Grant Information

Title of Project
Northern Colorado Big Game Critical Winter Range Restoration Project

Total Amount Requested	$ 328,875.00
Matching Contributions Proposed	$159,680.00
Proposed Grant Period	05/01/ 2022 - 11/30/ 2024

Project Description
The Northern Colorado Big Game Critical Winter Range Restoration Project seeks to restore 4,385 acres of mule deer and elk winter range habitat across Larimer, Boulder, and Jefferson counties at seven conserved foothills open space properties along Colorado's Front Range. This cross-jurisdictional project will significantly improve the vegetation communities needed to support healthy mule deer and elk populations on these lands by controlling and eradicating 4,385 acres of winter annual grasses (cheatgrass and feral rye) using a scientifically proven and widely accepted herbicide called, Rejuvra™. As a result, these open spaces will become more resistant and resilient to future disturbances (such as wildfire) and provide critical food resources, particularly in the form of winter browse, for mule deer and elk. The agency partners strongly believe that the treatment of annual invasives is the best way to improve overall native plant diversity and forage for big game species.

Project Abstract
This project is being submitted by Larimer County's Department of Natural Resources in partnership with Colorado Parks and Wildlife, City of Fort Collins Natural Areas, Boulder County Parks and Open Space, and Jefferson County Open Space. The five agency partners are putting forward this collaborative, landscape-scale project to restore big game winter range habitat across seven Front Range conserved open space properties which serve as critical winter range and concentration areas for mule deer and elk.

The foothills open space properties at Cherokee State Wildlife Area, Eagles Nest Open Space, Coyote Ridge Natural Area, Devil's Backbone Open Space, Bobcat Ridge Natural Area, Hall Ranch Open Space, and Matthews/Winters Park provide big game populations with high-quality forage and habitat. The primary project goal is to treat and eradicate 4,385 acres of invading cheatgrass and feral rye at these properties using Rejuvra™, an herbicide on the commercial market since 2017 and scientifically proven effective for long-term control through extensive research conducted by Colorado State University. The herbicide will be applied by helicopter given the difficult terrain and improved cost-effectiveness. The treatments will be monitored before and after application using line intercept cover monitoring and a GIS tool called, RangeView™. High-quality habitats on conserved lands are more important than ever as habitats on which big game species depend are becoming more fragmented.

Organization and Primary Contact Information

Organization	Larimer County Natural Resources
Organization Type	State or Local Government
City, State, Country	,,
Region (if international)	

Primary Contact Jennifer Almstead
Position/Title
Phone and E-mail x ; jalmstead@larimer.org

Additional Contacts

Role	Name

Project Location Information

Project Location Description	Cherokee State Wildlife Area and Eagles Nest OS, Livermore, Larimer County; Coyote Ridge Natural Area, Fort Collins, Larimer County; Bobcat Ridge Natural Area and Devil's Backbone OS, Loveland, Larimer County; Hall Ranch OS, Lyons, Boulder County; Matthews/Winter Park, Golden, Jefferson County
Project Country(ies)	North America - United States
Project State(s)	Colorado
Project Congressional District(s)	District 2 (CO)

Permits and Approvals

Permits/Approvals Description:

Permits/Approvals Status:

Permits/Approvals Agency-Contact Person:

Permits/Approvals Submittal-Approval Date:

Activities and Outcomes

Funding Strategy: Habitat Management
Metric: Restore CO - Improved management practices - Acres managed to treat annual invasive plants
Required: Recommended
Description: Enter the number of acres managed to treat annual invasive plants

Starting Value	0.00 Acres managed to treat annual invasive plants
Target value	4385.00 Acres managed to treat annual invasive plants

Note:

Funding Strategy: Planning, Research, Monitoring
Metric: Restore CO - Monitoring - # sites being monitored
Required: Recommended
Description: Enter the # sites being monitored

Starting Value	0.00 # sites being monitored
Target value	4385.00 # sites being monitored

Note:

I. PERSONNEL $0.00

Staff Name	Position	Annual Salary	Project Hours	Hourly Rate	LOE (%)	Project Salary	% Fringe	$ Fringe	Total Personnel
Totals						**$0.00**		**$0.00**	**$0.00**

II. TRAVEL $0.00

Domestic Airfare – Per Flight

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal **$0.00**

International Airfare – Per Flight

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal **$0.00**

Train – Per Ticket

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal **$0.00**

Rental Car – Per Day

Purpose/Destination	Days/Duration	Unit Cost	Quantity	Total Cost

SubTotal $0.00

Taxis – Per Trip

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal $0.00

Mileage – Per Mile

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal $0.00

Gasoline – Per Gallon

Purpose/Destination	Unit Cost	Quantity	Total Cost

SubTotal $0.00

Per Diem (M&IE) – Per Day

Purpose/Destination	Days/Duration	Unit Cost	Quantity	Total Cost

SubTotal $0.00

Lodging – Per Night

Purpose/Destination	Days/Duration	Unit Cost	Quantity	Total Cost

SubTotal **$0.00**

Meals (no M&IE) – Per Meal

Purpose/Destination	Days/Duration	Unit Cost	Quantity	Total Cost

SubTotal **$0.00**

III. EQUIPMENT	$0.00

Item Name	Description	Unit Cost	Quantity	Total Cost

IV. MATERIALS & SUPPLIES	$328,875.00

Type	Purpose	Unit of Measure	Unit Cost	Quantity	Total Cost
Rejuvra Herbicide	Ecosystem restoration	Acres	$40.00	4385	$175,400.00
Aerial Application, Surfactant, & Post-Emergence	Ecosystem restoration	Acres	$35.00	4385	$153,475.00

V. CONTRACTUAL SERVICES	$0.00

Subcontract/Contract – Per Agreement

Contractor Name	Description	Total Cost

SubTotal **$0.00**

Subgrant – Per Agreement

Subrecipient	Description	Total Cost

SubTotal **$0.00**

VI. OTHER DIRECT COSTS	$0.00

Type	Purpose	Unit of Measure	Unit Cost	Quantity	Total Cost

VII. TOTAL DIRECT COSTS	$328,875.00

VIII. INDIRECT COSTS	$0.00

Explanation of Modified Total Direct Cost Base(MTDC)	Rate Type	NICRA Expiration	$MTDC	Rate(%)	Total Cost

IX. TOTAL PROJECT COSTS	$328,875.00

Budget Narrative

Budget Narrative: add notes

1. Personnel

Personnel -

2. Travel

Domestic Airfare - Per Flight -
International Airfare - Per Flight -
Train - Per Ticket -
Rental Car - Per Day -
Taxis - Per Trip -
Mileage - Per Mile -
Gasoline - Per Gallon -
Per Diem (M&IE) - Per Day -
Lodging - Per Night -
Meals (No M&IE) - Per Meal -

3. Equipment

Equipment -

4. Materials and Supplies

Materials and Supplies - If awarded, 3,630 acres of Rejuvra™ herbicide treatment will be applied in 2022 and the remaining 750 acres will be applied in 2023. The treatments will be applied via helicopter due to the difficult foothills terrain of the sites.

5. Contractual Services

Subcontract/Contract - Per Agreement -

Subgrant - Per Agreement -

6. Other Direct Costs

Other Direct Costs -

7. Indirect Costs

Indirect Costs -

Matching Contributions

Matching Contribution Amount:	$52,375.00
Type:	Cash
Status:	Pledged
Source:	Larimer County Department of Natural Resources
Source Type:	Non-Federal
Description:	Follow up herbicide treatments at Devil's Backbone and Eagle's Nest open spaces. At $2.50 per acre, 1750 acres uploaded to RangeView for pre and post monitoring.

Matching Contribution Amount:	$16,745.00
Type:	In-kind
Status:	Pledged
Source:	Larimer County Department of Natural Resources
Source Type:	Non-Federal
Description:	Staff salary for project implementation, coordination, communications, monitoring, and grant administration.

Matching Contribution Amount:	$19,865.00
Type:	Cash
Status:	Pledged
Source:	Colorado Parks & Wildlife
Source Type:	Non-Federal
Description:	Follow up herbicide treatments at the Lower Range Unit at CPW's Cherokee State Wildlife Area. At $2.50 per acre, 750 acres were uploaded to RangeView for pre and post monitoring of cheatgrass.

Matching Contribution Amount:	$2,701.00
Type:	In-kind
Status:	Pledged
Source:	Colorado Parks & Wildlife
Source Type:	Non-Federal

Description:	Staff salary for project implementation, coordination, communications, and monitoring.

Matching Contribution Amount:	$22,875.00
Type:	Cash
Status:	Pledged
Source:	City of Fort Collins Natural Areas
Source Type:	Non-Federal
Description:	Follow up herbicide treatments at Bobcat Ridge and Coyote Ridge natural areas. At $2.50 per acre, 780 acres uploaded to RangeView for pre and post monitoring.

Matching Contribution Amount:	$9,562.00
Type:	In-kind
Status:	Pledged
Source:	City of Fort Collins Natural Areas
Source Type:	Non-Federal
Description:	Staff salary for project implementation, coordination, and monitoring.

Matching Contribution Amount:	$9,616.00
Type:	Cash
Status:	Pledged
Source:	Boulder County Parks and Open Space
Source Type:	Non-Federal
Description:	Follow up herbicide treatments at Hall Ranch Open Space. At $2.50 per acre, 350 acres uploaded to RangeView for pre and post monitoring.

Matching Contribution Amount:	$6,375.00
Type:	In-kind
Status:	Pledged
Source:	Boulder County Parks and Open Space
Source Type:	Non-Federal

Description:	Staff salary for project implementation, coordination, communications, and monitoring.

Matching Contribution Amount:	$9,616.00
Type:	Cash
Status:	Pledged
Source:	Jefferson County Open Space
Source Type:	Non-Federal
Description:	Follow up herbicide treatments at Matthews/Winters Park. At $2.50 per acre, 350 acres uploaded to RangeView for pre and post monitoring.

Matching Contribution Amount:	$9,950.00
Type:	In-kind
Status:	Pledged
Source:	Bayer U.S. LLC
Source Type:	Non-Federal
Description:	RangeView in-kind match at $2.50 per acre of 3,980 acres uploaded to RangeView for pre and post monitoring.

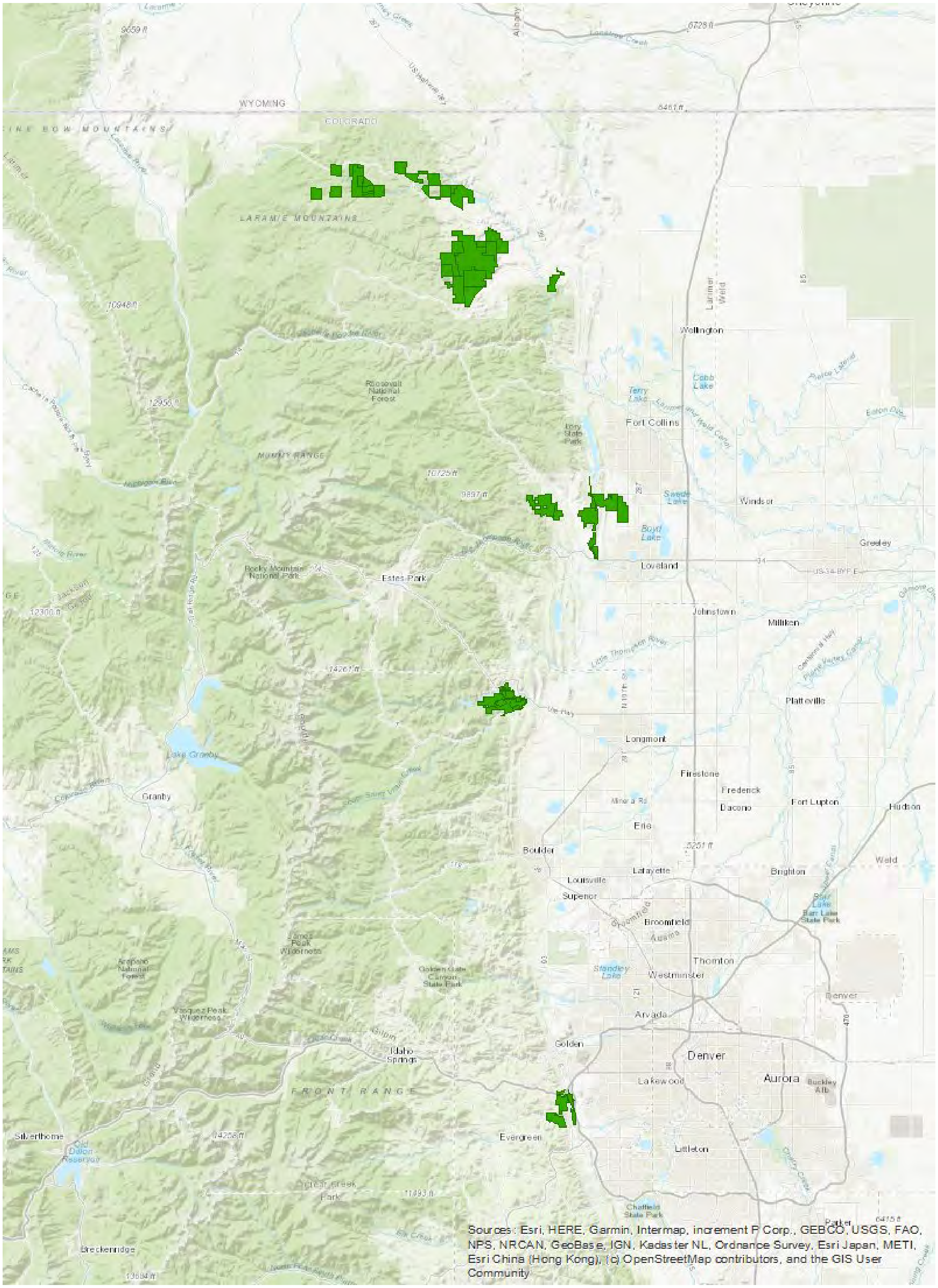
Total Amount of Matching Contributions:	$159,680.00

