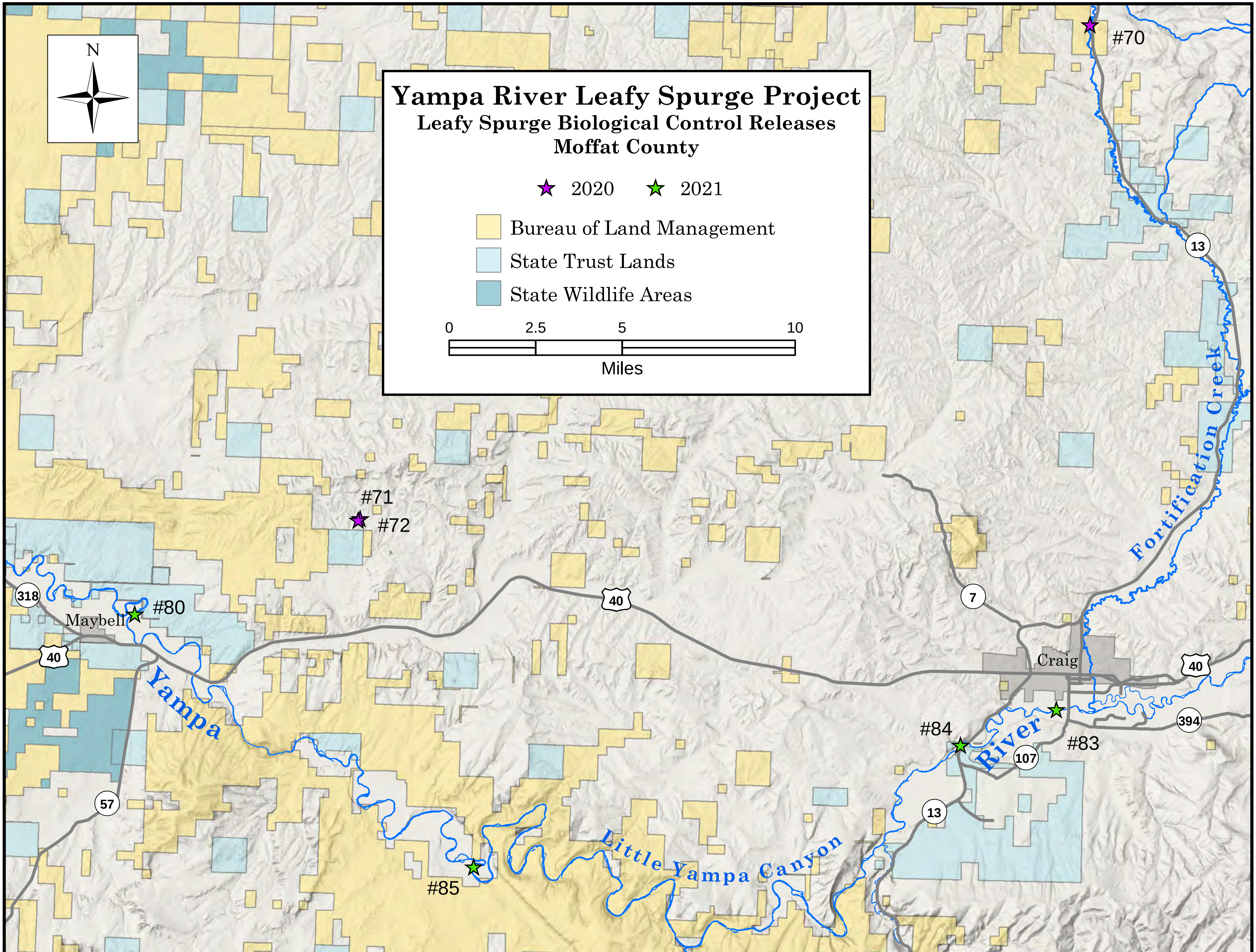
Source = CDA (no charge)

Source = CDA ($240 paid by YRLSP)

Source = Montana ($2,725 paid by CPW)



Yampa River Leafy Spurge Project
Leafy Spurge Biological Control Releases
Yampa River State Wildlife Area and Vicinity
2019
2020
2021
Yampa River SWA
Earle Properties
State Trust Lands
0
0.25
0.5
1
Miles
N
Yampa
River
Moffat County
Routt County
40
#54
#56
#57
#58
#59
#60
#61
#62
#63
#64
#65
#66
#67
#68
#69
#73
#74
#75
#76
#77
#78
#79
#81
#82



Yampa River Leafy Spurge Project
Leafy Spurge Biological Control Releases
Moffat County
2020
2021
Bureau of Land Management
State Trust Lands
State Wildlife Areas
0
2.5
5
10
Miles
N
#70
#71
#72
#80
#83
#84
#85
Maybell
Craig
Yampa
River
Little Yampa Canyon
Fortification Creek
318
40
57
7
13
107
394

References

Borchier, R. *et al*. 2006. Biology and biological control of leafy spurge. USDA Forest Service Publication FHTET-2005-07.

Lym, R.G. 2013. Leafy spurge control using flea beetles (*Aphthona* spp.). NDSU Extension publication W1183.

Progar, R.A. *et al*. 2010. Inundative release of *Aphthona* spp. Flea beetles (Coleoptera:Chrysomelidae) as a biological "herbicide" on leafy spurge (*Euphorbia esula* L.) in riparian areas. Environ. Entomology 103:242-248.

Progar, R.A. *et al*. 2011. Population dynamics and impacts of the red-headed leafy spurge stem-borer on leafy spurge (*Euphorbia esula*). Invasive Plant Science and Management 4:183-188.

Pacific NW Research Station. 2012. A barrage of beetles: controlling leafy spurge through beetle inundation. Science Findings 146, September 2012, USDA Forest Service.

Multispectral Satellite Remote Sensing and Predictive Modeling for Mapping of Presence and Invasion Risk of Leafy Spurge in Northwestern Colorado

Submitted by Chloe Mattilio
PhD Candidate, University of Wyoming
12 November 2021
In fulfillment of Task 1 Deliverable to Yampa River Leafy Spurge Project
(This document will serve as the basis for chapter 3 of her Doctoral Dissertation)

Introduction

Leafy spurge (*Euphorbia esula)* is a perennial invasive plant that is widespread in North America and is of high management concern in the north and central plains (Goodwin, Sheley, Nowierski, & Lym, 2001). With blue-green stems, linear leaves, small green flowers, and bright yellow bracts, mature leafy spurge is recognizable, and an additional identification characteristic is a milky white latex sap the plant releases when injured (Goodwin et al., 2001). Leafy spurge is commonly found in riparian areas in the Intermountain West, but can spread to neighboring grasslands, ridges, slopes, and upland areas, particularly where disturbance is frequent and soil moisture is low, allowing for less competition with other plants (Goodwin et al., 2001). Leafy spurge is particularly problematic in hayfields and pasture, as the ingenol content in the sap is toxic to cattle and horses, in addition to some wildlife (Goodwin et al., 2001). Individual stems can produce over 200 seeds annually, which can be expelled up to 15 feet from the plant when mature, and seeds are also buoyant and easily transported downstream (F . Larry Leistritz, 2004). Leafy spurge is also difficult to manage, as the extensive root system can sustain plants injured by even high rates of herbicides, targeted grazing, and biocontrol agents (Goodwin et al., 2001).

Remote sensing has been utilized for the detection and mapping of leafy spurge since 1995, using its distinct spectral characteristics (green-yellow bracts) to successfully distinguish infestations from surrounding vegetation using true color imagery alone (Anderson, Everitt, Escobar, Spencer, & Andrascik, 1996). Other remote detection attempts were made with this species, using sensors as simple as a multispectral sensor collecting reflectance in the visible and infrared portions of the spectrum (Hunt, Mcmurtrey, Williams, & Corp, 2004) up to hyperspectral sensors capable of recording reflectance in 224 bands, which resulted in a 84% - 95% detection rate of leafy spurge infestations at landscape scales (Williams & Raymond, 2002). One location where mapping of leafy spurge populations needs improvement is along the Yampa River, in Moffat and Routt Counties, Colorado, where leafy spurge is advancing away from the riverbanks and floodplains and into upland areas, so extent of the invasion is unknown.

In addition to desired maps of leafy spurge infestation along the Yampa River corridor, stakeholders are interested in understanding the risk of invasion for properties, recreational areas, and wildlife habitat adjacent to the river. One way to estimate future invasion risk of a study area is to apply ecological niche modeling/models (ENMs, also known as habitat suitability models) with presence data of the focal species and spatial data that covers the extent continuously with predictors variables that describe the habitat needs of the focal species (Bazzichetto et al., 2018; Peterson et al., 2001). ENMS work by training a machine learning classifier by extracting environmental predictor values at known presence locations, and then using those extracted values to describe the focal species ecological niche, which can be extended across all pixels of the spatial environmental predictors (Irzel, Ausser, & Hessel, 2002). In this research, a leafy spurge presence dataset from the Yampa River Corridor and an extensive leafy spurge presence dataset from Fremont County, Wyoming will be compared and combined to model ecological niche of leafy spurge populations to estimate leafy spurge invasion risk in Moffat and Routt Counties, Colorado.

Purpose and Research Questions

The purpose of this work is to map current distribution of leafy spurge along the Yampa River banks with satellite remote sensing. Additionally, based on presence of leafy spurge, spatial context, and a large dataset of leafy spurge populations from Fremont County, Wyoming, we will develop an invasion susceptibility model for predicting the likelihood of leafy spurge spread in Moffat and Routt Counties. We will address the following questions:

1. Where is leafy spurge present along the Yampa River corridor?
2. Based on current leafy spurge infestations, what is the risk of invasion spread in Moffat and Routt Counties?

Study Site and Methods

The stretch of the Yampa River we are focused on spans Moffat and Routt Counties, between Dinosaur National Monument and Hayden, Colorado. The Yampa River follows a free-flowing, turbid, and relatively slow meandering from the Park Range to a confluence with the Green River deep inside of Dinosaur National Monument. Flowing through mountain valleys, foothills, and down through arid sagebrush steppe, the Yampa provides habitat for fish, wildlife, and birds, and is well travelled for rafting trips. Leafy spurge has invaded riparian areas, riverbanks, and islands, and is now pushing into upland areas like the forest understory, rangelands, and agricultural areas.

The first step in developing an up-to-date presence map of leafy spurge infestations is to marry ground presence mapping efforts with remote sensing spectral detection of leafy spurge. Ground mapping of leafy spurge presence took place in the summers of 2019, 2020, and 2021 and covered the Yampa River Channel from Hayden, Colorado to Cross Mountain (east of Dinosaur National Monument). Mapping was conducted by volunteers from the Yampa River Leafy Spurge Project and took place largely by raft, with stops to map extent of infestations that extend beyond the immediate riverbanks. Presence of leafy spurge was recorded, as well as infestation characteristics like geomorphological type, vegetation type, proportional canopy coverage, proportional bare ground, size of leafy spurge infestation, and proportional leafy spurge abundance, which all may be important factors affecting imagery classification accuracy rates. These mapped presence sites were imported into a Geographic

Information System (GIS) and were used as interpretation and training areas for spectral profiling of leafy spurge for classification of satellite imagery.

We selected and purchased SPOT 7 satellite imagery from L3Harris Geospatial, which was collected in early July of 2019. The summer of 2019 was wet and cool, so this sampling date represents the late peak bloom of 2019. The spatial extent of this satellite imagery and resulting classification covers the area from Hayden, Colorado to Cross Mountain within 1.5 miles of the Yampa River channel (Figure 1). The imagery consists of 5 bands of light, one panchromatic (1.5m x 1.5m pixels) and four multispectral, red, green, blue, and near infrared (NIR) (6m x 6m pixels). The multispectral imagery was resampled to approximately 4m x 4m pixels using the finer resolution panchromatic band in ArcMap. Imagery was explored once pan-sharpened, and band combinations and representation were altered to highlight contrasts between ground mapped leafy spurge polygons and other recognizable land cover classes.

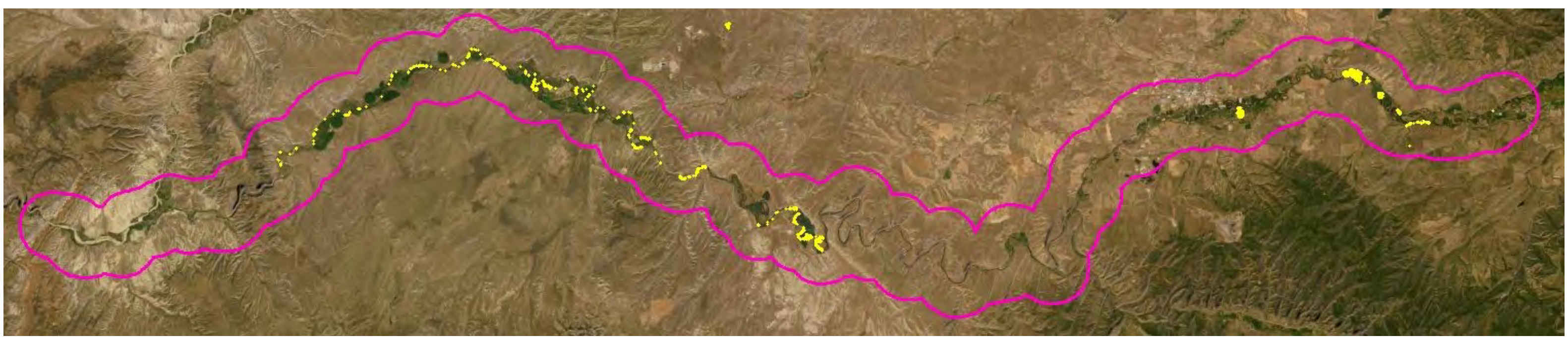


Figure 1. Map of YRLSP ground mapped leafy spurge presence data in yellow and the spatial extent of the satellite imagery and resulting classification outlined in magenta.