EasyGrantsID: 73930

National Fish and Wildlife Foundation – RESTORE Colorado 2022, Full Proposal

Title: Northern Colorado Big Game Critical Winter Range Restoration Project

Organization: Larimer County Natural Resources



The following pages contain the uploaded documents, in the order shown below, as provided by the applicant:

Upload Type	File Name	Uploaded By	Uploaded Date
RESTORE Full Proposal Narrative 2022	2022 RESTORE NOCO Grant Narrative_FINAL.pdf	Almstead, Jennifer	12/01/2021
Project Map	2022 RESTORE NOCO Project Maps-ALL.pdf	Almstead, Jennifer	12/01/2021
Letters of Support	NOCO Support Letters-ALL2.pdf	Almstead, Jennifer	12/01/2021
Statement of Litigation	Statement+of+Litigation.pdf	Almstead, Jennifer	12/01/2021
Board of Trustees, Directors, or equivalent	Board of Trustees.pdf	Almstead, Jennifer	12/01/2021
Applicant Controls Questionnaire	LCDNR-ApplicantControlsQuestionnaire.pdf	Almstead, Jennifer	12/01/2021
Other Documents	NOCO Site Photographs.pdf	Almstead, Jennifer	12/01/2021
Other Documents	RangeView-Demo-Info.pdf	Almstead, Jennifer	12/01/2021
Other Documents	Rejuvra Articles and Studies.pdf	Almstead, Jennifer	12/01/2021

The following uploads do not have the same headers and footers as the previous sections of this document in order to preserve the integrity of the actual files uploaded.

Full Proposal Project Narrative

Instructions: Save this document on your computer and complete the narrative in the format provided. The final narrative should not exceed five (5) pages; do not delete the text provided below. Once complete, upload this document into the on-line application as instructed.

1. **Grant Activities and Outcomes:** Elaborate on the primary activities that will be employed through the grant. Explain how these activities are expected to lead to the outcome(s). Discuss what makes these outcomes achievable and important. Describe how these activities relate to established plans (management, conservation, recovery, etc.) and priority conservation needs in the specific project location.

   The Northern Colorado Big Game Critical Winter Range Restoration Project aims to restore 4,385-acres of elk and mule deer winter range habitat across city, county, and state-owned open spaces. The project's seven (7) conserved properties are located in the state's northern foothills of Livermore, Fort Collins, Loveland, Lyons, and Golden, and serve as important winter migration and concentration areas for big game populations. This ecosystem restoration project takes a collaborative, landscape-scale approach by treating and eradicating the seed banks of invasive winter annual grasses such as cheatgrass (*Bromus spp.)* and feral rye (*Secale cereale)* to improve the biodiversity of native plant communities which support healthy mule deer and elk populations. The open spaces include Cherokee State Wildlife Area, Eagles Nest Open Space, Coyote Ridge Natural Area, Devil's Backbone Open Space, Bobcat Ridge Natural Area, Hall Ranch Open Space, and Matthews/Winters Park. As a result of this project, vegetation communities on these lands will become more resistant and resilient to future disturbances (such as wildfire and drought) and provide critical food resources, particularly in the form of winter browse, for mule deer and elk.

   According to the State's Demography Office, Colorado's population is projected to increase 1.8 million by 2050. From the 2020 U.S. Census, 84% of the state's population lives along the Front Range and accounts for nearly all of the population growth (94.8%) in the past 10 years. Colorado's foothills along the Front Range serve as critical habitat for big game winter range for mule deer and elk. Residential development has expanded into these areas leading to habitat loss, degradation, and fragmentation. In northern Colorado, a significant portion of the mapped big game winter range has been impacted by human encroachment, with some areas reaching up to 53% of the winter range developed.[1] These pressures place even greater importance on existing conserved lands to provide big game species with high-quality forage and cover, especially during the winter months.

   The proposed seven (7) properties in Larimer, Boulder, and Jefferson counties represent 40,000+ acres of protected open spaces within the Front Range foothills. These open spaces serve as important wildlife migration corridors and offer a safe haven in the winter for big game populations. Yet, like so many lands in the western U.S., the foothills properties are being invaded by winter annual grasses, such as cheatgrass (*Bromus spp.)* and feral rye (*Secale cereale)* which threaten a host of ecological functions and values. Cheatgrass is a Colorado List C noxious weed that degrades the entire ecosystem by removing fall and spring soil moisture and nutrients from native plant species, creating heavy thatch layers, and increasing fire return intervals. Habitats invaded by non-native invasives contain less plant diversity and produce less biomass than intact, native perennial plant dominated communities. Simultaneously, lands affected by cheatgrass are more susceptible to wildfire due to their fine fuel loads, which burn faster and hotter, and destroy big game winter browse habitat. If left untreated, these invasives will spread rapidly across these areas, reduce biomass, add finer fuels, displace native perennial plant species and deplete critical food resources that mule deer and elk depend on.

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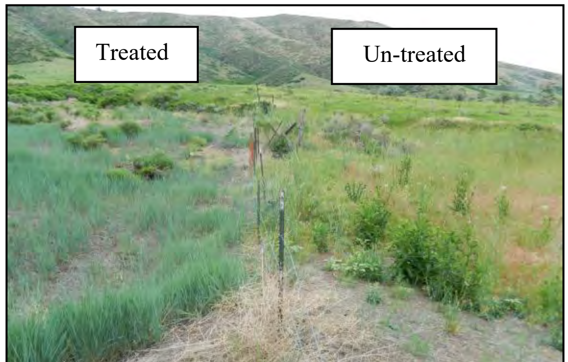
[1] Cooley, C. P., A. Holland, M. Cowardin, M. Flenner, T. Balzer, J. Stiver, E. Slezak, B. Marette, D. Neumann, T. Elm and J. Holst. 2020. Status Report: Big Game Winter Range and Migration Corridors. Colorado Parks and Wildlife.

Until recently, treatments for annual invasives only provided short-term control resulting in rapid re-invasion. In 2017, a new herbicide called, Rejuvra™, became available on the commercial market and after extensive research and treatments, provides a proven control method for winter annual invasives such as cheatgrass and feral rye.

How Rejuvra works: The chemical ***indaziflam*** (also referred to as Esplanade 200SC™, Specticle™, and Alion™) is a *seed root inhibitor,* providing long term invasive annual grass control (3+ years) with a single application of 5 oz/acre. This residual activity controls subsequent germination events that *deplete of the seedbank* with one or two applications, since cheatgrass seed viability in the soil is up to 5 years. Because this tool binds tightly to the upper soil profile it selectively controls the invasive annual grasses while promoting the growth of desirable perennial grasses, forbs, shrubs, and trees.



Devil's Backbone Open Space, Larimer County

Proven Results: Rejuvra Herbicide treatments have been scientifically proven through extensive research conducted and backed by Colorado State University[2]. The herbicide was first studied for rangeland-use in northern Colorado for research and early adoption of large-scale aerial applications of Rejuvra as a treatment for cheatgrass and feral rye. The results demonstrate the treatments offer long-term invasive winter annual grass control thereby allowing desirable perennial grasses, forbs and shrubs including winter browse species such as mountain mahogany, three leaf sumac, and antelope bitterbrush to return, thrive, and provide higher nutrition for big game species. All of the agency partners have had great success treating invasive winter annuals with Rejuvra™ and bring a wealth of technical expertise to the project to maximize project outcomes. **Indaziflam treatment is now widely accepted in Colorado and the West as a highly effective means for invasive annual grass control and ecosystem restoration.**

The Northern Colorado Big Game Critical Winter Range Restoration Project offers long-term control of invasive winter annuals by treating 4,385-acres of cheatgrass and feral rye across seven foothills open space properties owned and managed by Larimer County's Department of Natural Resources, Colorado Parks and Wildlife (CPW), the City of Fort Collins Natural Areas Department, Boulder County Parks and Open Space, and Jefferson County Open Space. The seven (7) properties were selected based upon their proximity to other open spaces across jurisdictional boundaries and to improve big game habitat along the state's northern Front Range foothills on a larger scale. The properties are located in elk and mule deer winter range and concentration areas, as identified by CPW. Agency partners strongly believe that the restoration of cheatgrass and feral rye-infested habitat is by far the best way to improve overall native plant diversity and cover, that in turn provides critical habitat for big game, as well as small mammals, reptiles, birds and insects. It is worth mentioning that the mountain mahogany plant communities found at these properties are extremely rare globally and found only in the northern part of the Colorado Front Range, southern Wyoming, and northern New Mexico. For this grant request, the partner agencies are pooling their resources to treat 4,385 acres of habitat invaded by cheatgrass and feral rye, utilizing an aerial herbicide application due to varying terrain limitations, application uniformity, and economy of scale. By combining resources, the cost per acre for the herbicide and aerial application have been reduced from $100 to $75 per acre. Refer to table below for the proposed herbicide treatment timeline. Due to cash flow constraints, Larimer County will treat Eagles Nest and Devil's Backbone open spaces in separate years.

Year	Acres	Location
2022 Spring/Summer	2,985 Acres	All properties, except Devil's Backbone OS
2023 Spring/Summer	1,400 Acres	Devil's Backbone OS Only
2024 Spring/Summer	Follow-up applications	All properties

[2] Sebastian, D.J., Fleming, M.B., Patterson, E.L., Sebastian, J.R., & Nissen, S.J. 2017. Indaziflam: a new cellulose biosynthesis-inhibiting herbicide provides long-term control of invasive annual grasses.

The activities proposed in this application are supported by established plans including CPW's 2020 Status Report: Big Game Winter Range, Larimer County's Habitat Partnership Program (HPP), CPW's State Wildlife Action Plan, Larimer County's Open Lands Master Plan (2015), Fort Collins Master and Foothills Management Plan, Boulder County Parks and Open Space Strategic Plan, Jefferson County Open Space Conservation Greenprint, and property management plans. This project also ties in directly with concepts brought forward by the Western Governors Association's Biosecurity and Invasive Species Initiative.

1. Cherokee State Wildlife Area (750 acre treatment site), Colorado Parks & Wildlife: Located in Livermore, CO, 35 miles northwest of Fort Collins, this SWA is the second largest in the state at approximately 28,000 acres. The property is divided into three units, with the Lower Pine Unit being proposed for 750 acres of cheatgrass treatment. Big game populations, especially deer and elk, depend on the area for wintering and calving/fawning grounds as verified from recent Colorado Parks and Wildlife collar studies. The rangeland integrity, including the Lower Pine Unit, is substantially compromised by cheatgrass and other invasive plant species. Due to the property's size, difficult terrain, and limited staff resources, CPW is falling behind the curve of invasive plant encroachment and this work will benefit the overall habitat and forage resources for big game.

2. Eagle's Nest Open Space (755 acre treatment site), Larimer County Department of Natural Resources: The property is located near Livermore, CO. The 755-acre property includes rolling foothills and rugged terrain, and nearly a mile of the N. Fork of the Cache la Poudre River. The open space supports a variety of native wildlife and is included in a large regional mule deer migration and winter concentration area that extends from Wyoming to Boulder County. The N. Fork of the Cache la Poudre River is designated as critical habitat for Preble's meadow jumping mouse. The open space has a high concentration of cheatgrass which is degrading the foothills grassland, mountain mahogany shrubland, and ponderosa pine woodland communities.

3. Coyote Ridge Natural Area (230 acre treatment site), City of Fort Collins Natural Areas: Coyote Ridge contributes to an attractive and ecologically diverse landscape that serves as the backdrop for the City of Fort Collins. The mountain mahogany shrubland, ponderosa pine forest, and grassland mosaic provides excellent wildlife and rare plant habitat, scenic views, and a treasured opportunity for the community to connect with nature. These plant communities are highly threatened due to residential development, fire suppression, overgrazing, and invasion by non-native species. The city has successfully treated over 600 acres of cheatgrass in this area since 2016 and the additional 230 acres of treatment will take place adjacent to these treatments.

4. Devil's Backbone Open Space (1,400 acre treatment site), Larimer Department of Natural Resources: Devil's Backbone Open Space is located west of the City of Loveland. This 3,000-acre open space offers towering rock formations and intact native foothills grasslands and shrublands. The Devil's Backbone rock outcrop is one of the most impressive and visible geologic landmarks in Larimer County. To-date, the county has successfully treated 350 acres with Rejuvra™ which are already showing remarkable signs of regrowth of native perennial plant community composition. The proposed 1,400-acre treatment site is adjacent to previous treatments on the western side of the property where feral rye and cheatgrass occupy >90% of the ground canopy.

5. Bobcat Ridge Natural Area (550 acre treatment site), City of Fort Collins Natural Areas: Bobcat Ridge provides 2,606 acres of diverse wildlife habitat, including grassland, shrubland and ponderosa forest foothills ecosystems. Currently, the Natural Areas Department is focusing on the reduction of cheatgrass cover at Bobcat Ridge through aerial herbicide treatments in conjunction with an on-site post Cameron Peak Fire (2020) restoration effort. The proposed treatment will be focused within the Ponderosa pine forest and grasslands surrounding them.

6. Hall Ranch Open Space (350 acres treatment site), Boulder County Parks and Open Space: This 3,205-acre open space is located west of Lyons, Colorado. The property is representative of Front Range foothills steppe shrublands and lowlands, with a large, resident mule deer herd. This area has long been recognized as significant big game habitat by CPW with portions of Hall Ranch located in habitat deemed critical for mule deer and elk. Since 2017, Boulder County Parks and Open Space has treated 300 acres of winter annual grasses on the property

and the proposed 350 acres will applied in fire sensitive Bitterbrush sites and connect to previous treatment areas. An additional 600 acres of treatment is planned in 2022, outside of this grant request.

7. Matthews/Winter Park (350 acres treatment site), Jefferson County Open Space (JCOS): Located south and north of I-70, west of C-470, and adjacent to Denver's Red Rocks Mountain Park, Matthews/Winters Park includes 2,446 acres of forested ridges, shrublands, and prairie grasslands. These high-quality habitats are currently functional but threatened due to encroaching invasive species. The proposed treatment area is located in the southern portion of the park adjacent to the City of Denver's Red Rocks Park and the steep terrain has proven difficult for JCOS to manage invasives until now. If funded, this project will serve as a pilot project for JCOS to demonstrate that aerial herbicide applications are great for treating large contiguous areas of invasive species.

2. **Tracking Metrics:** Indicate how the project will monitor, assess progress and report on the metrics selected previously in the application. Discuss any challenges or limitations anticipated with tracking the metrics.

Three methods will be used to monitor treatment sites on all properties before and after treatment. (1) Installation of long-term random transects inside the treated areas. Within each individual property there will be at least three 100' transects installed. Line intercept canopy cover monitoring will be conducted along the transect for cheatgrass and all desirable perennial vegetation before the treatment and every year for four years, following treatment. (2) All partners will utilize RangeView™, a new innovative GIS tool which detects the levels of cheatgrass infestation before and after the herbicide treatments using remote sensing. Ninety percent (90%) of the proposed 4,385 acres treatment area have already been analyzed for cheatgrass to provide the baseline data and uploaded to the project partners' accounts within the RangeView™ tool (rangeview.bayer.us). (3) Photo points will capture the images of the habitat changes on the landscape. Maps, quantitative data, and results on the amount of cheatgrass present before and after herbicide treatments will be provided as part of the project deliverables to NFWF and included in the final report.

3. **Project Team:** List key individuals and describe their qualifications relevant for project implementation.

Casey Cisneros, Land Stewardship Manager, Larimer County Department of Natural Resources – Mr. Cisneros completed a Master's in Natural Resources Stewardship with a focus in ecological restoration from Colorado State University. He has 17 years of experience as a practitioner of weed management and ecological restoration.

Bernadette Kuhn, Environmental Planner/Restoration Project Manager, City of Fort Collins Natural Areas – For the past 13 years, Ms. Kuhn has led restoration and vegetation ecology projects in Colorado and Wyoming. She is a contributing author for the Colorado State Wildlife Action Plan and the Climate Change Vulnerability Assessment for Colorado Bureau of Land Management.

Steve Murdock, Natural Resources Team Lead, Jefferson County Open Space – Mr. Murdock has over 10 years of weed management and ecological restoration experience. In his current role, he oversees invasive species management, forest management, and habitat restoration across over 50,000 acres of open space. He is a Certified Ecological Restoration Practitioner through the Society of Ecological Restoration.

Seth Schwolert, Property Technician IV, Colorado Parks and Wildlife – Mr. Schwolert currently manages Cherokee SWA and eight other SWAs in Larimer County. Seth graduated from the University of Wyoming with a bachelor's degree in Rangeland Ecology/Watershed Management and a minor in Wildlife and Fisheries Biology Management. As an active partner with Muley Fanatics, Mule Deer Foundation, Larimer County Habitat Partnership Program, and other NGO groups, Seth has overseen numerous grant funded habitat improvement projects.

Derek Sebastian, Vegetation Management Areas Sales Manager, Bayer U.S. LLC – Dr. Sebastian completed his MS and PhD at Colorado State University with research focused on development of tools such as Rejuvra (indaziflam) for long-term invasive winter annual grass control. He has been involved with research studies (~50) and invasive grass treatments with county and city agencies and published the aforementioned long-term results of indaziflam treatments on invasive annual grass control and subsequent habitat benefits.

Joe Swanson, Senior Weed Specialist/County Weed Coordinator, Boulder County Parks and Open Space – Joe has served 8 years with Boulder County Parks and Open Space overseeing invasive plant and noxious weed management on over 45,000 acres of open space property. Joe has 15 years of experience in rangeland management, collectively overseeing 140,000 acres of property in his career. Joe has been on the forefront of Boulder County Parks and Open Space Rejuvra applications working with aerial and ground applications of approximately 3,000 acres since 2014.

4. **Project Demographics:** Describe the community(ies) where the project will take place, who will benefit from the project, and how they were or will be engaged in project development and implementation. Provide demographic information on the community(ies), including but not limited to age, race and ethnicity, and household income.

   According to 2020 Census Data, Larimer, Boulder, and Jefferson counties, located in north-central Colorado, are home to 1.27 million people and continually top countless charts as the best places to live in the west. Collectively, the counties' median age is 37.8 years old, median household income is $77,154, and race is represented by 79% White, 14% Hispanic or Latino, 3.5% Asian, 1.2% African American, and less than 1% Pacific Islander. The seven conserved foothills open space properties, totaling 40,000+ acres, are located near major cities and rural communities along the Northern Front Range and offer ample recreational opportunities for residents and visitors to enjoy year-round. At Matthews/Winter Park in Golden, the property is within walking distance from an RTD station that serves the Denver metro area. There are low economic barriers for access, in fact, five of the open space properties have no entrance fee. While the Devil's Backbone will require a $10 entrance fee per vehicle beginning in 2022 to support ongoing management efforts, Larimer County provides Discover Packs which allows local library patrons to visit any of the county's fee based properties, up to one week, for free. CPW's Cherokee State Wildlife Area requires a valid hunting or fishing license or State Wildlife Area (SWA) pass. Income from SWA pass sales are directed back to help manage and maintain SWA's. Conserving ecological sensitive lands, such as significant wildlife habitat, was identified as the single most important priority by citizens who participated in a Northern Colorado regional study called, Our Lands Our Future. All five agencies sought extensive input from residents, user groups, and advisory boards through their master planning efforts which ultimately inform the management of these properties.

5. **Leverage**: To better demonstrate the full scope and community involvement of this project, please describe any additional funding sources pledged or committed not previously described in the matching contributions section of this proposal.

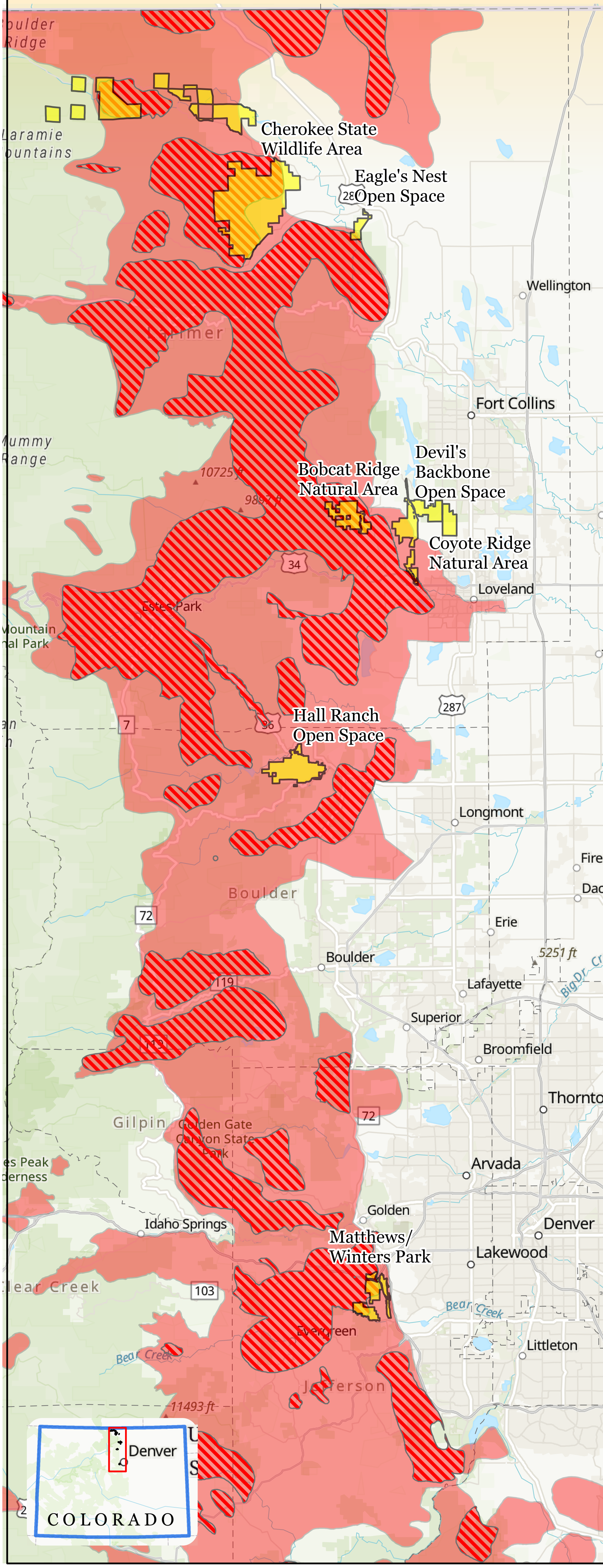
   All five agency partners have all committed significant cash and in-kind contributions demonstrating each agency's commitment and support to the project. As part of Colorado Parks and Wildlife's cash match are $12,990 in funds secured from Muley Fanatics Foundation and Larimer County's Habitat Partnership Program. If awarded, funding will leverage $159,680 in local cash and in-kind funds to restore an additional 2,870-acres of valuable mule deer and elk habitat, representing a 190% increase beyond what the agency partners would be able to do on their own treating only 1,510 acres across the seven properties.