Polygons of leafy spurge were digitized based on interpretation of the imagery in ArcMap, and polygons were also developed for other land cover classes, which were combined to create a dataset of "not leafy spurge." Once these spatial datasets were completed, polygons were imported into Program R where spectral reflectance values were extracted to train the classification algorithm. The classification method utilized was a machine learning technique known as random forest, from the randomForest package in Program R. This sorts all pixels in the imagery into the classes “leafy spurge” or “not leafy spurge” using decision trees where two of our four bands of light are tried at each “branch.” A “forest” of these decision trees is grown to user-determined size and the class results are pooled across all trees for a final class result. For this classification, 101 trees were grown, and half of the training samples were reserved for an internal validation of results. Two classification maps were developed with both binary classification of “leafy spurge” and “not leafy spurge” classes and with a probabilistic scale from 0 to 1, from least likely to be leafy spurge to most likely to be leafy spurge. An accuracy assessment of the binary leafy spurge classification was conducted using a confusion matrix of classified and ground truthed data, and users’ accuracy, producer’s accuracy, overall accuracy, and the kappa coefficient were calculated. To investigate differences in reflectance for red, green, blue, and NIR light, correctly and incorrectly classified ground mapped leafy spurge presence polygons were selected from the classification map, and reflectance values were extracted for all four bands for each class. To test differences in reflectance for detected and missed leafy spurge polygons, a two-way analysis of variance (ANOVA) was conducted in Program R for each band of light.

To better understand classification performance, ground validation of results was conducted by locating accessible areas within and outside of ground mapped leafy spurge polygons in the study area. These locations were selected to cover a broad range of habitat types, classification results, anomalies, and areas of interest. Within these locations, four or more pixels of the same class were generalized to make polygons of the same class, and to avoid GPS inaccuracies, validation points were placed within these polygon centroids. In June of 2021, these validation points were visited by river and on foot to verify the presence or absence of leafy spurge. These points were scattered from the Yampa River State Wildlife Area, through Craig, Colorado, in the Little Yampa Canyon, and through Axial Basin. In addition to confirming leafy spurge presence and absence, binary classification performance (correct or incorrect), geomorphologic type, vegetation type, count of other species present, inundation frequency, proportional leafy spurge cover, proportional canopy cover, and proportional bare ground were all recorded.

Classification maps were exported back to ArcMap where classification values were extracted for both classification methods for leafy spurge presence polygons from YRLSP ground mapping and from 2021 validation points. Proportion of correctly identified leafy spurge polygons were calculated for each level of each infestation characteristic (e.g. trace, low, moderate, and high levels for characteristic canopy cover). Binary classification results (leafy spurge vs not leafy spurge) were fit to a logistic regression to determine effect of infestation characteristics (geomorphological type, vegetation type, proportional canopy cover, proportional bare ground, size of leafy spurge infestation, and proportional coverage of leafy spurge) on classification accuracy from ground mapped data. Additionally, These same analysis methods were applied for the 2021 validation point, with proportional correct classification recorded for each level of each infestation characteristic and binary classification results fit to a logistic regression model to determine which infestation characteristics (geomorphologic type, vegetation type, count of other species present, inundation frequency, proportional leafy spurge cover, proportional canopy cover, and proportional bare ground) affect classification accuracy within the validation dataset.

Finally, habitat suitability for leafy spurge was estimated for Moffat and Routt Counties using the ENMTML package in Program R (Andrade, Velazco, & De Marco Júnior, 2020) for ENM and the following environmental predictors: Soil proportional clay, soil proportional sand, soil proportional silt, soil proportional organic matter, soil pH, and hydric condition, temperature and precipitation annual and monthly means, temperature and precipitation annual and monthly variation, average daily solar radiation, slope, and aspect. Collinearity between these 27 predictor variables was reduced using aPrincipal Component Analysis (PCA), and the components that describe 95% of total predictor variance were used as the final model predictors. The modeling algorithms used were support vector machine, maximum entropy, and random forest, three classification techniques compatible with presence-only distribution modeling, and all three algorithms were combined to create an ensemble model. Two ENMs were estimated, one with leafy spurge presence locations from YRLSP ground mapping and extensive leafy spurge presence locations from Fremont County, Wyoming (ENM3), and one with Yampa leafy spurge presence locations alone (ENM4). These resulting ENMs were exported to ArcMap, where final ENM maps were made that predict leafy spurge ecological suitability for Moffat and Routt Counties at 1 km x 1km pixels with three different suitability scales: A continuous ecological suitability prediction on a scale of 0 to 1 (least suitable for leafy spurge to most suitable for leafy spurge), a binary ecological suitability prediction (suitable for leafy spurge or not suitable for leafy spurge), and a categorical ecological suitability prediction (low suitability for leafy spurge, moderate suitability for leafy spurge, and high suitability for leafy spurge), grouped by evenly splitting the distributions of the continuous suitability predictions. These binary and categorical ecological suitability models were summarized by calculating the proportional study area occupied by each class of leafy spurge suitability, examining principal component coefficients, and evaluating model performance.

Results and Discussion

Classification of Imagery for Leafy Spurge Mapping

The random forest classification of multispectral satellite imagery resulted in an overall accuracy rate of 91.3%, with a 94.7% user's accuracy rate (how often ground leafy spurge was correctly classified in the map) and an 89.9% producer's accuracy rate (how often classified leafy spurge will be present on the ground) for leafy spurge. The final accuracy metric calculated for the remote sensing classification was the kappa coefficient (ranges from -1 to 1, with values close to 0 showing that the classification performed no better than random and 1 describing the data perfectly), which was equal to 0.902, indicating that our remote sensing classification performed significantly better than random. Correctly classified leafy spurge mean spectral 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) (Table 1). Overall, this classification method worked well, but if mapping was to take place again, satellite imagery with additional wavelengths of near infrared light may be useful for distinguishing leafy spurge from other land cover types.

		Data from training set			
				Totals	User's Accuracy
Class assigned from imagery classification		214 Correctly classed leafy spurge	12 Not-spurge incorrectly classed as spurge	226 (214 + 12)	94.7 (214 / 226) *100
		24 Spurge, incorrectly classed as not-spurge	165 Not-spurge correctly classed as not-spurge	189 (24 + 189)	87.3 (165 / 189) * 100
	Totals	238 (214 + 24)	177 (12 + 165)	415	
	Producer's Accuracy	89.9 (214 / 238) *100	93.2 (165 / 177) *100		Total Accuracy 91.3%

Kappa = **0.902**

$$\frac{415 * \text{correct classification } (214 + 165) - \text{ref vs class } ((238 * 226) + (177 * 189))}{415^2 - \text{ref vs class } ((238 * 226) + (177 * 189))}$$

Figure 2. Confusion matrix of binary random forest classification of training polygons classified as leafy spurge vs not leafy spurge. Class accuracies for producer's and user's accuracy are calculated, with 12 false positives, where not-leafy spurge was incorrectly classified as leafy spurge and 24 false negatives, where leafy spurge was incorrectly classified as not leafy spurge. The kappa coefficient is also calculated, with a value of 0.902 indicating that the classification model describes the dataset fairly well.

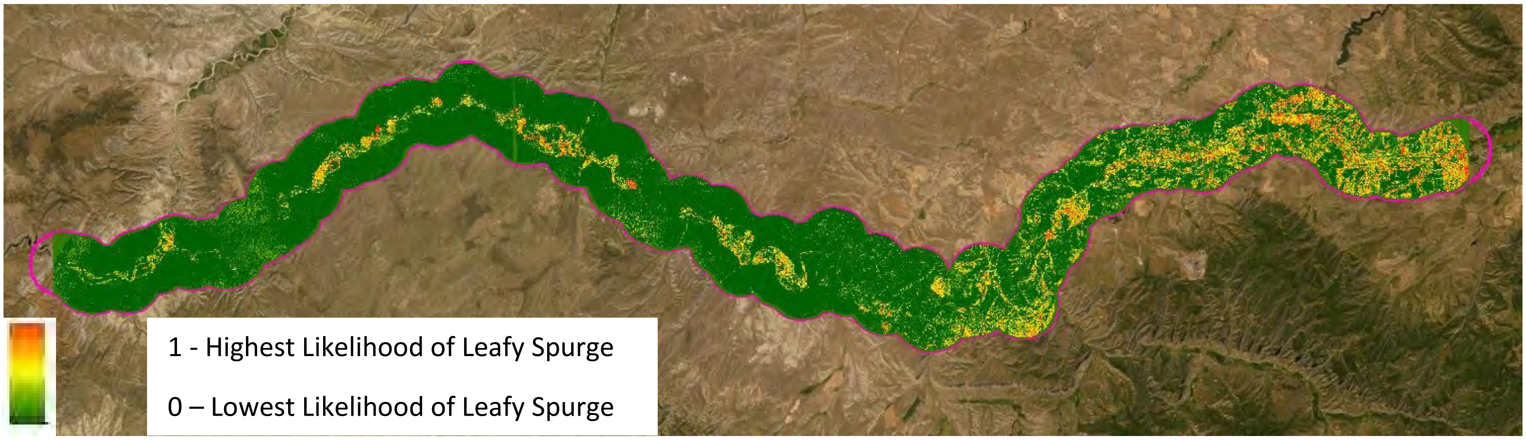


Figure 3 and Figure 4. Random forest classification predictions for imagery study area (magenta outline) for a binary classifier (top) and a probabilistic classifier (bottom). The binary map shows pixels classified as not leafy spurge as colorless and pixels classified as leafy spurge in yellow. The probabilistic model represents values from 0 to 1, for least likely to be leafy spurge in dark green and most likely to be leafy spurge in red.

Table 1. Results table from two-way t-test comparing 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 (Mean) and p-values (p-value) testing the differences between the class means for each band of light. P-values marked with * are significantly different.

	Band of Light of Multispectral Imagery							
	Red		**Green**		**Blue**		**Near Infrared**	
Class	Mean	p-value	Mean	p-value	Mean	p-value	Mean	p-value
Spurge	308	0.8	434	0.1	368	0.6	1359	0.03*
Missed Spurge	309		433		367		1323	

Classification Results of Ground Mapped Leafy Spurge Polygons

Across a range of environmental and infestation conditions, the number of correctly identified leafy spurge polygons and missed leafy spurge polygons varies within the 314 presence locations in the dataset (Figure 5). Generally, single leafy spurge populations, populations with high bare ground coverage, and populations located on banks were misclassified more frequently. Binary classification results of leafy spurge vs not leafy spurge was fit to an additive logistic regression model:

Logit1 <- glm(spurge ~ geomorph + bareground + vegetation + abundance + canopycover + area)

Based on odds ratios of coefficients, single leafy spurge populations, populations with high bare ground coverage, and populations growing in on banks and riparian shrub vegetation cover types were more likely to be missed by our random forest imagery classification (Table 2). This may be due to single leafy spurge populations being harder to detect with our limited spatial resolution (4m x 4m pixels), banks changing with seasonal flooding between satellite imagery collection and validation mapping, and dense shrub cover obscuring leafy spurge invasions beneath their canopy, though canopy coverage itself was not a significant predictor of leafy spurge classification accuracy.