6. **Context:** Please provide a brief progress update on any past or current NFWF grants that are directly applicable to this proposal.
   There are no past or current NFWF grants which are applicable to this request.

7. **Other (Optional):** Provide any further information important for the review of this proposal.

   On behalf of the project partners, we are grateful to NFWF for the opportunity to apply to the Colorado RESTORE Program for this important restoration project. Thanks to Seth Gallagher for joining us on a site visit to view portions of the project in September 2021. Below is a link to a short, 2-minute video on the Rejuvra™ herbicide treatment for your reference. https://www.youtube.com/watch?v=JvQLJhoSJUY&t=2s

Northern Colorado Big Game
Critical Winter Range Habitat Restoration Project



Elk Winter Range & Concentration

- 2022 RESTORE Properties
- Elk: Winter Concentration
- Elk: Winter Range

0 5 10 20 Miles

N W E S

JEFFERSON COUNTY COLORADO Open Space

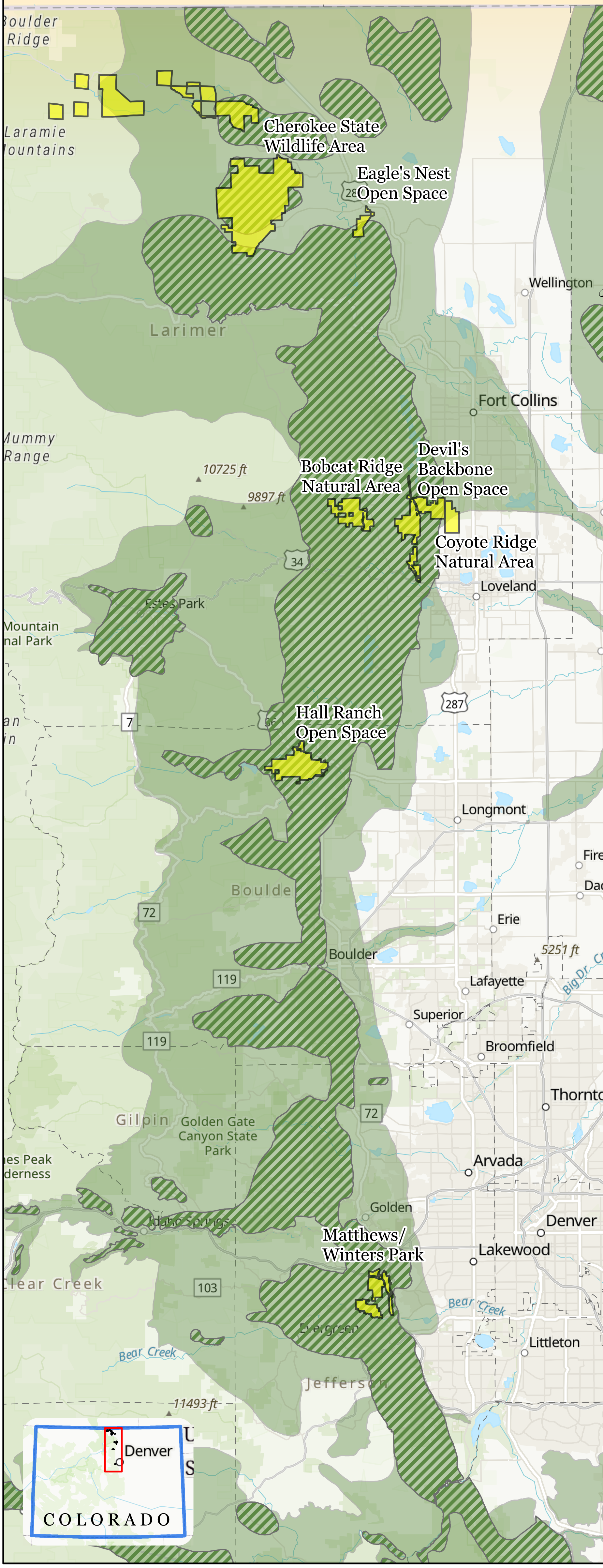
City of Fort Collins Natural Areas

LARIMER COUNTY

Boulder County Parks & Open Space

COLORADO PARKS • WILDLIFE

Northern Colorado Big Game Critical Winter Range Habitat Restoration Project



Mule Deer Winter Range & Concentration

- 2022 RESTORE Properties
- Mule Deer: Winter Concentration
- Mule Deer: Winter Range

0 5 10 20 Miles

N W E S

JEFFERSON COUNTY COLORADO Open Space

City of Fort Collins Natural Areas

LARIMER COUNTY

Boulder County Parks & Open Space

COLORADO PARKS & WILDLIFE

LARIMER COUNTY | NATURAL RESOURCES

1800 S. County Road 31, Loveland, CO 80537, (970) 619-4570, larimer.org/naturalresources

November 27, 2021

Mr. Seth Gallagher
National Fish and Wildlife Foundation
Grasslands and Mountain West Program Director
Rocky Mountain Regional Office

Re: NOCO Big Game Critical Winter Range Restoration Project

Dear Mr. Gallagher:

On behalf of Larimer County's Department of Natural Resources, I am writing to convey our strong support for the Northern Colorado Big Game Critical Winter Range Restoration Project. This project is in partnership with Colorado Parks and Wildlife, the City of Fort Collins Natural Areas, Boulder County Parks and Open Space, and Jefferson County Open Space and exemplifies the spirit of partnership and strong collaboration in our region.

As northern Colorado's population continues to soar, the habitats where big game populations depend are becoming increasingly fragmented or lost altogether. The proposed seven foothills open space properties serve as critical winter refuge areas for mule deer and elk. Through the assistance of this grant, the proposed treatment will eradicate over four-thousand acres of winter annual grasses that are invading their habitat. Our department has been using Rejuvra™ treatment over the past several years to treat cheatgrass and feral rye at Devil's Backbone. The treatment is extremely effective, and we have been absolutely amazed by how quickly the native plant communities are returning.

Our department has committed $52,375 in funds toward the treatment of 2,150-acres of winter annual grasses at Devil's Backbone and Eagles Nest open spaces and also includes $16,745 of in-kind match. If awarded, NFWF funds will be leveraged many times over and significantly improve 4,380-acres of elk and mule deer critical winter range and winter concentration along Colorado's Front Range foothills for decades to come.

Sincerely,

Daylan Figgs
Director

COLORADO
Parks and Wildlife
Department of Natural Resources

11/18/2021

Mr. Seth Gallagher

National Fish and Wildlife Foundation

Program Director, Grasslands and Mountain West

Rocky Mountain Regional Office

Re: 2022 RESTORE Grant Application

Dear Mr. Gallagher:

On behalf of Colorado Parks and Wildlife, I am writing to offer my strong support of the Northern Colorado Big Game Critical Winter Range Restoration Project. The invasion of cheat grass (downy brome) along the Front Range of Northern Colorado has negatively influenced rangeland integrity, leading to a significant decline in forage quality and quantity for wild grazing ungulates like deer and elk. Because of this, Colorado Parks and Wildlife is a committed partner of the Northern Colorado Big Game Critical Winter Range Restoration Project, committing matching funds totaling $22,566($19,865 cash match and $2,701 in kind).

The proposed project would counteract negative rangeland health impacts from cheat grass on city, county, and state owned properties which are known to serve as wildly important big game habitat. Through collar data and annual aerial surveying, CPW has identified these areas as important winter range for big game. The Cherokee State Wildlife Area for example, has been proven in recent years to serve as heavily utilized wintering and calving/fawning grounds.

This proposal seeks to utilize Rejuvra to combat cheat grass invasion and restore rangelands to their native state. Rejuvra has been proven to eradicate downy brome in the seedbank and increase vegetative production significantly in following years. Treatment of these properties will bolster forage resources for wintering wildlife and greatly improve big game habitat conditions.

I want to urge the National fish and Wildlife Foundation to support this landscape scale multi-agency project which would provide direct benefit to 4,380 acres of invaluable deer and elk winter range. Thank you for your consideration.

Brandon Muller

Assistant Area Wildlife Manager

970-692-9530

Brandon.Muller@state.co.us

1745 Hoffman Mill Road
PO Box 580, Fort Collins, CO 80522-0580

ZOE SHARK / Interim Direct
NATURAL AREAS DEPARTMENT

970-221-6311 /P
zshark@fcgov.com /E

December 1, 2021

Mr. Seth Gallagher
National Fish and Wildlife Foundation
Program Director, Grasslands and Mountain West
Rocky Mountain Regional Office

Dear RESTORE Grant Selection Committee,

I am writing on behalf of the City of Fort Collins Natural Areas to express our support for the Northern Colorado Big Game Critical Winter Range Restoration Project grant. As a collaborating partner, the Natural Areas has committed $22,875 in cash match toward the project which will be used to treat 250 acres of cheatgrass. The Natural Areas is also committing $9,651 in in-kind match for staff time on the project. Funding from RESTORE will allow our staff to treat 780 acres at Bobcat Ridge and Coyote Ridge Natural Areas. These areas are in critical need of treatment to improve winter range and production areas for elk and mule deer. Aerially cheatgrass treatments will also restore habitat for hundreds of other native species that occur at the site.

The Natural Areas has a long history of applying integrated pest management techniques to restore habitat. Aerially spraying is a major restoration priority at Coyote Ridge and Bobcat Ridge. A total of 1,150 acres have been treated in the past using Plateau, Rejuvra and Esplanade. Our staff have been amazed at the cover and diversity of native plants that rebound these treatments. With funding from RESTORE, Natural Areas staff will be able to scale up these efforts considerably.

The impact of the Natural Areas, along with three counties and CPW treating 4,380 acres will achieve landscape-scale restoration that will benefit mule deer, elk, and hundreds of other native species across our precious open spaces on the Front Range. We encourage the RESTORE committee to approve this grant request. Please feel free to contact me if you have any questions.

Sincerely,

Zoe Shark

DocuSigned by:
Zoe Shark 12/1/2021
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Interim Director

Parks & Open Space
5201 St. Vrain Road • Longmont, CO 80503
303-678-6200 • POSinfo@bouldercounty.org
www.BoulderCountyOpenSpace.org

Date: November 29, 2021

Mr. Seth Gallagher
National Fish and Wildlife Foundation
Program Director, Grasslands and Mountain West
Rocky Mountain Regional Office

Re: 2022 RESTORE Grant Application

On behalf of Boulder County Parks and Open Space (BCPOS), I am writing to offer my strong support of the Northern Colorado Big Game Critical Winter Range Restoration Project. BCPOS is an original Rejuvra research site and has been actively treating cheatgrass for 6 years. The use of Rejuvra has the full support of BCPOS and its Resource Management Division for control of cheatgrass on BCPOS properties and to assist in restoring our native ecosystems.

As a partner in this grant, BCPOS is preparing to expand our proposed 2022 treatments by approximately 350 acres on Hall Ranch OS. Hall Ranch OS is located just south and west of Lyons, Colorado and is one of BCPOS's premier open space properties. This OS has tremendous ecological diversity and is home to our largest Mule Deer herds. Furthermore, to show our commitment to this project BCPOS will be providing a cash match of $9,616 and an in-kind match of $6,375 which includes, monitoring and research of the site, and any potential re-treatments of the site as needed.

Currently, approximately 300 acres of Rejuvra treatments have occurred in Mule Deer management areas since 2016. Monitoring and data collection has been ongoing, post treatments. Data collection includes; plant species diversity and richness, biomass measurements, Mule Deer utilization, and browse studies to determine impacts on shrub growth of 7 species. Many of these treatment areas are now into their 4th and 5th season of monitoring. (Please see attached documents). These studies show a potential urgency to increase the treated area on Hall Ranch OS to prevent overuse of those areas by resident Mule Deer herds.

BCPOS is committed to an active Rejuvra treatment program, allocating money each fiscal year for annual applications. It is for this, and reasons stated above, that we are pursuing your help via this Restore Grant. These grant dollars will greatly enhance are current program and provide big game habitat for years to come.

Sincerely,

Therese Glowacki, Director

JEFFERSON
COUNTY COLORADO
Open Space

November 16, 2021

Mr. Seth Gallagher
National Fish and Wildlife Foundation
Program Director, Grasslands and Mountain West
Rocky Mountain Regional Office

Re: 2022 RESTORE Grant Application

Dear Mr. Gallagher,

Jefferson County Open Space (JCOS) strongly supports the Northern Colorado Big Game Critical Winter Range Restoration Project to fund treatment of 350 acres of Matthews/Winters Park in Morrison, Colorado and will be committing $9,616 as cash match towards the project. Managing invasive species is a major priority for JCOS as part of our Conservation Greenprint goal, and this project will aid us in achieving our goal of treating 48,000 acres of land impacted by invasive species by the end of 2025.

This cross-boundary collaborative project will utilize a new tool for managing invasive annual grasses that has been proven through research and several previous JCOS treatments, offering long-term invasive species control and restoration of desirable native plants, including those that improve critical big game winter range habitat.

As the Denver metro area population continues to exponentially grow, the habitat that big game species such as elk and mule deer depend on to overwinter is becoming increasingly fragmented, putting added pressure on our Open Space properties. Land management organizations like ours need act quickly to ensure that our properties can support these big game populations when they need it most.

For these reasons, JCOS respectfully asks the National Fish and Wildlife Foundation to support this multi-agency project to restore 4,380-acres of mule deer and elk winter range habitat across seven foothills Open Space properties. Please do not hesitate to contact Steve Murdock, Natural Resources Team Lead for JCOS, at 303.271.5998 or smurdock@jeffco.us if you have questions or need additional information. Thank you for your consideration.

Sincerely,

Tom Hoby

Tom Hoby, CPRE
Director

Colorado State University
Department of Bioagricultural
Sciences and Pest Management
Fort Collins, Colorado 80523-1177

November 18, 2021

RESTORE Colorado Grants Program

To Whom It May Concern:

I am writing this letter in support of a grant submission entitled "Northern Colorado Big Game Critical Winter Range Restoration Project". Invasive winter annual grasses, primarily downy brome (aka cheatgrass) are rapidly destroying landscapes across Colorado's Front Range and in many other western ecosystems. These invasive grasses fill an open niche in our shortgrass prairie and montane ecosystems leading to increased fire frequency and reductions in species diversity.

The proposed project (Northern Colorado Big Game Critical Winter Range Restoration Project) represents the next logical step in the implementation of scientifically validated cheatgrass management across Front Range landscapes. Over the last seven years, I have directed the research of two Ph.D. and one M.S. student examining the impacts of cheatgrass and determining the resilience of these ecosystems once cheatgrass competition is removed. The tool we have identified as the most useful in this process is the herbicide, indaziflam (Rejuvra™). My students and I were the first to determine the potential of this soil active herbicide to provide three plus years of downy brome control under rangeland conditions with a single application. We have published five journal manuscripts describing the length of cheatgrass control, the response of the remnant plant communities, and the interactions with prescribed burning. In almost every case, controlling cheatgrass with Rejuvra has resulted in increased species diversity, increased grass biomass, and improved ecosystem services such as wildlife and pollinator habitat.

The proposed project seeks to implement cheatgrass management across multiple counties and open space programs to improve important winter range for mule deer and other wildlife. A study at Hall Ranch, in Boulder County, demonstrated that controlling cheatgrass with Rejuvra significantly increased browse for mule deer, increasing winter survival.

The successful implementation of the project could serve as a model for other parts of Colorado and other western states. I recommend that this project be funded to the fullest extent possible.

Sincerely,

Scott Nissen

Dr. Scott Nissen, Professor, Weed Science

Encompassing Entomology, Plant Pathology, and Weed Science
Phone (970) 491-5261 ~ bspm@lamar.colostate.edu ~ FAX (970) 491-3862
http://www.colostate.edu/depts/bspm/index.html

Statement of Litigation

Instructions: Save this document on your computer and complete. The final narrative should not exceed two (2) pages; do not delete the text provided below. Once complete, upload this document into the on-line application as instructed.

Litigation: In the space provided below, state any litigation (including bankruptcies) involving your organization and either a federal, state, or local government agency as parties. This includes anticipated litigation, pending litigation, or litigation completed within the past twelve months. Federal, state, and local government applicants are not required to complete this section. If your organization is not involved in any litigation, please state below.

Larimer County's Department of Natural Resources (applicant) is a local government and is not required to complete this section, as stated above.

Board of Trustees, Directors, or equivalent are not applicable. The property improvements proposed in this application are government-owned by a city, county, or state agency.

APPLICANT CONTROLS AND CAPABILITIES QUESTIONNAIRE

NFWF

INSTRUCTIONS: THIS QUESTIONNAIRE IS REQUIRED WITH THE SUBMISSION OF A NFWF FULL PROPOSAL, FOR THE FOLLOWING ORGANIZATION TYPES: NONPROFIT ORGANIZATIONS, STATE, LOCAL & MUNICIPAL GOVERNMENTS, INDIAN TRIBAL GOVERNMENTS, FOR-PROFIT ENTITIES, AND INCORPORATED INDIVIDUALS. PLEASE COMPLETE AND SIGN THE QUESTIONNAIRE IF YOUR ORGANIZATION DOES NOT HAVE A RECENT (WITHIN TWO YEARS) SINGLE AUDIT UPLOADED TO THE FEDERAL AUDIT CLEARINGHOUSE. DO NOT INCLUDE COPIES OF POLICIES OR PROCEDURES WITH THE SUBMISSION OF THIS FORM.

NFWF UTILIZES THIS QUESTIONNAIRE ALONG WITH A RISK ASSESSMENT TO DETERMINE ELIGIBILITY AND COMPLIANCE REQUIREMENTS, WHICH MAY INCLUDE THE FOLLOWING: ADDITIONAL REPORTING, RESTRICTION OF ADVANCE FUNDING, AND/OR SUBMISSION OF SUPPORTING DOCUMENTATION.

ORGANIZATION LEGAL NAME

ORGANIZATION EIN

1. Does your organization have a Single Audit (within the last 2 years) on file with the Federal Audit Clearinghouse?

   ✓ If yes, sign and upload form.

   If no, complete form in its entirety.

2. Has your organization been suspended or debarred from any government contracting process? If yes, please explain.

   Enter response here.

3. Has your organization managed a federal award before? If yes, provide a summary of your most recent award, including period of performance, awarding agency, federal program, and size of award.

   Enter response here.

4. Does your organization maintain written accounting policies and procedures applicable to headquarters and, if applicable, field offices? If no, please explain.

   Enter response here.

5. Does your accounting system (or other management system) allow organizational expenses to be tracked: (a) to a specific project on which your organization is working; (b) to specific tasks within that project; and, (c) to specific cost-type categories within each task (e.g., materials, supplies, travel, etc.)? If no, please explain.

   Enter response here.

6. Does your organization have a timekeeping system or documented process that allows staff time to be tracked: (a) to a particular project on which staff spends time; and, (b) to specific tasks within each project? If no, please explain.

   Enter response here.

7. Does your organization maintain employment agreements or other formal evidence of employment between it and its employees? If no, please explain.
   Enter response here.

8. Does your organization maintain basic property and casualty insurance? If no, please explain.
   Enter response here.

9. Is your organization able to perform the project on a cost-reimbursable basis? If no, please explain by providing the following information: (a) current cash on hand amount, (b) advance funding required, and (c) purpose of advance funding.
   Enter response here.

10. Does your organization maintain a written policy on conflict of interest? Would your organization's policy on conflict of interest operate to notify NFWF if your organization finds that it is unable to satisfactorily manage a conflict of interest pertinent to a NFWF-funded project? If no, please explain.
    Enter response here.

11. Does your organization maintain a written procurement policy? If no, please explain.
    Enter response here.

12. If your proposed project includes contracts and/or sub-recipients, has an appropriate legal/contracting officer at your organization reviewed such elements and confirmed that your organization indeed has the ability to implement the arrangements as envisioned?
    Enter response here.

13. Does your organization maintain written policies for property management and do you perform inventory audits/verifications on a regular basis? [APPLICABLE TO PROJECTS WITH EQUIPMENT OR SUPPLIES BUDGETED]
    Enter response here.

14. In the past three (3) years has your organization, or any unit or office thereof, been audited by an external donor/funder or agent thereof? (This would include, but not be limited to, audits of your organization by governmental entities.) If yes, please list all such audits.
    Enter response here.

I certify to the best of my knowledge and belief that the above information is true, complete, and accurate and that I am authorized to submit on behalf of the organization represented above.

SIGNATURE

NAME AND TITLE Thanh Matthews
Accountant II

Northern Colorado Big Game Critical Winter Range Restoration Project
Site Photographs of Proposed Treatment Areas

Colorado Parks and Wildlife - Cherokee State Wildlife Area (Livermore, CO)

The proposed 750 acre treatment area will occur in the Lone Pine Unit within the Cherokee State Wildlife Area. More specifically, the treatment will take place along the Lone Pine Creek Drainage just north of Red Feather Lakes Road.

Larimer County Department of Natural Resources - Eagle's Nest Open Space (Livermore, CO)

The proposed 755 acre treatment area includes all of Eagle's Nest Open Space which has a high concentration of cheatgrass which is degrading the foothills grassland, mountain mahogany shrubland, and ponderosa pine woodland communities.

City of Fort Collins Natural Areas - Coyote Ridge Natural Area (Fort Collins, CO)

The proposed 230 acre treatment area will be focused on the mountain mahogany and sumac shrublands in the higher elevations and represent the most threatened and cheatgrass infested communities at this natural area.

Larimer County Department of Natural Resources - Devil's Backbone Open Space (Loveland, CO)

The proposed 1,400 treatment area is immediately adjacent to previous treatments on the west side of the property where feral rye and cheatgrass occupy more than 90% of the ground canopy.

Boulder County Parks and Open Space - Hall Ranch Open Space (Lyons, CO)

The proposed 350 acre treatment area will include cheatgrass infested ares in fire sensitive Bitterbrush habitat and connect to to the other treated acres at Hall Ranch Open Space.

City of Fort Collins Natural Areas - Bobcat Ridge Natural Area (Loveland, CO)

The proposed 550 acres will be focused within the ponderosa pine forests and grasslands that are being threatened by cheatgrass and directly adjacent to treatments completed in previous years.

Jefferson County Open Space - Matthews/Winter Park (Golden, CO)

The proposed 350 acre treatment area is located in the southern portion of Matthews/Winter Park which shares a property boundary with the City of Denver's Red Rocks Park.