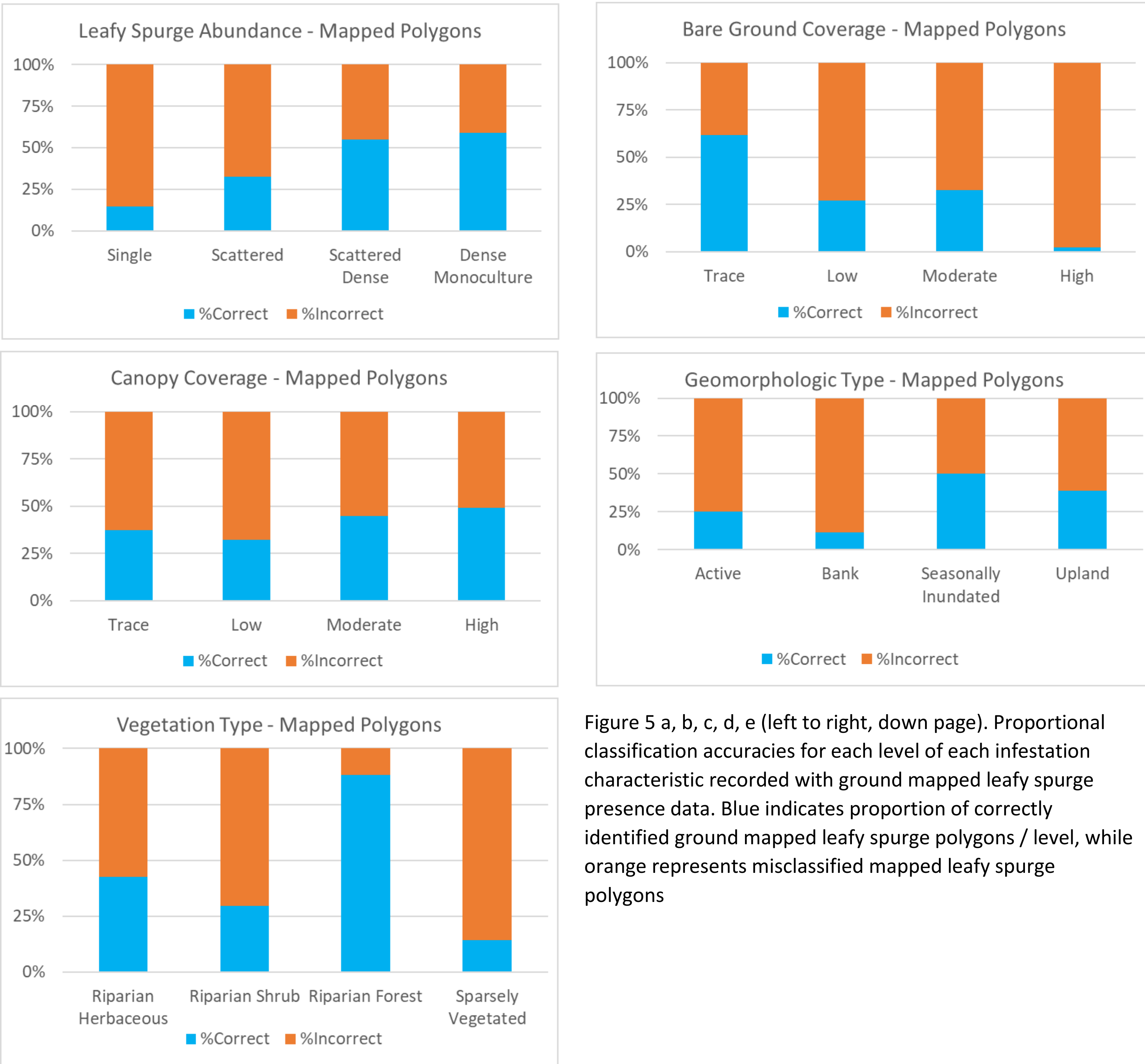


Figure 5 a, b, c, d, e (left to right, down page). Proportional classification accuracies for each level of each infestation characteristic recorded with ground mapped leafy spurge presence data. Blue indicates proportion of correctly identified ground mapped leafy spurge polygons / level, while orange represents misclassified mapped leafy spurge polygons

Table 2. Logistic regression output for significant predictors of leafy spurge classification of ground mapped leafy spurge polygons with odds ratio (values <1, decrease odds of correctly classifying leafy spurge, values >1, increase odds of correctly classifying leafy spurge), impact on leafy spurge prediction rates, and p-values of logistic regression.

	Odds Ratio	**Leafy Spurge Prediction**	**p-value**
Geomorphology - Bank	0.1803	-	0.0359
Bare Ground - Low	23.5146	+	0.0100
Bare Ground -Moderate	38.3177	+	0.0029
Bare Ground - Trace	89.3340	+	0.0002
Vegetation – Riparian Shrub	0.1061	-	0.0212
Leafy Spurge Abundance – Single	0.1361	-	0.0108
Polygon Area	1.0002	+	0.0180

Classification Results of Leafy Spurge Ground Validation Points

Much like the ground mapped leafy spurge dataset, the 271 ground validation points visited spanned a range of environmental and infestation conditions. Of these 271 points, 190 points were classified as leafy spurge (70% predicted leafy spurge), 81 were classified as not leafy spurge (30% not spurge), and 159 of the 271 points were correctly classified (59% overall accuracy rate). Of these validation points that were classified as leafy spurge, 102 out of 190 were correctly classified (54%). These validation accuracy rates are not encouraging because validation locations were chosen based on anomalies or features of interest from the classification prediction (e.g. a series of validation points were set in a seasonal Yampa tributary, to see if positive leafy spurge classified pixels were really spurge, or misclassification of riparian plants away from the main channel of the Yampa). The number of correctly identified leafy spurge locations and missed leafy spurge locations varies out of the 190 leafy spurge presence locations within the dataset (Figure 6). The random forest classification method was more accurate at identifying leafy spurge populations growing as discrete patches rather than scattered populations (Figure 6c). Binary classification results of correctly classified leafy spurge vs not leafy spurge was fit to an additive logistic regression model:

```
Logit2 <- glm(TRUE ~ geomorph + bareground + vegetation + abundance + canopycover + area + inundation + count_other_species)
```

Based on odds ratios of coefficients, discrete patches of leafy spurge and locations with higher leafy spurge percent cover were more likely to be correctly classified by the random forest imagery classification (Table 3). Dense populations with high leafy spurge cover may have more recognizable spectral signatures than sparse populations, and discrete boundaries of leafy spurge patches may be more identifiable, as scattered populations might share pixel space with other land cover types though validation locations, though number of additional species present at validation locations did not significantly influence classification accuracy of leafy spurge (Figure 6d).

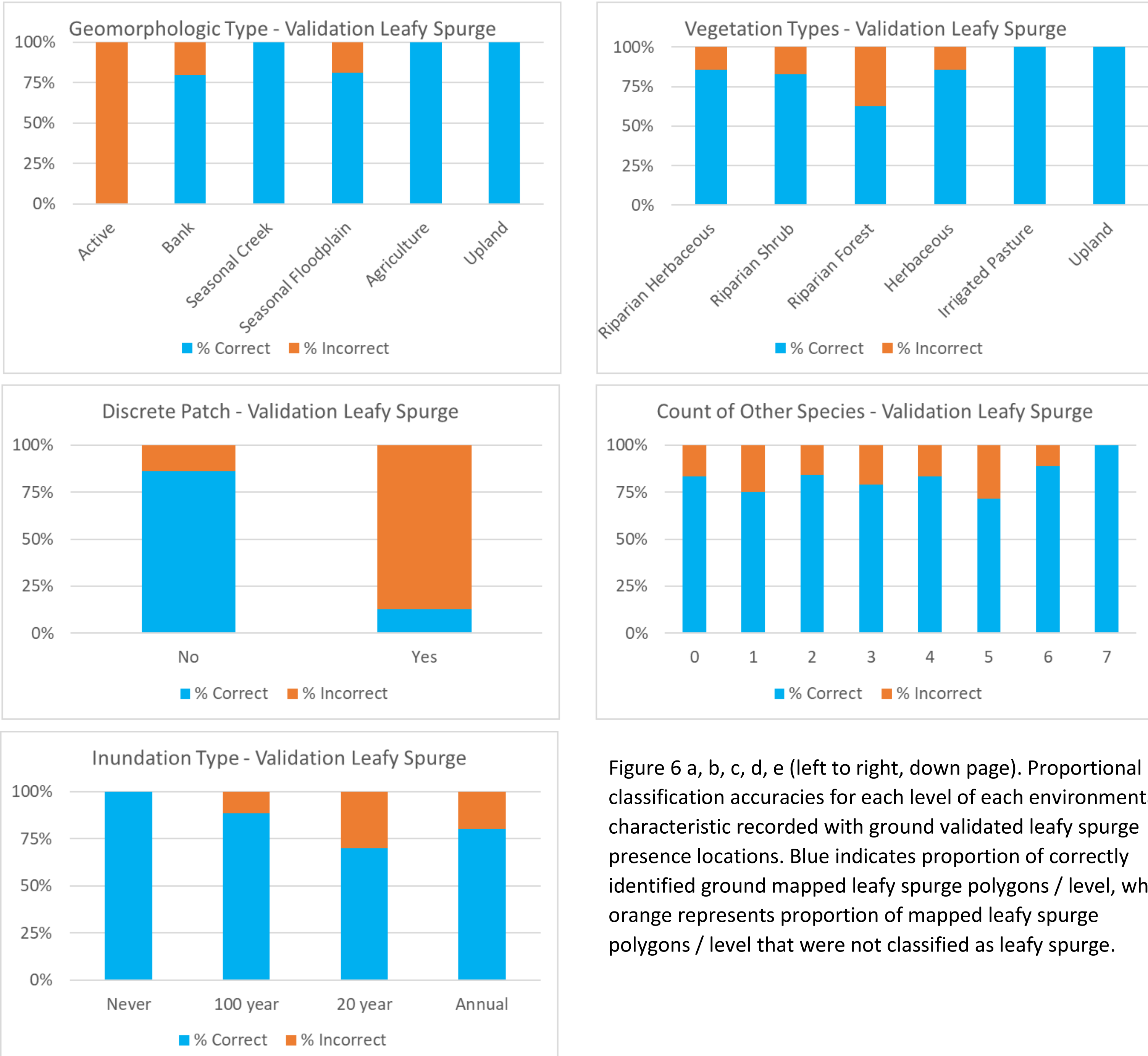


Figure 6 a, b, c, d, e (left to right, down page). Proportional classification accuracies for each level of each environmental characteristic recorded with ground validated leafy spurge presence locations. Blue indicates proportion of correctly identified ground mapped leafy spurge polygons / level, while orange represents proportion of mapped leafy spurge polygons / level that were not classified as leafy spurge.

Table 3. Logistic regression output for <u>significant predictors</u> with odds ratio (values <1, decrease odds of correctly classifying leafy spurge, values >1, increase odds of correctly classifying leafy spurge), impact on leafy spurge prediction rates, and p-values of logistic regression.

	Odds Ratio	**Odds of Spurge**	**p-value**
Discrete Patch	8.128695	+	0.0480
Leafy Spurge Percent Cover	1.555534	+	5.8e-08

Ecological Niche Modeling of Leafy Spurge

Invasion risk of leafy spurge was predicted for Moffat and Routt Counties using ecological niche models trained with either YRLSP ground mapped leafy spurge data and a large dataset of leafy spurge presence locations from Fremont County, Wyoming (Full model, ENM3, n = 17,721 points) or YRLSP ground mapped data only (ENM4, n = 314). Continuous suitability predictions were grouped into three equally distributed classes according to their values to create final maps of low, medium, and high leafy spurge suitability for both models (Figures 7 and 8) and amount of the study area was calculated for proportional area and acreage for each class (Tables 4 and 6). Generally, both models follow similar spatial patterns, but the Yampa only model, ENM4, predicted more acreage of invasion risk than the full Fremont and Yampa model, ENM3. This is somewhat unexpected, as a wider range of values for environmental predictors from a separate extent should theoretically increase the variability of predicted habitat suitability.

For each model, the final environmental predictors used were principal components developed from covarying factors from the full predictor set, and significant factors and the first five components for each model are summarized (Tables 5 and 7). For the full Fremont, Moffat, and Routt model, ENM3, soil textural properties, pH, and hydric rating, and slope were important. For the Yampa only model, precipitation and annual mean temperature are important. Aspect, mean temperature of the coldest quarter, and the precipitation of the warmest quarter were important in both models

(Tables 5 and 7). Slope and soil properties could have larger affects on leafy spurge habitat suitability in the full spatial extent because there is a wider range of values of both, as there are a lot more topographic types and soil orders represented in the full extent of environmental predictors. Mean temperature of the coldest temperature and precipitation of the warmest quarter would be hypothesized to influence leafy spurge ecological niche, as harsh winter cold and summer drought conditions stress plants across the region.

Model performance for the maximum entropy, support vector machine, random forest, and ensemble algorithms were evaluated based on their kappa values, which describe how well the habitat suitability classifier describes the leafy spurge data. The best fit for the full Fremont Yampa model, ENM3 is the ensemble model (kappa = 0.9697) while the best fit for the Yampa only model, ENM4 is a tie between the maximum entropy model and the random forest model (kappa of both = 0.9286) (Tables 6 and 9). The ensemble model underperforming the other two algorithms for the ENM4 was unexpected but may be an example of overfitting the ecological niche model. With either model describing current leafy spurge with over 90% accuracy, both models are valid, but the more conservative full Fremont and Yampa ENM3 leafy spurge ecological nice model should be a good tool for management and monitoring of leafy spurge in the Yampa River Watershed.

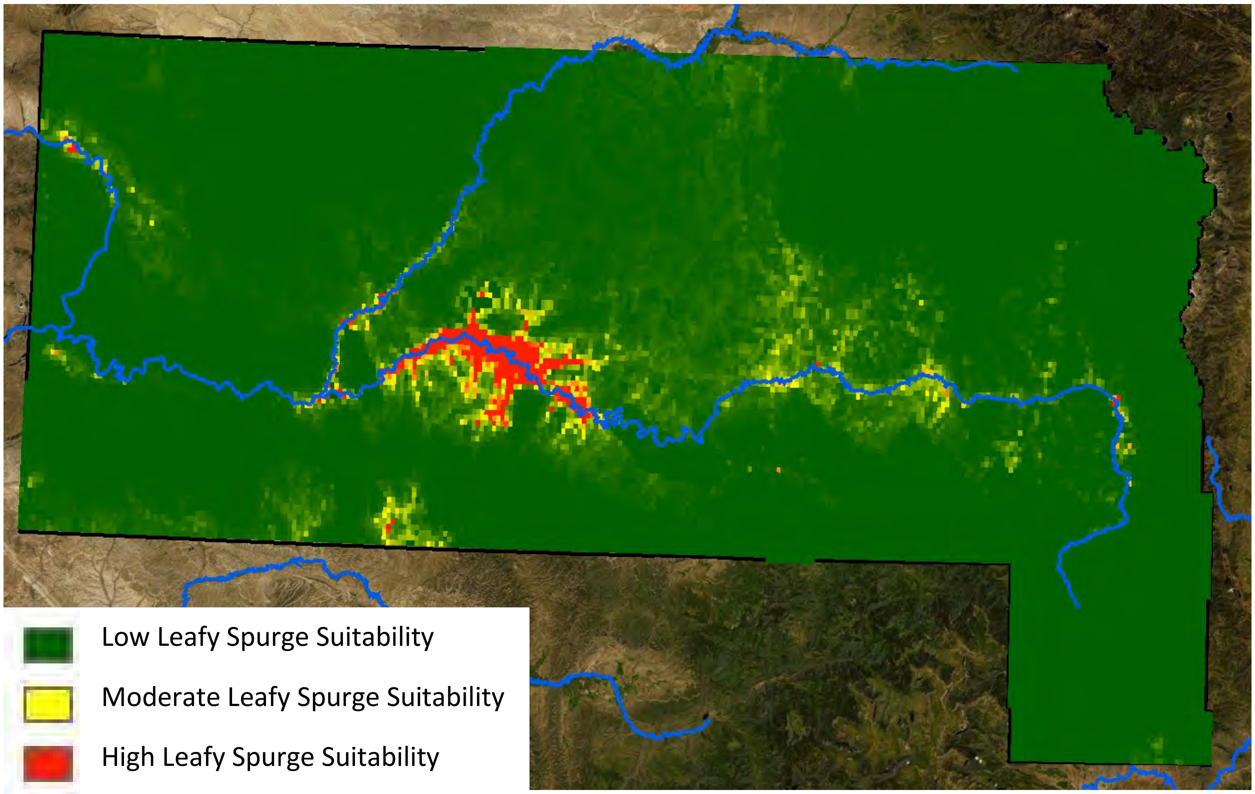


Figure 7. Categorical map of the full Fremont Yampa ENM3 leafy spurge habitat suitability model for Moffat and Routt Counties, with low, moderate, and high leafy spurge habitat suitability shown as green, yellow, and red, respectively.

Table 4. Output of full Fremont and Yampa ENM3 leafy spurge suitability classes, split into three classes (low, medium, and high) with proportional coverage and acreage within the Moffat-Routt County study area.

Class	% of Study Area	Class Acreage
Low	47.7	2173440
Medium	44.4	2023040
High	7.9	359680
		Total - 4556160

Table 5. Coefficient table of eigenvalues of significant environmental predictors from the full Fremont Yampa ENM3 for the first five components of the principal component analysis for reducing predictor collinearity.

ENM3	Name	Comp1	Comp2	Comp3	Comp4	Comp5
Clay	**% Soil Clay**	-0.0060		-0.0246	-0.0246	
OM	**% Soil Organic Matter**			-0.0160	-0.0370	
Sand	**% Soil Sand**	0.0090	-0.0466		-0.0499	0.0403
Silt	**% Soil Silt**	-0.0076				
BIO1	**Annual Mean Temperature**			-0.0320		
BIO12	**Annual Precipitation**			-0.0263		
Aspect	**Aspect**	0.044012		0.0292		
Hydric	**Hydric Rating of Soil**	0.0111	0.0405	0.0014		
BIO3	**Isothermality**			0.0030		
BIO5	**Max Temperature Warmest Month**			-0.0067		0.0042
Radiance	**Mean Daily Radiance**			-0.0002		
BIO2	**Mean Diurnal Range**			0.0400		
BIO11	**Mean Temperature Coldest Quarter**		0.0493			
BIO10	**Mean Temperature Warmest Quarter**			-0.0192		0.0118
BIO8	**Mean Temperature Wettest Quarter**			-0.0035		
BIO19	**Precipitation Coldest Quarter**			-0.0109		0.0384
BIO14	**Precipitation Driest Month**			-0.0214		0.0094
BIO17	**Precipitation Driest Quarter**			-0.0547		0.0294
BIO18	**Precipitation Warmest Quarter**		0.0235	-0.0404		
BIO13	**Precipitation Wettest Month**					-0.0380
BIO16	**Precipitation Wettest Quarter**					-0.0484
Slope	**Slope**		-0.0483	-0.0163		-0.0134
pH	**Soil pH**		0.0056			0.0046
BIO7	**Temperature Annual Range**			0.0331		
BIO4	**Temperature Seasonality**			0.0160		

Table 6. Model evaluation table from full Fremont Yampa ENM3 model, showing the area under the curve (AUC) and kappa (estimate of classification accuracy of the model), with values closer to 1 indicating the model that better describes the data.

Algorithm	AUC	Kappa
Ensemble	0.9953	0.9697
Random Forest	0.9958	0.9611
Support Vector Machine	0.9930	0.9496
Maximum Entropy	0.9950	0.9438

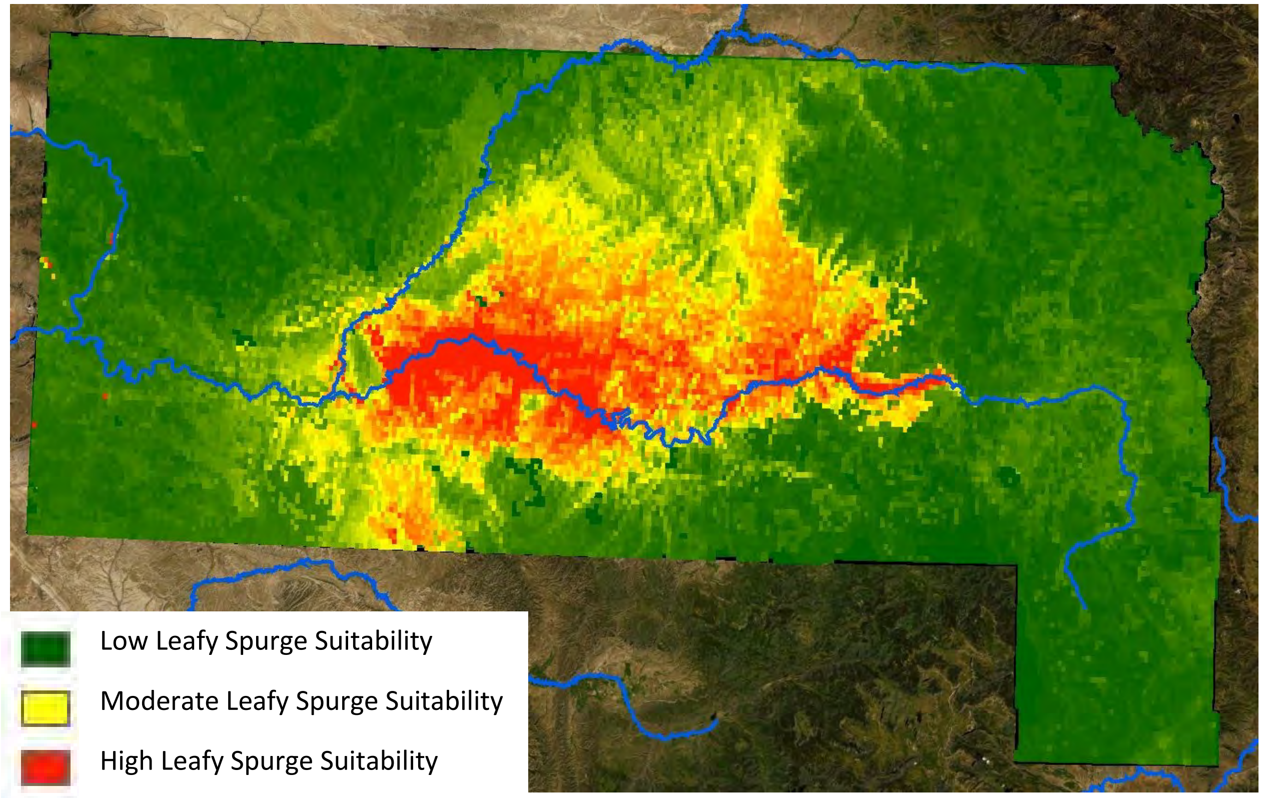


Figure 8. Categorical map of the Yampa only ENM4 leafy spurge habitat suitability model for Moffat and Routt Counties, with low, moderate, and high leafy spurge habitat suitability shown as green, yellow, and red, respectively.

Table 7. Output of Yampa only ENM4 leafy spurge suitability classes, split into three classes (low, medium, and high) with proportional coverage and acreage within the Moffat Routt County study area.

Class	% of Study Area	Class Acreage
Low	38.6	1758720
Medium	41.7	1900160
High	19.7	897280
		Total - 4556160

Table 8. Coefficient table of eigenvalues of significant environmental predictors from the Yampa only ENM4 for the first five components of the principal component analysis for reducing predictor collinearity.

ENM4	Name	Comp1	Comp2	Comp3	Comp4	Comp5
%Clay	**% Soil Clay**					-0.0241
%OM	**% Soil Organic Matter**			0.0316	-0.0086	
BIO1	**Annual Mean Temperature**			-0.0017		-0.0146
BIO12	**Annual Precipitation**			0.0484		-0.0329
Aspect	**Aspect**	0.0285	0.0078	0.0457		
BIO5	**Max Temp Warmest Month**			0.0494		-0.0100
BIO2	**Mean Diurnal Range**				0.0176	
BIO11	**Mean Temperature Coldest Quarter**		-0.0358			0.0052
BIO9	**Mean Temperature Driest Quarter**			0.0482		
BIO10	**Mean Temperature Warmest Quarter**			0.0119		-0.0359
BIO14	**Precipitation Driest Month**			0.0473		
BIO17	**Precipitation Driest Quarter**			0.0481		-0.0427
BIO18	**Precip Warmest Quarter**		0.0207	0.0246	-0.0438	0.0391
BIO13	**Precipitation Wettest Month**			0.0446	0.0396	-0.0148
BIO16	**Precipitation Wettest Quarter**			0.0437	0.0387	-0.0198
pH	**Soil pH**				-0.0276	-0.0438

Table 9. Model evaluation table from Yampa only ENM4 model, showing the area under the curve (AUC) and kappa (estimate of classification accuracy of the model), with values closer to 1 indicating the model that better describes the data.

Algorithm	AUC	Kappa
Maximum Entropy	0.9866	0.9286
Random Forest	0.9936	0.9286
Ensemble	0.9917	0.9107
Support Vector Machine	0.9898	0.9107

References

Anderson, G. L., Everitt, J. H., Escobar, D. E., Spencer, N. R., & Andrascik, R. J. (1996). Mapping leafy spurge (euphorbia esula) infestations using aerial photography and geographic information systems. *Geocarto International*, *11*(1), 81–89. https://doi.org/10.1080/10106049609354526

Andrade, A. F. A. de, Velazco, S. J. E., & De Marco Júnior, P. (2020). ENMTML: An R package for a straightforward construction of complex ecological niche models. *Environmental Modelling and Software*, *125*(March), 104615. https://doi.org/10.1016/j.envsoft.2019.104615

Bazzichetto, M., Malavasi, M., Bartak, V., Acosta, A. T. R., Rocchini, D., & Carranza, M. L. (2018). Plant invasion risk: A quest for invasive species distribution modelling in managing protected areas. *Ecological Indicators*, *95*, 311–319. https://doi.org/10.1016/j.ecolind.2018.07.046

F . Larry Leistritz, D. A. . B. and N. M. . H. (2004). Assessing the Economic Impact of Invasive Weeds : The Case of Leafy Spurge (Euphorbia esula). *Weed Technology*, *18*(2004), 1392–1395.

Goodwin, K., Sheley, R., Nowierski, R., & Lym, R. (2001). Leafy Spurge: Biology , Ecology and Management, 1–24. Retrieved from http://store.msuextension.org/publications/AgandNaturalResources/EB0134.pdf

Hunt, E. R., Mcmurtrey, J. E., Williams, A. E. P., & Corp, L. A. (2004). *Spectral characteristics of leafy spurge (Euphorbia esula) leaves and flower bracts*. Retrieved from https://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=10282&content=PDF

Irzel, A. H. H., Ausser, J. H., & Hessel, D. C. (2002). Ecological-Niche Factor Analysis: How To Compute Habitat-Suitability Maps Without Absence Data ? *Ecology*, *83*(7), 2027–2036. https://doi.org/10.1890/0012-9658(2002)083[2027:ENFAHT]2.0.CO;2

Peterson, T. A., Vieglais, D., Approach, N. E. W., Ecological, T. O., On, B., Tools, N. E. W., … Potential, P. (2001). Predicting Species Invasions Using Ecological Niche Modeling: New Approaches from Bioinformatics Attack a Pressing Problem. *BioScience*, *51*(5), 363. https://doi.org/10.1641/0006-3568(2001)051[0363:PSIUEN]2.0.CO;2

Williams, A. P., & Raymond, E. (2002). *Estimation of leafy spurge cover from hyperspectral imagery using mixture tuned matched filtering*. Retrieved from www.elsevier.com/locate/rse

To the University of Wyoming:

The members of the Committee approve the thesis of Hannah Kuhns presented on July 28, 2021.

Dr. Andrew Kniss, Chairperson

Dr. John Derek Scasta, Outside Member

Dr. Daniel Tekiela, External Member

APPROVED:

Dr. Andrew Kniss, Head, Department of Plant Sciences.

Dr. Barbara Rasco, Dean, College of Agriculture and Natural Resources

Kuhns, Hannah, A. D., Leafy Spurge (*Euphorbia esula* L.) seed production, germination, and vegetative propagule potential in a riparian ecosystem, Master of Science, Department of Plant Sciences, August, 2021.