Proposed Treatment Area

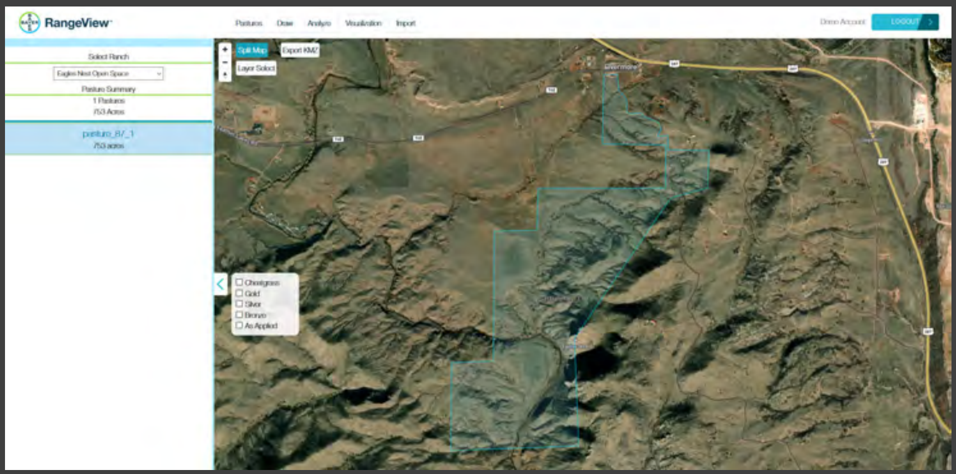
RangeView Cheatgrass Analysis

Cherokee Park Wildlife Area

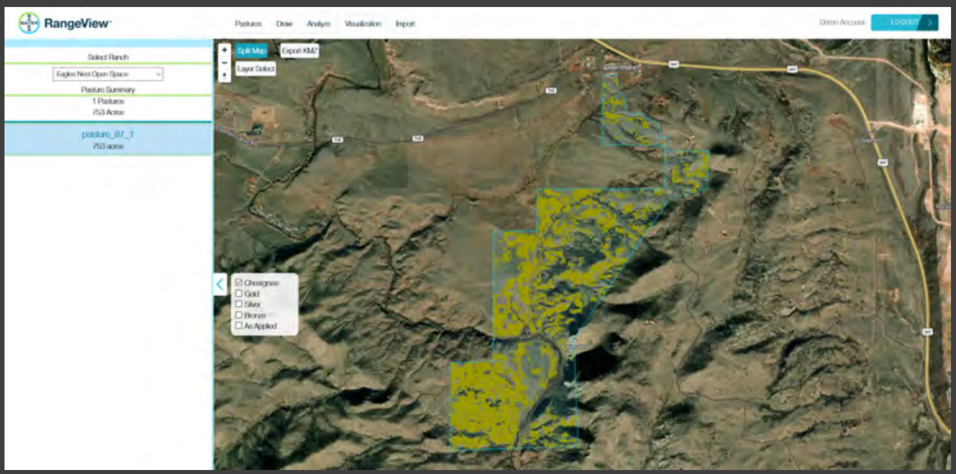
https://rangeview.bayer.us/

RESTRICTED

Proposed Treatment Area



RangeView Cheatgrass Analysis



Eagle’s Nest Open Space

https://rangeview.bayer.us/

RESTRICTED

RangeView™

Spot cheatgrass from above.
And stop it for the long haul.

Cheatgrass infestations reduce rangeland productivity by stealing water and nutrients from native plants. This lack of desirable forage limits livestock grazing and dramatically affects the overall bottom line for ranchers across the western United States. But recent breakthroughs in technology and herbicide chemistry offer a new opportunity to confidently eliminate cheatgrass for the long haul.

Take back your rangelands with innovation guaranteed to work.

Combine the satellite imagery and digital planning tools of **RangeView™** with the long-lasting effects of **Rejuvra® herbicide** for unrivaled cheatgrass control. This powerful approach can restore your rangelands, reestablish native species and increase forage quality for overall better ROI in four easy steps:

1. Identify

/// Pinpoint cheatgrass infestations on your land with our proprietary algorithm and receive a refundable analysis.*

2. Plan

/// Calculate the gallons of Rejuvra needed for your application and easily share the treatment and boundary file with your applicators.

3. Apply

/// Break the cycle of germination and future seed production in cheatgrass and receive up to four years of protection with Rejuvra.

4. Compare

/// Review performance by using historical satellite imagery to compare treated areas against untreated areas.

4 Year RangeView Restoration Pledge

*After applying Rejuvra, upload your applied treatment file in the RangeView application to receive four years of guaranteed performance as well as a $5/acre rebate to cover your analysis cost.

Sign up to restore your rangeland for the long haul at ***RangeView.us*** *or call toll-free at 844.229.3721.*

//// Get a stronger return on investment.

After planning your treatment approach with RangeView™, Rejuvra® herbicide breaks the cycle of germination and stops cheatgrass seed production. This unique mode of action offers four years of protection to restore rangeland and improve your bottom line. Here's how:

A stronger return on your land:

/// By stopping cheatgrass at the seed level, Rejuvra allows desirable forage to return with up to **3.5x more perennial grass production.** This reduces grazing pressure and improves the overall ecosystem.

A stronger return on your herd:

/// Rejuvra increases forage quality so you can produce more pounds of beef per acre. That means you can improve pasture rotation, reduce feed costs by up to **$50-$100 per head** and increase production gains.

A stronger ROI for your operation:

/// Reduce operational costs and grow your business potential with Rejuvra. Apply now to set yourself up for the long haul by reducing labor costs by up to **$15-$25 per head** and providing the lowest cost per pound of gain.

//// *Start planning your strong return today and sign up for the long haul at* **RangeView.us** *or call toll-free at* **844.229.3721.**

ALWAYS READ AND FOLLOW LABEL INSTRUCTIONS.
Bayer Environmental Science, a Division of Bayer CropScience LP, 5000 CentreGreen Way, Suite 400, Cary, NC 27513. For additional product information, call toll-free 1-800-331-2867. www.environmentalscience.bayer.us. Not all products are registered in all states.

BAYER Rejuvra®

RESEARCH BRIEF

Restoring Cheatgrass Invaded Rangelands Decreases Wildfire Risk and Increases Wildlife Browse

Jim Sebastian, Dr. Shannon Clark, Dr. Derek Sebastian, Steve Sauer

Management Implications:

- Rejuvra® (indaziflam, Bayer) is a pre-emergence restoration herbicide that provides multi-year invasive annual grass control with a single application, while allowing for the re-establishment of desirable perennial grasses, forbs, and shrubs.
- Cheatgrass treatments on Boulder County Open Space properties resulted in increased leader growth (1.5X-2.8X) on shrub species in treated areas compared to non-treated areas, increasing critical winter forage for mule deer and elk.
- Cheatgrass treatments resulted in 4-5X increase in perennial grass biomass.
- Cheatgrass litter was reduced by 92% 8 months after treatment, and eliminated by 20 months after treatment.
- This research suggests that Rejuvra could be a useful tool in wildlife habitat improvement projects, specifically in critical winter range areas that are at high risk for cheatgrass-fueled wildfires.

The Number One Threat to Mule Deer and Sage-Grouse in the West is Cheatgrass Fueled Wildfires[1]

Invasive winter annual grasses such as cheatgrass, medusahead, and ventenata continue to spread at an alarming rate. These invasive species are one of the largest threats to western rangeland, and the wildlife that depend on these shrubland communities for survival. The fine-fuels that accumulate from annual grasses significantly alter the fire regime by increasing wildfire frequency, and facilitating the conversion to invasive annual grass monocultures. Between 2000 and 2018, more than 15 million acres of sagebrush steppe have burned by wildfire, and although fire is a natural part of the sagebrush steppe, its frequency and severity are increasing far above natural levels[2]. Hundreds of wildlife species across 14 states are dependent on intact shrub communities such as sage-grouse, mule deer, pronghorn, and elk; however, once these sites convert to invasive grass monocultures, it is nearly impossible to restore these critical shrub-dominated plant communities.

Restoring cheatgrass infested critical winter range

Boulder County Open Space (BCOS) manages properties in the lowland, foothills and mountains of Colorado that provide critical overwintering habitat for mule deer, elk, and other

wildlife. A major concern of BCOS ecologists and wildlife biologists is the loss of critical wildlife habitat areas due to cheatgrass-fueled wildfires.

In winter 2017 and 2018, six sites were treated with indaziflam (Rejuvra®, Bayer) (7 oz/ac) plus glyphosate (12 oz/ac), while desirable shrub species were in dormancy and no leaves were present. These sites were 2 to 20 acres in size with dense stands of mountain mahogany, four-lobed sumac, antelope bitterbrush, winterfat, rubber rabbitbrush, four-winged saltbush, and fringed sage.

Permanent random transects (3 X 200') were created inside cheatgrass-treated, and immediately adjacent, non-treated plots. Data collection included line intercept canopy cover for cheatgrass and all desirable perennial vegetation. In addition, biomass was collected for all species including cheatgrass litter to determine fine-fuel weights in treated vs. non-treated plots. This provided an indication of how quickly cheatgrass fine-fuel litter degrades after indaziflam (Rejuvra®) treatments. Shrub measurements including longest leader growth were collected along the entirety of the transect. Data were collected over two consecutive summers, at approximately 8 and 20 months after treatment (MAT).

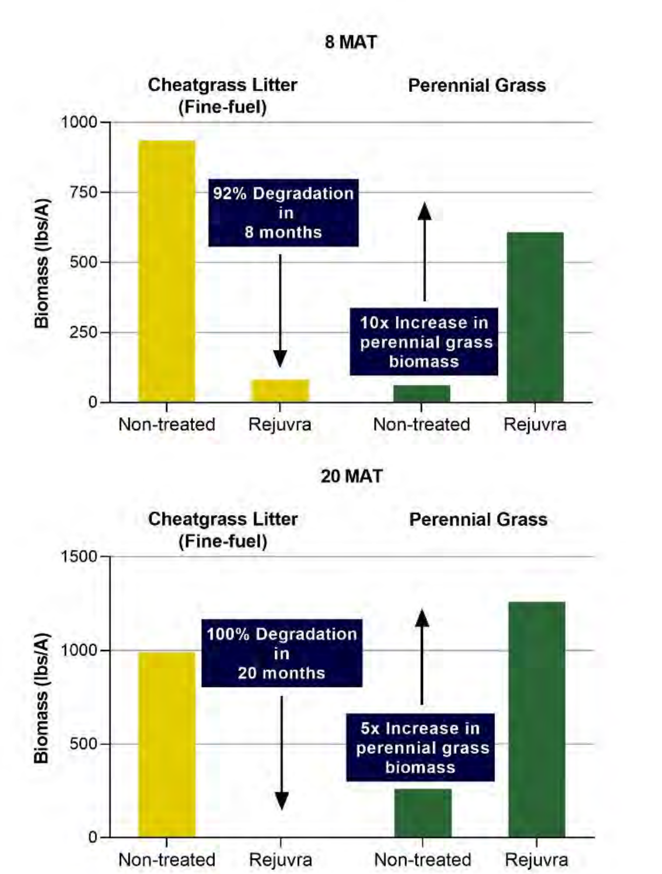


Figure 1. Cheatgrass litter and perennial grass biomass 8 and 20 months after treatment (MAT) in non-treated vs. indaziflam (Rejuvra®) treated areas.

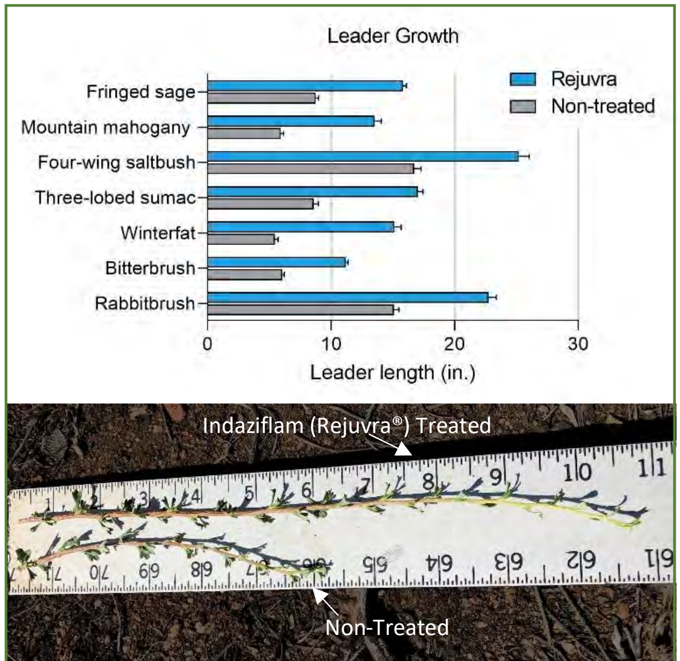


Figure 2. Average longest leader growth on shrubs in treated (Indaziflam, Rejuvra®) vs. non-treated areas across 6 sites at 20 months after treatment. Not all shrubs occurred in every site. Bottom image showing representative antelope bitterbrush leader growth comparing treated and non-treated growth.

The first summer after application, cheatgrass litter biomass averaged 935 lb/A in non-treated areas compared to 82 lb/A in treated areas, a 92% degradation of cheatgrass litter in areas treated with indaziflam (Rejuvra®). By the second summer after application, cheatgrass litter had completely degraded in indaziflam (Rejuvra®) treated sites. Perennial grass at the sites responded positively to the treatments, with an average 5x increase in biomass by 20 MAT (Figure 1).

New growth measurements on shrubs spanning the transect lines revealed increased leader growth and shrub canopy volume in the treated areas for all seven shrub species evaluated. New leader growth was 1.5x to 2.8x longer on shrubs in areas treated for cheatgrass compared to non-treated areas (Figure 2), while shrub canopy volume increased 120% to 400% with cheatgrass treatments (data not shown).