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

Hannah Kuhns

A thesis submitted to the Department of Plant Sciences

and the University of Wyoming

in partial fulfillment of the requirements

for the degree of **Master of Science**

in

Plant Sciences

Laramie, Wyoming

August 2021

ACKNOWLEDGEMENTS

I would like to thank Dr. Dan Tekiela for guiding me through this process and for challenging and supporting me in equal measure. Thank you to Dr. Andrew Kniss for your time and support and constant patience in your answers of all of my statistically-related questions, as well as for your guidance through the home stretch of this process. Thank you to Dr. Derek Scasta and Dr. Debalin Sarangi for providing invaluable insight during the proposal process and helping me to develop my ideas. To Dr. Albert Adjesiwor – thank you for sharing your insight and expertise with me.

I would like to thank Chloe Mattilio, Emily Repas, Claire Visconti, Nick Race, Axl Taylor, and Caz Norton for endless assistance in the field, lab, and greenhouse, as well as for general motivation and moral support.

A special thanks to everyone who is a part of and/or associated with the Yampa River Leafy Spurge Project – this would not have been possible without your knowledge and local connections and community spirit. Thank you to Ryan Pendleton who was a wonderful resource at the greenhouse and to Ellyn Sturgeon for much needed moral support and general logistic assistance.

To my family – thank you for your love and support and belief in me. I could not have done it without you. Finally, Jackson Rambough, thank you for being my best friend and the most giving and strong and selfless partner through all of this.

TABLE OF CONTENTS

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.

Literature Cited

Alley, H. P., C. G., Messersmith. 1985. Chemical control of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*. Chapter 7, 65 – 78.

Bangsund, D. A., J. F. Baltezore, J. A. Leitch, F. L. Leistritz. 1993. Economic impact of leafy spurge on wildland in Montana, South Dakota, and Wyoming. *Agricultural Economics Report*, 304: No. 1189-2016-94289.

Dunn, P. H. 1979. The distribution of leafy spurge (*Euphorbia esula*) and other weedy *Euphorbia* spp. in the United States. *Weed Science*. 27(5): 509 – 516.

Hanson, H. C., V. E. Rudd. 1933. Leafy spurge: Life history and habitats. *Agricultural Experiment Station Bulletin 226, Fargo: North Dakota Agricultural College*.

Leistritz, F. L., D. A. Bangsund, N. M. Hodur. 2004. Assessing the economic impact of invasive weeds: The case of leafy spurge (*Euphorbia esula*). *Weed Technology*. 18: 1392 – 1395.

Messersmith, C. G., R. G. Lym, D. S. Galitz. 1985. Biology of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 5(3): 42 – 56.

Selleck, G. W., R. T. Coupland, C. Frankton. 1962. Leafy spurge in Saskatchewan. *Ecological Monographs*. 32(1): 1 – 29.

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.

Literature Cited

Alley, H. P., C. G., Messersmith. 1985. Chemical control of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*. Chapter 7, 65 – 78.

Anderson, G. L., E. S. Delfosse, N. R. Spencer, C. W. Prosser, R. D. Richard. 1999. Biological control of leafy spurge: An emerging success story. *USDA, ARS, Montana State University-Bozeman*. 15 – 25.

Bangsund, D. A., J.A. Leitch, F. L. Leistritz. 1996. Economics of herbicide control of leafy spurge (*Euphorbia esula* L.). *Journal of Agricultural and Resource Economics*. 21(2): 381 – 395.

Detling, J. K., M. I. Dyer, D. T. Winn. 1979. Net photosynthesis, root respiration and regrowth of *Bouteloua gracilis* following simulated grazing. *Oecologia*. 41(2): 127 – 134.

Frost, R. A., K. L. Launchbaugh. 2003. Prescription grazing for rangeland weed management: A new look at an old tool. *Rangelands*. 25(6): 43 – 47.

Goodwin, K., R. L. Sheley, R. Nowierski, R. Lym. 2003. Leafy spurge: Biology, ecology and management. *Montana State University, Extension Service Bulletin*, EB 124.

Hanson, H. C., V. E. Rudd. 1933. Leafy spurge: Life history and habitats. *Agricultural Experiment Station Bulletin 226, Fargo: North Dakota Agricultural College*.

Helgeson, E. A., J. H. Longwell. 1942. Control of leafy spurge by sheep. *Bimonthly Bulletin. North Dakota Agricultural Experiment Station*. 4: 10 – 12.

Hilbert, D. W., D. M. Swift, J. K. Detling, M. I. Dyer. 1981. Relative growth rates and the grazing optimization hypothesis. *Oecologia*. 51(1): 14 – 18.

Johnston, A., R. W. Peake. 1960. Effect of selective grazing by sheep on the control of leafy spurge (*Euphorbia esula* L.). *Journal of Range Management*. 13(4): 192 – 195.

Kirby, D. R., R. B. Carlson, K. D. Krabbenhoft, D. Mundal, M. M. Kirby. 2000. Biological control of leafy spurge with introduced flea beetles (*Aphthona* spp.). *Journal of Range Management*. 53(3): 305 – 308.

Landgraf, B. K., P. K. Fay, K. M. Havstad. 1984. Utilization of leafy spurge (*Euphorbia esula*) by sheep. *Weed Science*. 32(3): 348 – 352.

Leitch, J. A., F. L. Leistritz, D. A. Bangsund. 1996. Economic effect of leafy spurge in the Upper Great Plains: Methods, models, and results. *Impact Assessment*. 14(4): 419 – 433.

Leistritz, F. L., D. A. Bangsund, N. M. Hodur. 2004. Assessing the economic impact of invasive weeds: The case of leafy spurge (*Euphorbia esula*). *Weed Technology*. 18: 1392 – 1395.

Lym, R. G. 1998. The biology and integrated management of leafy spurge (*Euphorbia esula*) on North Dakota Rangeland. *Weed Technology*. 12(2): 367 – 373.

Lym, R. G., C. G. Messersmith. 1983. Control of leafy spurge with herbicides. *North Dakota Farm Research*. 16 – 19.

Lym, R. G., C. G. Messersmith. 1985. Cost effectiveness of leafy spurge control during a five-year management program. *North Dakota Farm Research Bimonthly Bulletin*. 43(1): 7 – 9, 14.

Lym, R. G., C. G. Messersmith. 1994. A decade of herbicide treatments controlled leafy spurge. *North Dakota Farm Research Bulletin, ND Agricultural Experiment Station*. 50(3): 9 – 12.

Lym, R. G., D. R. Kirby. 1987. Cattle foraging behavior in leafy spurge (*Euphorbia esula*)-infested rangeland. *Weed Technology*. 1(4): 314 – 318.

Messersmith, C. G., R. G. Lym, D. S. Galitz. 1985. Biology of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 5(3): 42 – 56.

Nelson, D., D. Hirsch. 1999. Biological control of leafy spurge using flea beetles in North Dakota. *North Dakota Department of Agriculture*. 11 pp.

Noble, D. L., P. H. Dunn, L. A. Andres. 1979. The leafy spurge problem. *Proceedings: Leafy Spurge Symposium*. 8 – 15.

Nowierski, R. M., R. W. Pemberton. 2002. Leafy spurge. *In: Van Driesche, R. et al. 2002, Biological Control of Invasive Plants in the Eastern United States*. Chapter 14: 181 – 194.

Olson, B. E., J. R. Lacey. 1994. Sheep: A method for controlling rangeland weeds. *Sheep Research Journal, Special Issue*. 105 – 112.

Paige, K. N., T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory: The advantage of being eaten. *The American Naturalist*. 129(3): 407 – 416.

Rees, N. E. 1994. The *Aphthona* pilot study. *Leafy Spurge Symposium, Bozeman, MT*. p. 28.

Sedivec, K. K., R. P. Maine. 1993. Angora goat grazing as a biological control for leafy spurge: A three-year summary. *Great Plains Agricultural Council Leafy Spurge Task Force*. 1 – 5.

Sedivec, K. K., T. Hanson, C. Heiser. 1995. Controlling leafy spurge using goats and sheep. *Extension Publication R-1093, North Dakota State University, Fargo, ND.*

Selleck, G. W., R. T. Coupland, C. Frankton. 1962. Leafy spurge in Saskatchewan. *Ecological Monographs*, 32(1): 1 – 29.

Sheley, R. L., B. H. Mullin, P. K. Fay. 1995. Managing riparian weeds. *Rangelands*. 17(5): 154 – 157.

Chapter 2 figures and tables

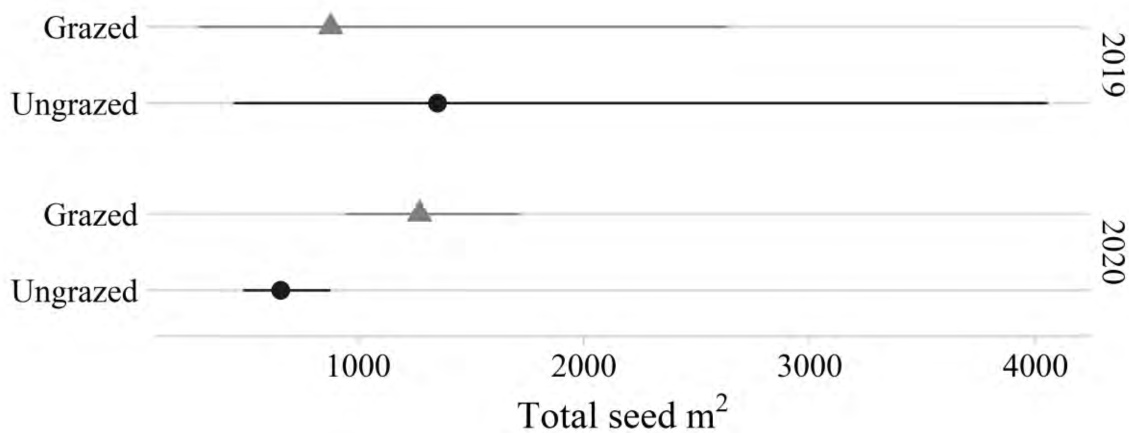


Figure 1. Within treatment season (2019) and one-year post-treatment season (2020) effect of grazing on leafy spurge total seed production (error bars represent 95% confidence intervals). Grazed plots had a reduction in leafy spurge seed production in 2019 ($p = 0.0203$) and an increase in leafy spurge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

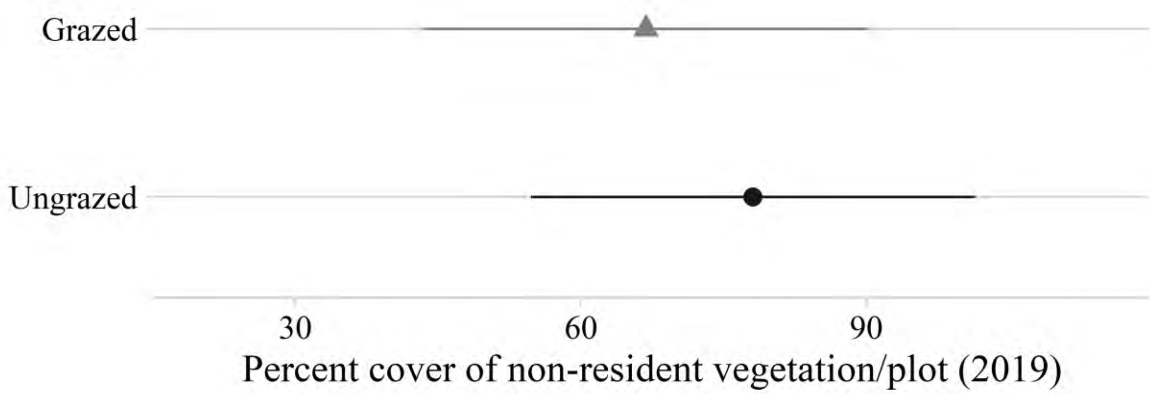


Figure 2. Within treatment season (2019) effect of grazing on non-resident vegetation cover (error bars represent 95% confidence intervals). Non-resident vegetation cover was reduced in the plots that were grazed ($p = 0.0009$).

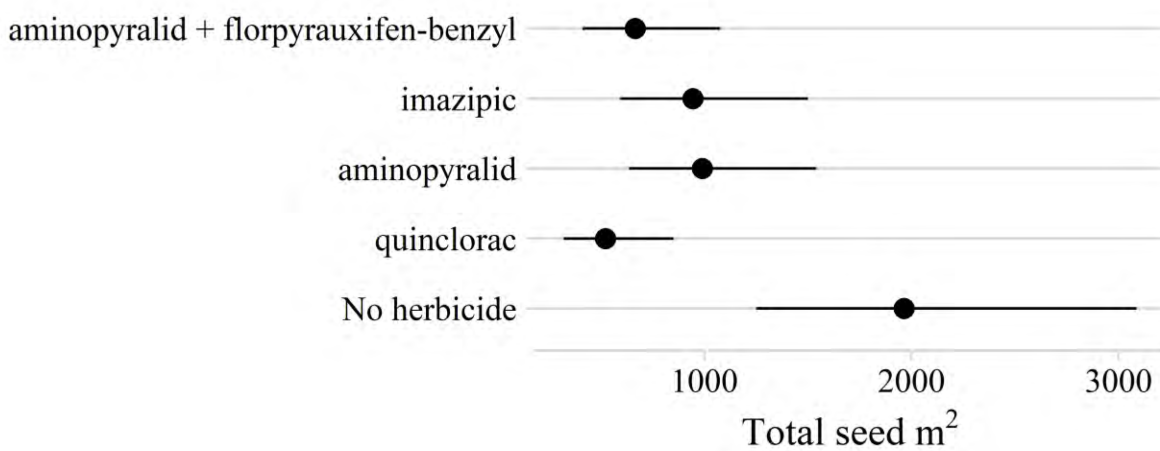


Figure 3. One-year post-treatment season (2020) effect of herbicide on leafy spurge total seed production (error bars represent 95% confidence intervals). Herbicide treatments significantly reduced the total leafy spurge seed production compared to plots that did not receive applications ($p = 0.0013$).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

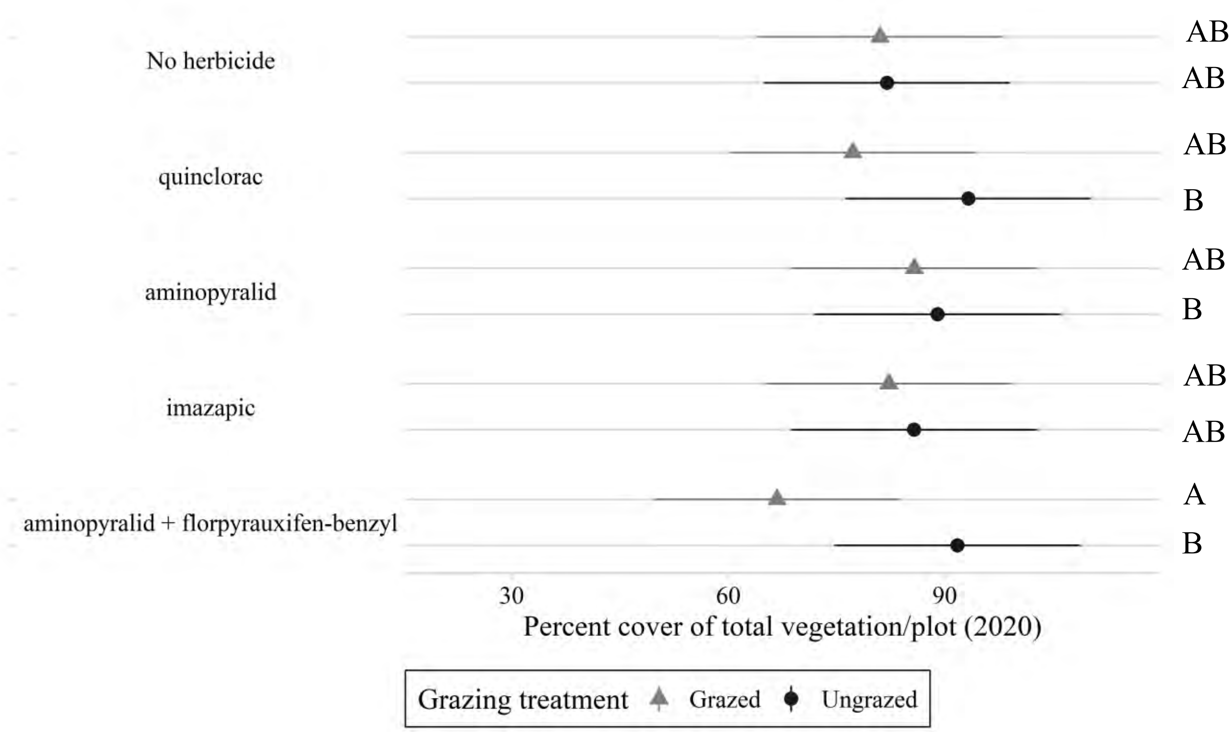


Figure 4. One-year post-treatment season (2020) effect of grazing and herbicide interaction on total vegetation cover (p = 0.0459), percentages with the same letter are not significantly different (error bars represent 95% confidence intervals). The combination of grazing and aminopyralid + florpyrauxifen-benzyl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

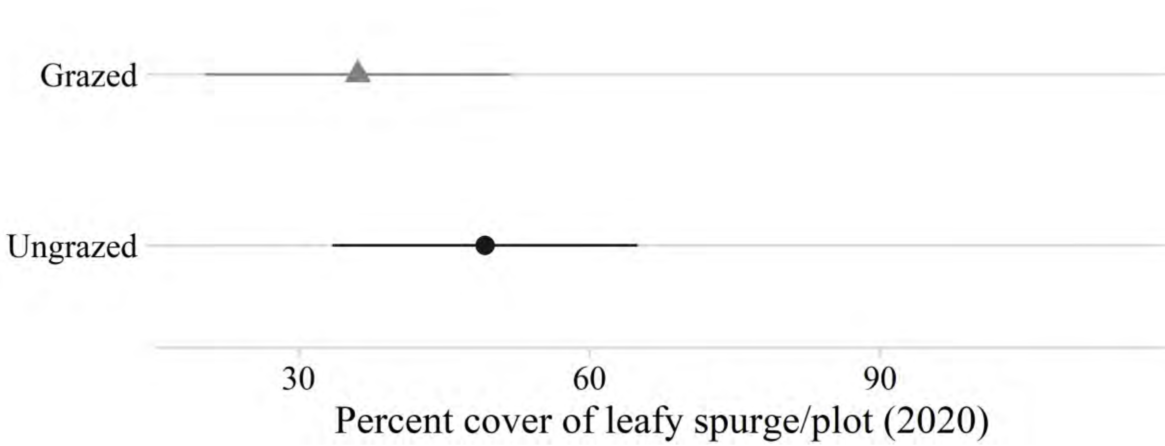


Figure 5. One-year post-treatment (2020) effect of grazing on leafy spurge cover (error bars represent 95% confidence intervals). Grazed plots had a greater reduction in percent cover of leafy spurge compared to plots that were not grazed (p = 0.0044).

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):

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

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.

Literature Cited

Bakke, A. L. 1936. Leafy spurge, *Euphoriba esula* L. *Research Bulletin: Iowa Agriculture and Home Economics Experiment Station*, 17(198): 1.

Best, K. F., G. G. Bowes, A. G. Thomas, M. G. Maw. 1980. The biology of Canadian weeds. 39. *Euphorbia esula* L. *Canadian Journal of Plant Science*, 60: 651 – 553.

Bowes, G. G., A. G. Thomas. 1978a. Leafy spurge (*Euphorbia esula* L.) control based on a population model. *Proceedings of the 1st International Rangelands Congress*, 254 – 256.

Bowes, G. G., A. G. Thomas. 1978b. Longevity of leafy spurge seeds in the soil following various control programs. *Journal of Range Management*, 31: 137 – 140.

Brown, E. O., R. H. Porter. 1942. The viability and germination of seeds of *Convolvulus arevensis* L. and other perennial weeds. *Iowa Agriculture and Home Economics Experimental Station Research Bulletin*, 25(294): 475 – 504.

Dunn, P. H. 1979. The distribution of leafy spurge (*Euphorbia esula*) and other weedy *Euphorbia* spp. in the United States. *Weed Science*. 27(5): 509 – 516.

Foley, M. E. 2004. Leafy spurge (*Euphorbia esula*) seed dormancy. *Weed Science*, 52(1): 74 – 77.

Foley, M. E. 2008. Temperature and moisture status affect afterripening of leafy spurge (*Euphorbia esula*) seeds. *Weed Science*, 56(2): 237 – 243.

Foley, M. E., W. S. Chao. 2008. Growth regulators and chemicals stimulate germination of leafy spurge (*Euphorbia esula*) seeds. *Weed Science*, 56(4): 516 – 522.

Hanson, H. C., V. E. Rudd. 1933. Leafy spurge: Life history and habitats. *Agricultural Experiment Station Bulletin 226, Fargo: North Dakota Agricultural College*.

Messersmith, C. G., R. G. Lym, D. S. Galitz. 1985. Biology of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 5(3): 42 – 56.

Michel, B. E. 1983. Evaluation of water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology*, 72: 66 – 70.

Selleck, G. W., R. T. Coupland, C. Frankton. 1962. Leafy spurge in Saskatchewan. *Ecological Monographs*, 32(1): 1 – 29.

Watson, A. K. 1985. Introduction – The leafy spurge problem. *Leafy Spurge, Monograph series of the Weed Science Society of America*, Chapter I(3): 1 – 6.

Wicks, G. A., Derscheid, L. A. 1964. Leafy spurge seed maturation. *Weeds*, 12(3): 175 – 176.

Chapter 3 figures and tables

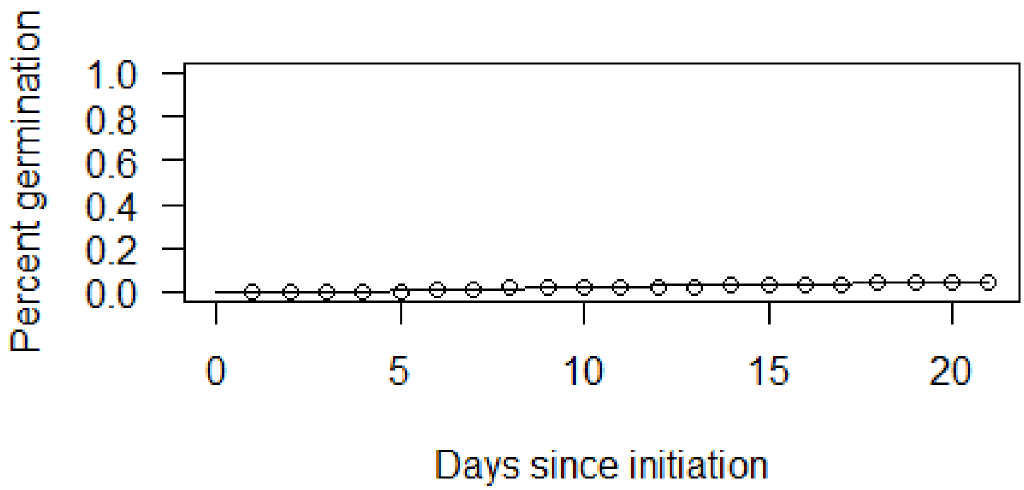


Figure 1. Percent germination of leafy spurge seeds over 21 days based on dose-response analysis model output for 0 Ψ and 30 °C treatment combination. The d parameter represents the upper asymptote, or the proportion of seeds that can germinate at the longest interval (d = 0.05, p-value < 0.001). The 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.

<u>Change in root buds analysis</u>

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.

Literature Cited

Bakke, A. L. 1936. Leafy spurge, *Euphoriba esula* L. *Research Bulletin: Iowa Agriculture and Home Economics Experiment Station*, 17(198): 1.

Bowes, G. G., A. G. Thomas. 1978. Leafy spurge (*Euphorbia esula* L.) control based on a population model. *Proceedings of the 1st International Rangeland Congress. Denver, CO*: 254 – 256.

Coupland, R. T., J. F. Alex. 1954. Distribution of the underground parts of leafy spurge (*Euphorbia esula* L.). *Canadian Journal of Agricultural Science*, 34(2): 161 – 176.

Coupland, R. T., J. F. Alex. 1955. Distribution of vegetative buds on the underground parts of leafy spurge (*Euphorbia esula* L.). *Canadian Journal of Agricultural Science*, 35(1): 76 – 82.

Dunn, P. H. 1979. The distribution of leafy spurge (*Euphorbia esula*) and other weedy *Euphorbia* spp. in the United States. *Weed Science*. 27(5): 509 – 516.

Hanson, H. C., V. E. Rudd. 1933. Leafy spurge: Life history and habits. *Agricultural Experiment Station Bulletin 226, Fargo: North Dakota Agricultural College*.

Hayden, A. 1934. Distribution and reproduction of Canada thistle in Iowa. *American Journal of Botany*. 355 – 373.

Heidel, B. 1982. Leafy spurge – A challenge in natural areas management. *Natural Areas Journal*. 2(2): 10 – 13.

Messersmith, C. G., R. G. Lym, D. S. Galitz. 1985. Biology of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 5(3): 42 – 56.

McIntyre, G. I. 1979. Developmental studies on *Euphorbia esula*. Evidence of competition for water as a factor in the mechanism of root bud inhibition. *Canadian Journal of Botany*, 57(22): 2572 – 2581.

Progar, R. A., G. Markin, J. Milan, T. Barbouletos, M. J. Rinella. 2010. Inundative release of *Apthona* spp. flea beetles (Coleoptera:Chrysomelidea) as a biological "herbicide" on leafy spurge in riparian areas. *Journal of Economic Entomology*, 103(2): 242 – 248.

Raju, M. V. S. 1985. Morphology and anatomy of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 4(3): 26 – 41.

Raju, M. V. S., R. T. Coupland, T. A. Steeves. 1966. On the occurrence of root buds on perennial plants in Saskatchewan. *Canadian Journal of Botany*, 44(1): 33 – 37.

Raju, M. V. S., T. A. Steeves, R. T. Coupland. 1963. Developmental studies on *Euphorbia esula* L. Morphology of the root system. *Canadian Journal of Botany*, 41(5): 579 – 589.

Raju, M. V. S., T. A. Steeves, R. T. Coupland. 1964a. On the regeneration of root fragments of leafy spurge (*Euphorbia esula* L.). *Weed Research*, 4(1): 2 – 11.

Raju, M. V. S., T. A. Steeves, J. M. Naylor. 1964b. Developmental studies on *Euphorbia esula* L: Apices of long and short roots. *Canadian Journal of Botany* 42(12): 1615 – 1628.

Selleck, G. W., R. T. Coupland, C. Frankton. 1962. Leafy spurge in Saskatchewan. *Ecological Monographs*. 32(1): 1 – 29.