Conclusions:

This research suggests that indaziflam (Rejuvra®) could be a useful tool in wildlife habitat improvement projects on invasive winter annual grass dominated sites. Sites responded favorably to the removal of cheatgrass and benefits to wildlife habitat were realized in a relatively short time-frame (8 to 20 MAT). Within indaziflam (Rejuvra®) treated sites, the fine-fuel created by cheatgrass litter was completely degraded within 20 months after application, significantly reducing the risk of habitat loss from cheatgrass-fueled wildfires. Wildlife browse was increased for seven different shrub species utilized by mule deer, elk and other browse species during winter months, indicating a substantial improvement to critical winter range in Boulder County, CO.

Our findings reinforce the findings of field managers, that cheatgrass and other invasive annual grasses pose a significant threat to the habitat and population of browse species. For land managers, this management tool provides a long-term control option to reduce wildfire risk and begin the restoration process on the millions of infested acres within critical habitat areas

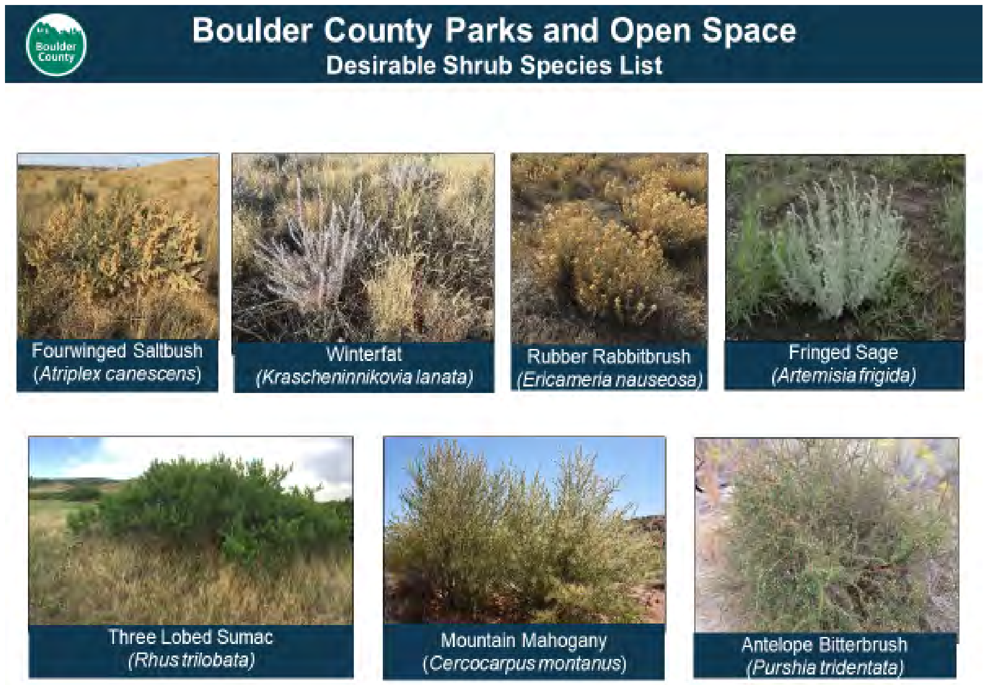
FOR FURTHER READING

1. Nikonow, H (2019). Cheating the Sage. Mule Deer Foundation Journal, Sept/Oct Issue. https://www.partnersinthesage.com/blog/2019/cheating-the-sage-mdf
2. Audobon Society https://rockies.audubon.org/sagebrush/cheatgrass-fire
3. Sebastian DJ, Sebastian JR, Nissen SJ, Beck KG, (2016). A potential new herbicide for invasive annual grass control on rangeland. *Rangeland Ecology and Management*, 69(3): 195-198. doi: 10.1016/j.rama.2015.11.001
4. Clark SL, Sebastian DJ, Nissen SJ, Sebastian JR, (2019). Effect of indaziflam on native species in natural areas and rangeland. *Invasive Plant Science and Management*, 12: 60-67. doi: 10.1017/inp.2019.4
5. Rejuvra Plot Tour Video Series, Utah, Colorado, & Montana, 2019: : https://www.environmentalscience.bayer.us/vegetation-management/range-and-pasture/portfolios-and-solutions/rejuvra/plot-tour

Appendix C, Rejuvra Mule Deer Data, Boulder County

Boulder County POS, Rejuvra Mule Deer Research

Jim Sebastian, Boulder County, Joe Swanson, Boulder County

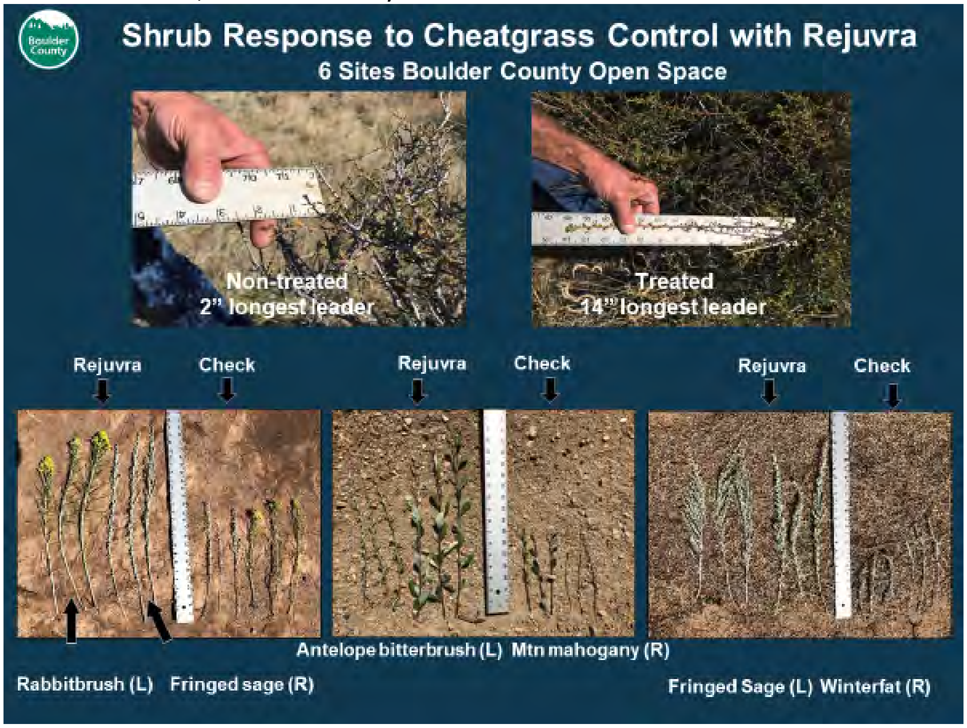


Study Summary: In 2018, Boulder County Parks and Open Space conducted a "Deer browse" study on 6 different open space sites including Hall Ranch OS. The study utilized 200-meter-long transects in Rejuvra applied sites, with the controls occurring on adjacent untreated sites. The study focused on growth response to the removal of cheatgrass from the eco-system. Methods consisted of linear 200-meter-long transects and measuring new growth of leaders/stems on shrubs that fell within a 6-foot horizontal distance to the transect line. This was done on treated and untreated sites.

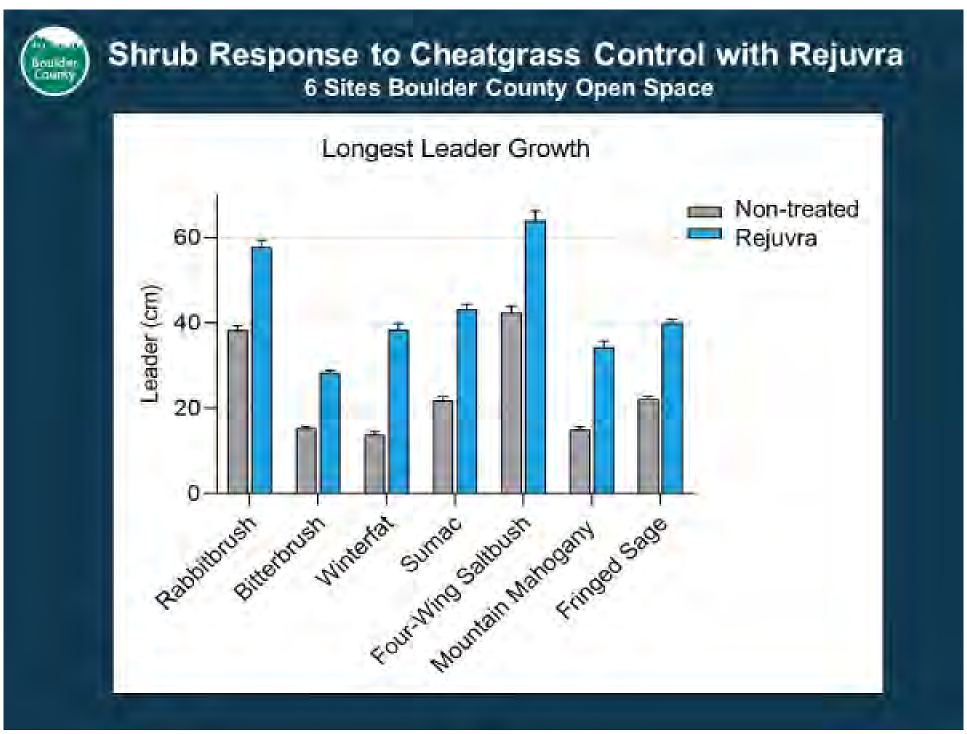
In addition to this study, in 2020, Boulder County POS Invasive Plants Department started to conduct a deer utilization study on the same sites on Hall Ranch OS. The hope was to provide research data that would confirm our assumption deer were utilizing treated areas for forage more than untreated areas. As a result, visitation data was tracked through game cameras and track counts on treated versus untreated sites.

Subsequently, we have also started to look at the amount of leaf biomass on shrubs from treated versus untreated sites and potentially forage quality of browse species as well. The leaf biomass data is very early in its collection and we hope to learn more in the coming months. If you have any questions concerning this data, please contact Joe Swanson, County Weed Coordinator, Boulder County, 303.594.0163 or, Jim Sebastian, Resource Specialist Weeds Dept. 303.817.2725.

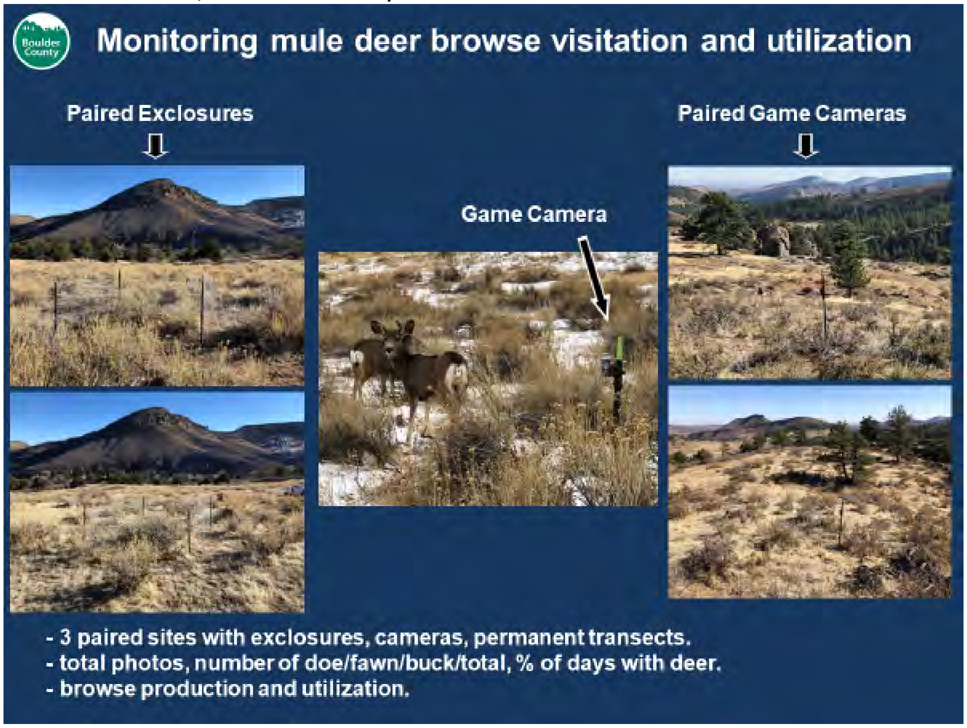
Appendix C, Rejuvra Mule Deer Data, Boulder County