Chapter 4 figures and tables

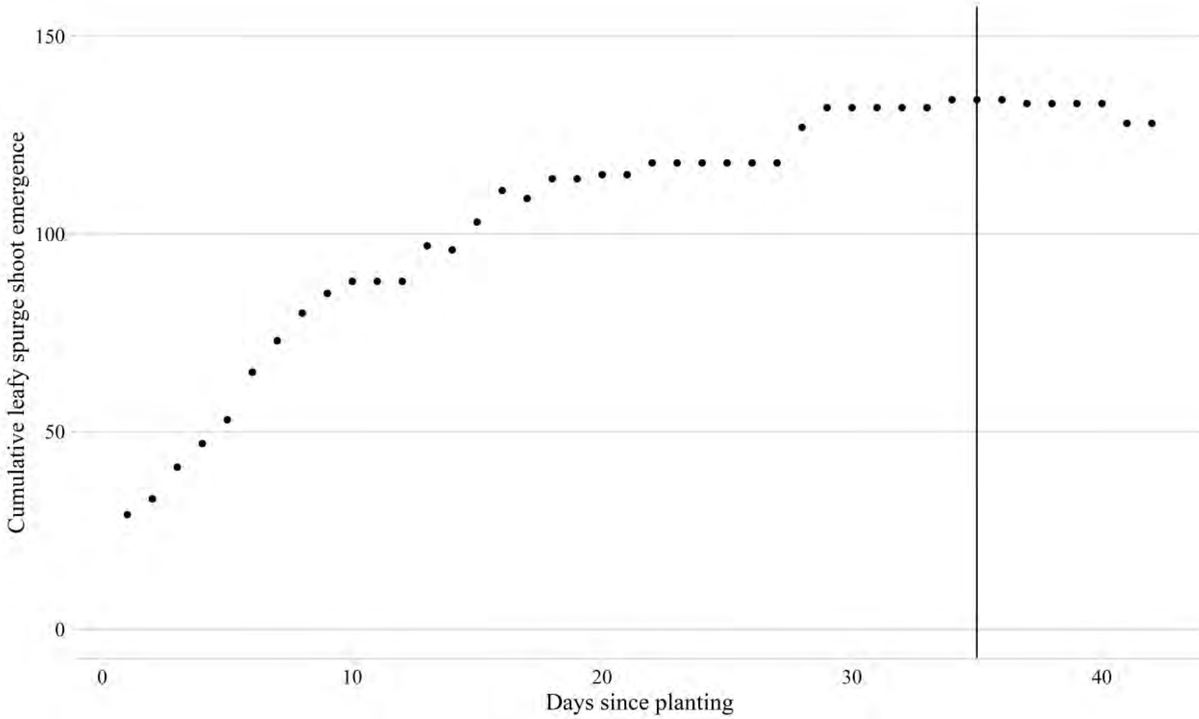


Figure 1. Cumulative distribution function for leafy spurge shoot emergence from root fragments after planting in the greenhouse. Based on the curve, root fragments were left planted for a minimum of 35 days (vertical line) before being dug up and quantified for buds and shoot emergence.

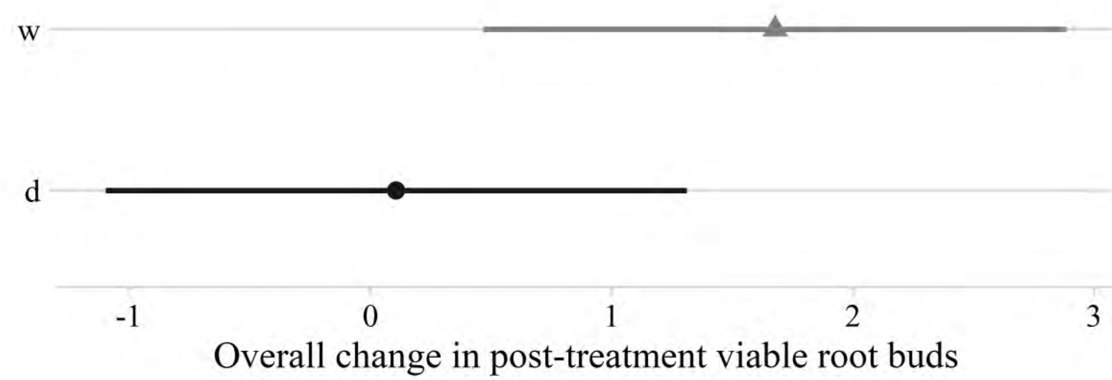


Figure 2. Overall change in post-treatment viable root buds for wet (w) and dry (d) treatments. Root fragments exposed to the wet treatment had a 1.6% increase in viable buds compared to the root fragments exposed to the dry moisture treatment (p = 0.07).

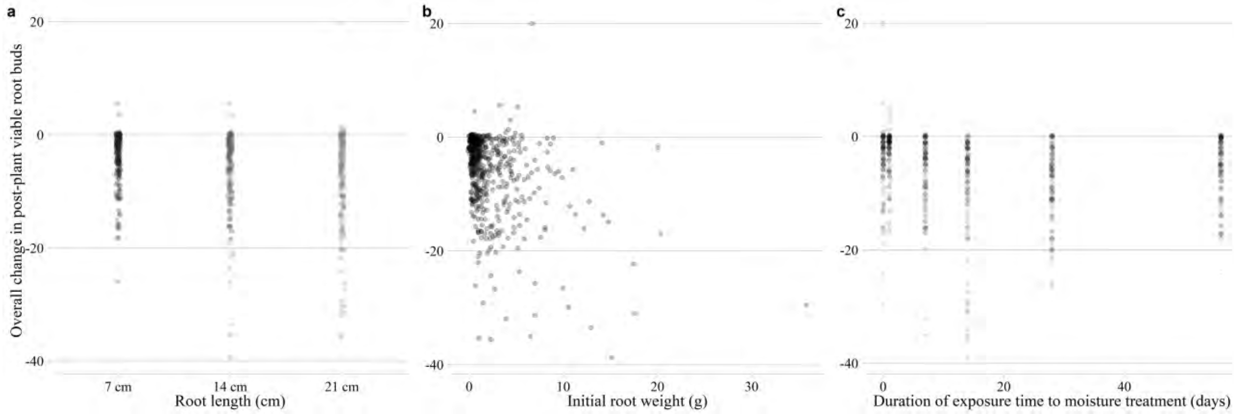


Figure 3. Overall change in post-plant viable root buds for root length (a), initial root weight (b), and duration of exposure time to moisture treatment (c). Longer root fragments had a 0.1% reduction in viable buds for every centimeter of increased root length ($p = 0.053$). Root fragments with heavier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

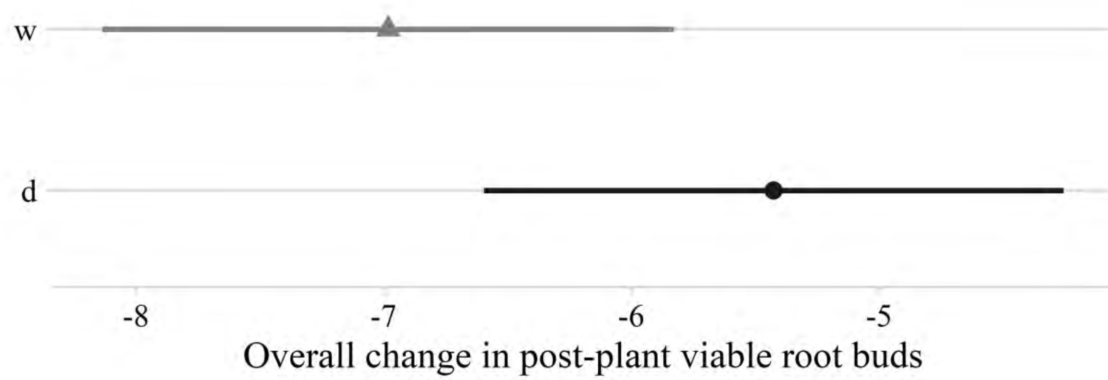


Figure 4. Overall change in post-plant viable root buds for wet (w) and dry (d) treatments. Root fragments exposed to the wet treatment had a 2% decrease inviable buds compared to the root fragments exposed to the dry treatment (p = 0.064).

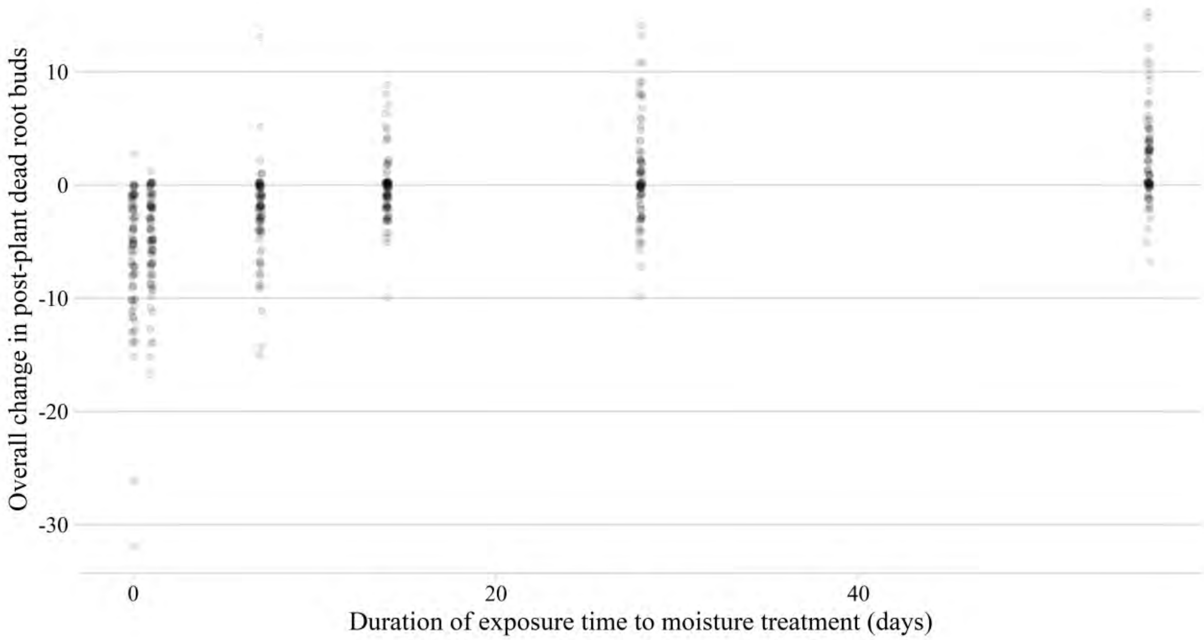


Figure 5. Overall change in post-plant dead root buds for the duration of exposure to the moisture treatments. Root fragments exposed to the moisture treatments for a longer duration of time had a 0.1% increase in dead buds for every additional day of exposure ($p < 0.001$).

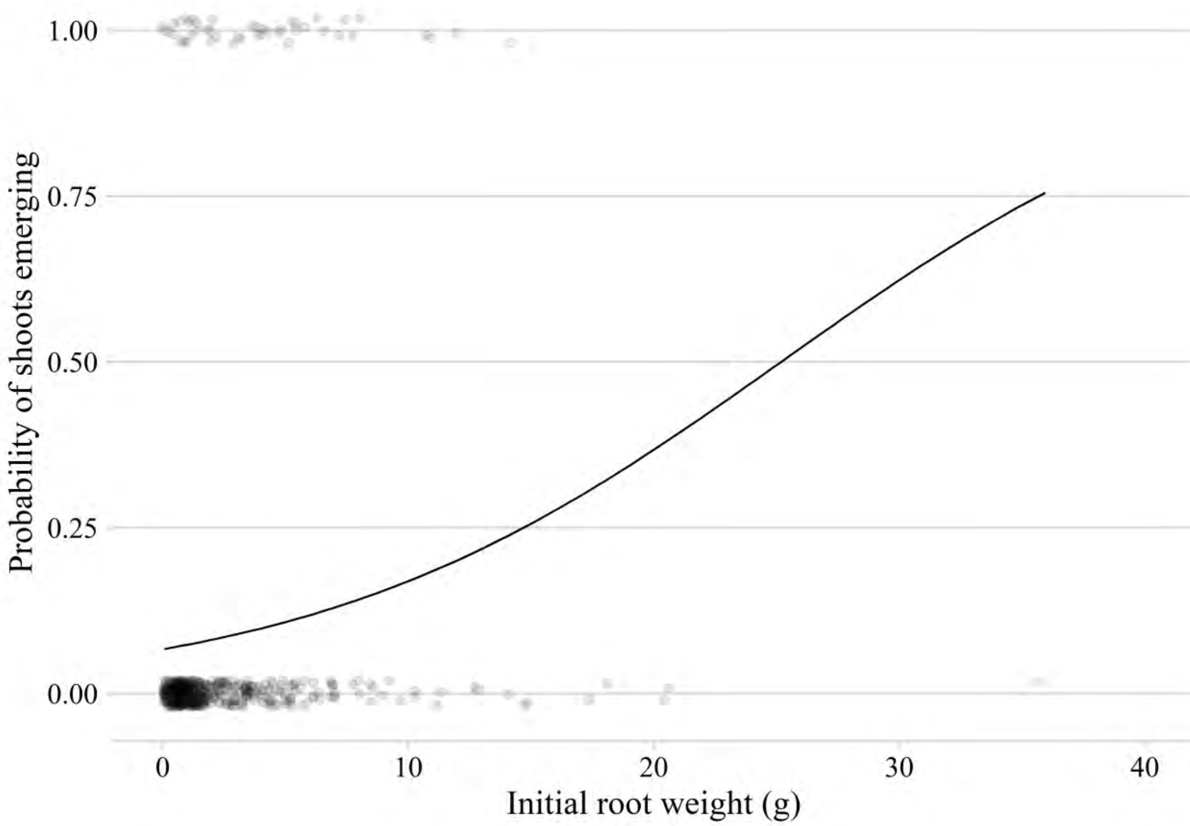


Figure 6. Probability of shoots emerging based on initial root weight (g). Root fragments with heavier initial root weights were more likely to produce shoots ($p = 0.007$).

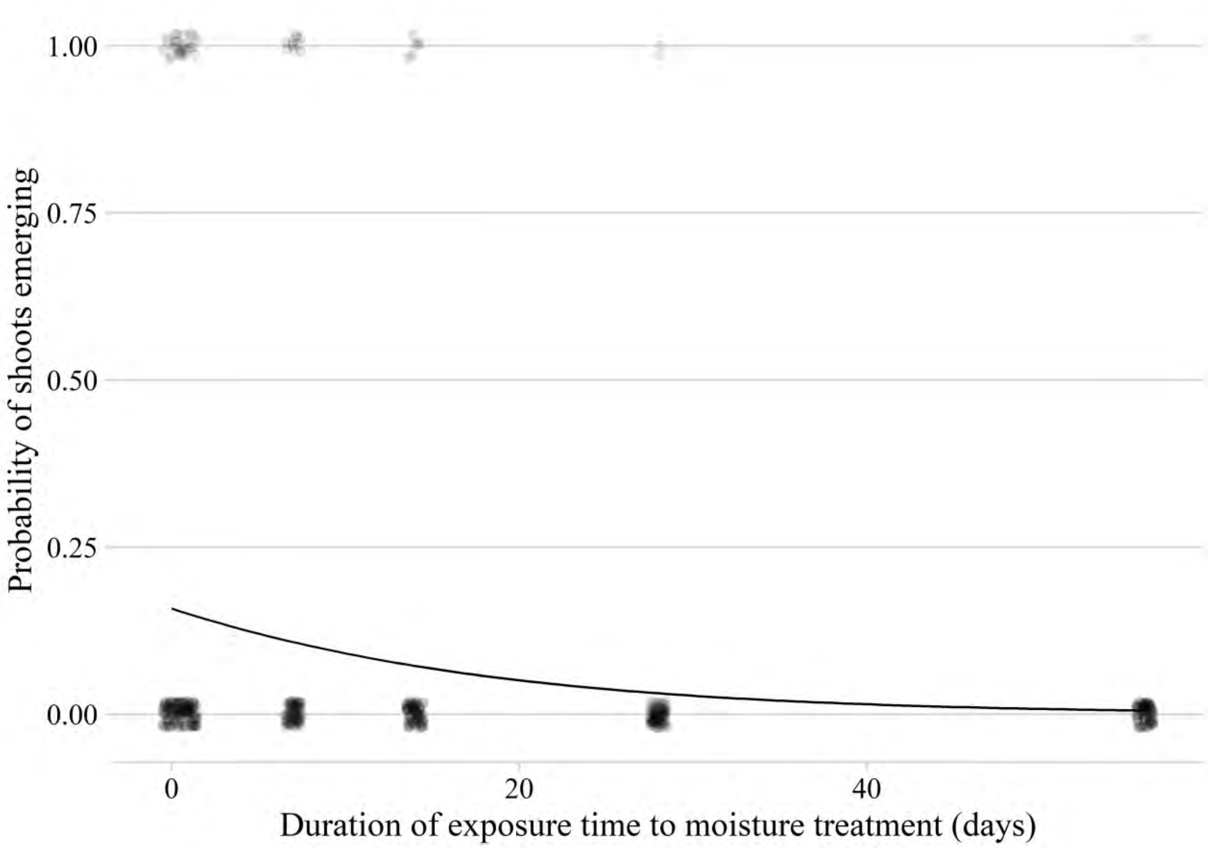


Figure 7. Probability of shoots emerging based on duration of exposure time to moisture treatment (days). Root fragments exposed to a moisture treatment for a shorter period of time were more likely to produce shoots ($p < 0.001$).

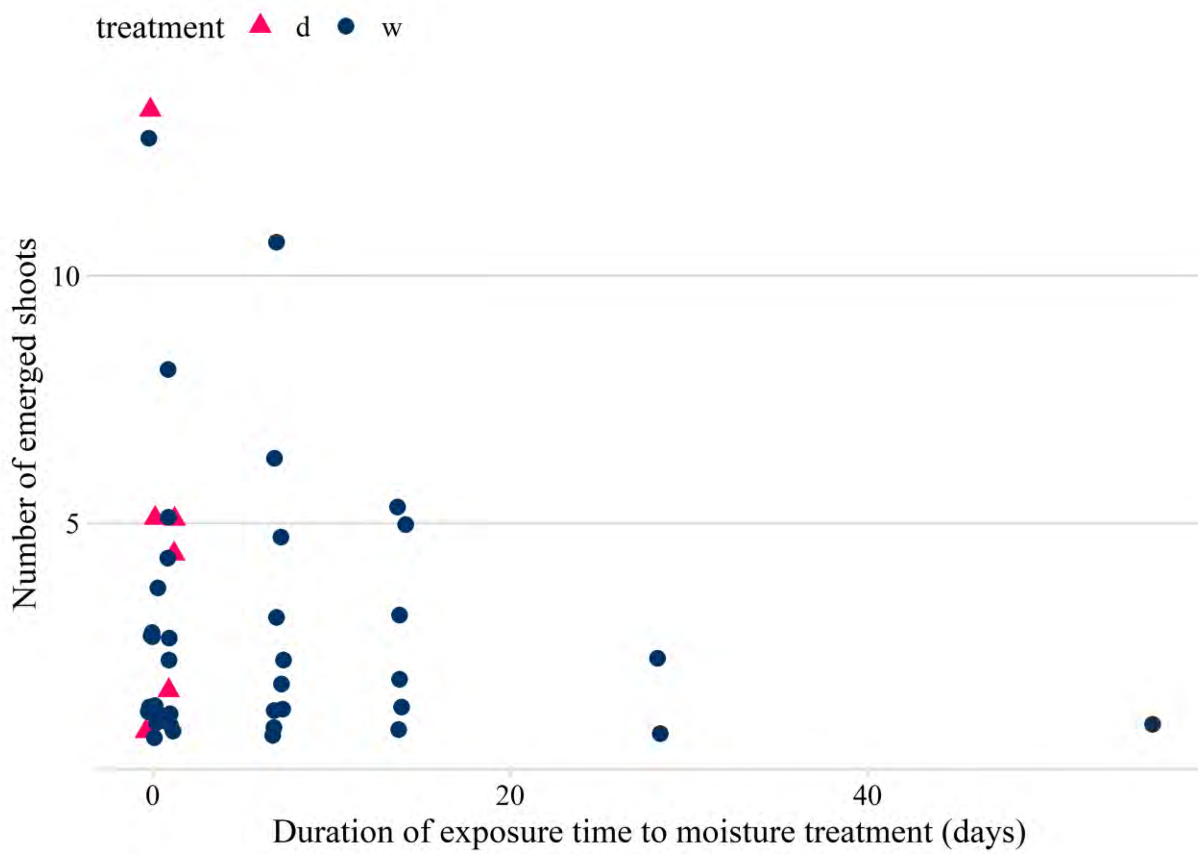


Figure 8. Number of emerged shoots based on an interaction between duration of exposure time and moisture treatment, either wet (w) or dry (d). Results based on the subset of root fragments that had shoots emerge. Root fragments produced more shoots when exposed to the wet moisture treatment for a short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

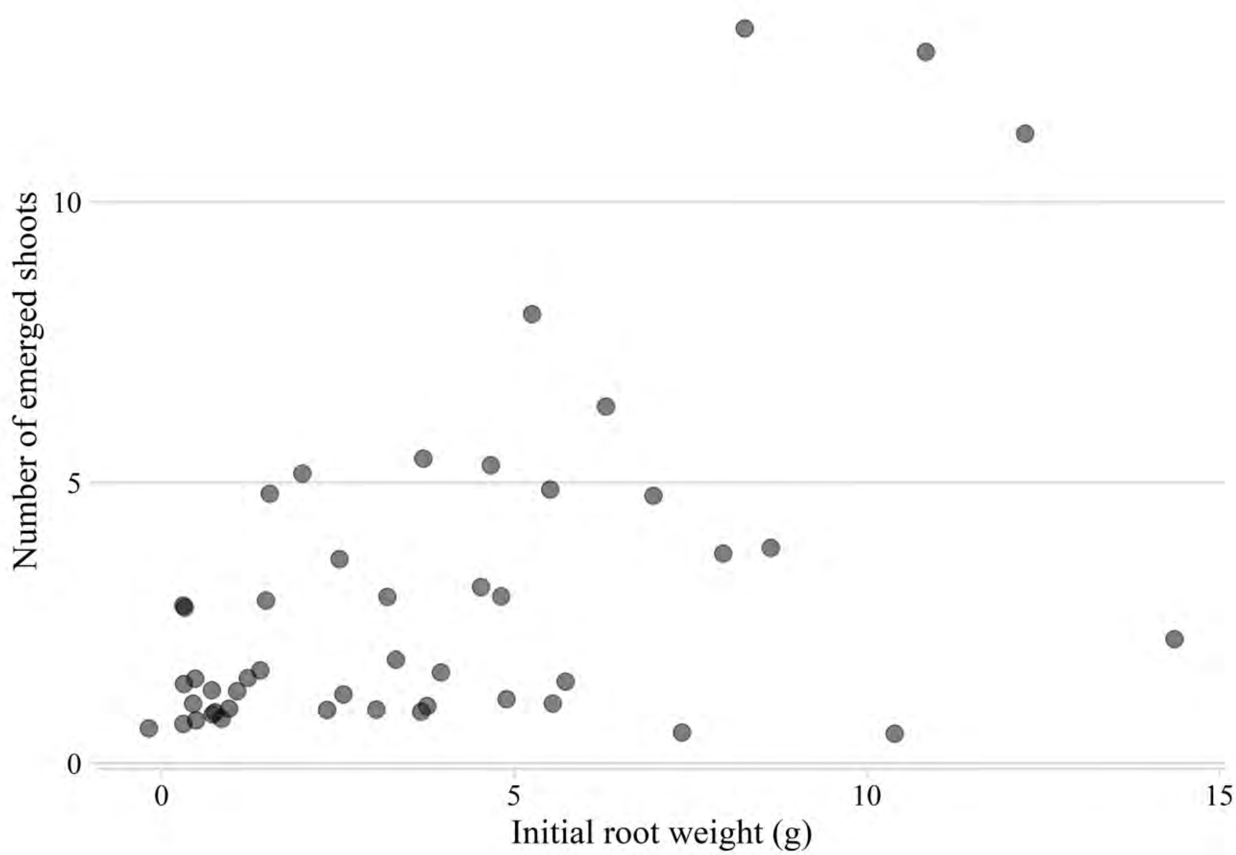


Figure 9. Number of emerged shoots based on initial root weight (g). Root fragments with heavier initial root weights were able to produce more shoots ($p < 0.001$).

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.

Literature Cited

Bakke, A. L. 1936. Leafy spurge, *Euphoriba esula* L. *Research Bulletin: Iowa Agriculture and Home Economics Experiment Station*, 17(198): 1.

Best, K. F., G. G. Bowes, A. G. Thomas, M. G. Maw. 1980. The biology of Canadian weeds. 39. *Euphorbia esula* L. *Canadian Journal of Plant Science*, 60: 651 – 553.

Hanson, H. C., V. E. Rudd. 1933. Leafy spurge: Life history and habitats. *Agricultural Experiment Station Bulletin 226, Fargo: North Dakota Agricultural College*.

Leitch, J. A., F. L. Leistritz, D. A. Bangsund. 1996. Economic effect of leafy spurge in the Upper Great Plains: Methods, models, and results. *Impact Assessment*. 14(4): 419 – 433.

Leistritz, F. L., D. A. Bangsund, N. M. Hodur. 2004. Assessing the economic impact of invasive weeds: The case of leafy spurge (*Euphorbia esula*). *Weed Technology*. 18: 1392 – 1395.

Lym, R. G. 1998. The biology and integrated management of leafy spurge (*Euphorbia esula*) on North Dakota Rangeland. *Weed Technology*. 12(2): 367 – 373.

Lym, R. G., C. G. Messersmith. 1983. Control of leafy spurge with herbicides. *North Dakota Farm Research*. 16 – 19.

Lym, R. G., C. G. Messersmith. 1994. A decade of herbicide treatments controlled leafy spurge. *North Dakota Farm Research Bulletin, ND Agricultural Experiment Station*. 50(3): 9 – 12.

Messersmith, C. G., R. G. Lym, D. S. Galitz. 1985. Biology of leafy spurge. *Leafy Spurge, Monograph series of the Weed Science Society of America*, 5(3): 42 – 56.

Noble, D. L., P. H. Dunn, L. A. Andres. 1979. The leafy spurge problem. *Proceedings: Leafy Spurge Symposium*. 8 – 15.

Selleck, G. W., R. T. Coupland, C. Frankton. 1962. Leafy spurge in Saskatchewan. *Ecological Monographs*, 32(1): 1 – 29.

Watson, A. K. 1985. Introduction – The leafy spurge problem. *Leafy Spurge, Monograph series of the Weed Science Society of America*, Chapter I(3): 1 – 6.

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 ($\alpha = 0.05$)

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 ($\alpha = 0.05$)

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 (α = 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**