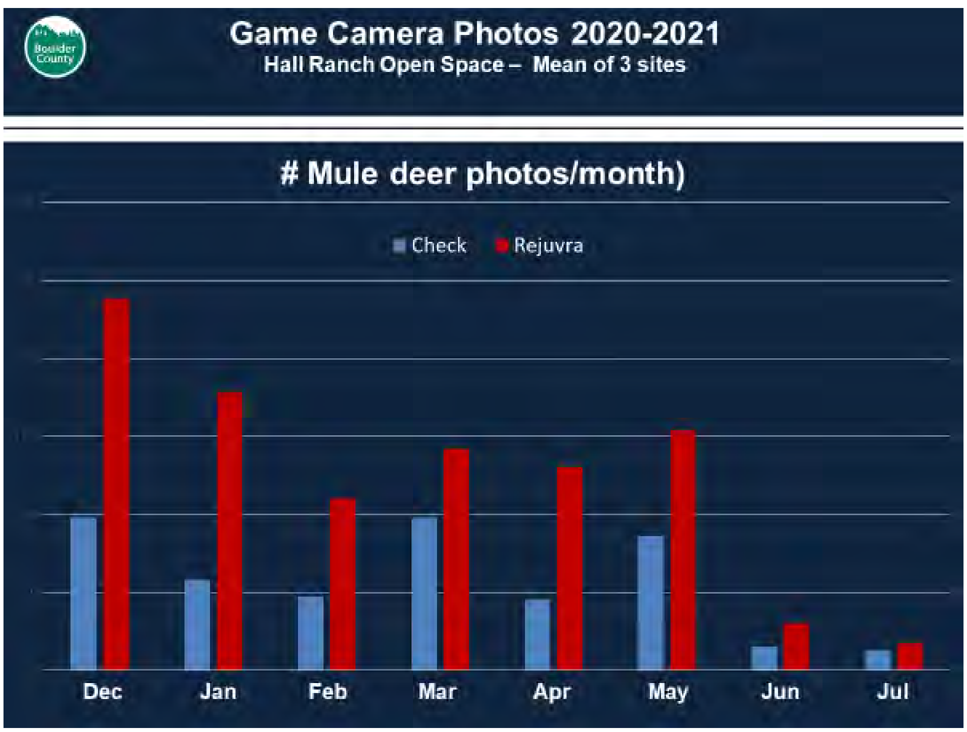
With the removal of cheatgrass from these study sites, you can clearly see the increased growth of shrubs in those areas versus untreated sites. In many cases new growth in treated areas exceeded untreated areas by 2x to 3x the length in one growing season. All of this leads to more forage availability in critical winter months.



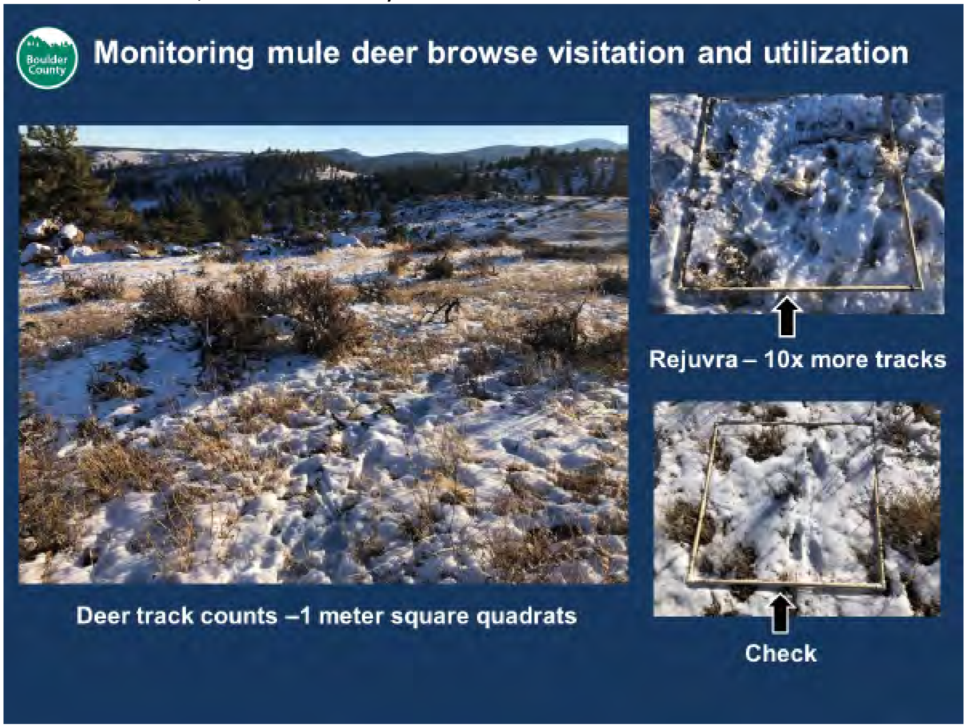
Appendix C, Rejuvra Mule Deer Data, Boulder County

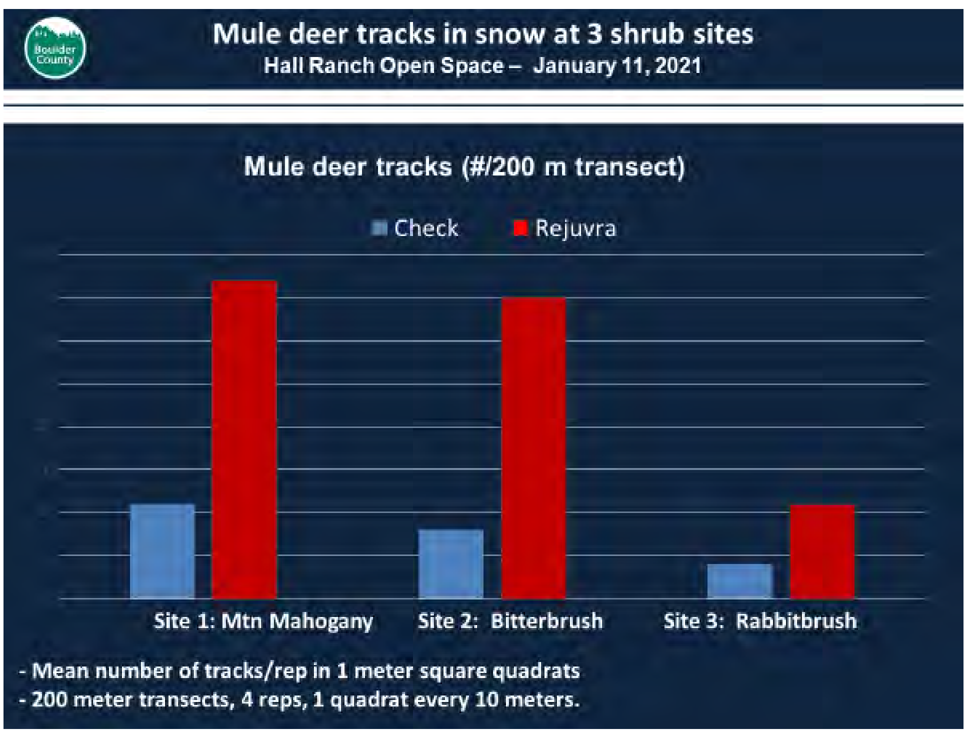


Game camera, deer visitation data was also collected on treated vs. untreated sites, as well as deer track data. This data can be reviewed in the following slides, and once again, data clearly indicates a higher usage of treated sites over untreated sites throughout this data.



Appendix C, Rejuvra Mule Deer Data, Boulder County





Appendix C, Rejuvra Mule Deer Data, Boulder County

Mule Deer Habitat Summary

- Cheatgrass significantly competes directly with both native browse shrub and forb species.

- Longest leader growth of all 6 browse species monitored in this study doubled where cheatgrass was controlled with Rejuvra.

- Mule deer game camera photos nearly doubled in Rejuvra sprayed vs immediately adjacent untreated sites.

- There were 2 to 3x more mule deer tracks inside quadrats where Rejuvra was sprayed vs checks.

- Although nutritional values of browse was not collected, browse species are more robust and likely provide higher nutrition where cheatgrass is controlled.

- Healthier habitat equates to healthier does, fawns, and bucks with possibly higher fawn recruitment rates.

- Cheatgrass leads to higher fire frequency and high intensity wildfires that destroys mule deer browse and habitat.

- Wildfires burn a mosaic pattern through shrubs and forbs where cheatgrass is controlled.

Research Article

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Indaziflam: a new cellulose-biosynthesis-inhibiting herbicide provides long-term control of invasive winter annual grasses

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Abstract

BACKGROUND: Indaziflam is a cellulose-biosynthesis-inhibiting (CBI) herbicide that is a unique mode of action for resistance management and has broad spectrum activity at low application rates. This research further explores indaziflam's activity on monocotyledons and dicotyledons and evaluates indaziflam's potential for restoring non-crop sites infested with invasive winter annual grasses.

RESULTS: Treated Arabidopsis, downy brome, feral rye and kochia were all susceptible to indaziflam in a dose-dependent manner. We confirmed that indaziflam has increased activity on monocots (average $GR_{50} = 231$ pM and 0.38 g AI ha^{-1}) at reduced concentrations compared with dicots (average $GR_{50} = 512$ pM and 0.87 g AI ha^{-1}). Fluorescence microscopy confirmed common CBI symptomologies following indaziflam treatments, as well as aberrant root and cell morphology. Across five application timings, indaziflam treatments resulted in superior invasive winter annual grass control 2 years after treatment (from 84 ± 5.1% to 99 ± 0.5%) compared with imazapic (36% ± 1.2%). Indaziflam treatments significantly increased biomass and species richness of co-occurring species 2 years after treatment.

CONCLUSION: Indaziflam's increased activity on monocots could provide a new alternative management strategy for long-term control of multiple invasive winter annual grasses that invade >23 million ha of US rangeland. Indaziflam could potentially be used to eliminate the soil seed bank of these invasive grasses, reduce fine fuel accumulation and ultimately increase the competitiveness of perennial co-occuring species.

Keywords: cellulose biosynthesis inhibitor; cellulose synthase; downy brome; feral rye; imazapic; indaziflam; pre-emergence

1 INTRODUCTION

Herbicide discovery has slowed drastically with no new major mode of action introduced in the last 20 years.[1] As herbicide resistance continues to spread,[2−4] there is a need for compounds with new target sites.[1] It is more important than ever for land managers to take a stewardship approach with their herbicide tools by incorporating multiple modes of action;[5,6] however, limited herbicide alternatives can make this difficult. Herbicides used for cropland weed management should be evaluated for use in non-crop markets so that new herbicide modes of action and weed management solutions are available for non-cropland weed management. While land managers rely on the chemical industry to provide weed management solutions via new chemistries, it is equally important that land managers continually challenge their current weed management strategies and reduce selection pressure by using herbicide alternatives. Indaziflam, *N*-[(1*R*,2*S*)-2,3-dihydro-2,6-dimethyl-1*H*-inden-1-yl]-6-[(1*RS*)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine, first released in 2010, is a relatively new cellulose-biosynthesis-inhibiting (CBI) herbicide that is an underutilized tool for weed control and resistance management in non-crop markets.[7−9]

Indaziflam is registered in the United States for use in several perennial cropping systems, including established citrus, grape and tree nut crops, and was recently registered in Brazil for use in sugar cane, eucalyptus and pines.[8,10−13] Labeled non-crop application sites include rights-of-way, turf and ornamentals.[8,11] A recently established non-crop label for the release or restoration of desirable vegetation in natural areas, open spaces, wildlife management areas and fire rehabilitation areas is the focus of this research.[8,11,14]

Indaziflam represents a resistance management alternative with a unique mode of action and application timing.[9,14,15] Indaziflam provides broad-spectrum pre-emergence control of several annual grasses and broadleaf weeds.[8] Indaziflam is lipophilic (log $K_{ow} = 2.8$) and has low water solubility (2.8 mg L^{-1}), which

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could partially explain its increased residual soil activity compared with other commonly recommended herbicides.[8,16] Indaziflam is applied at low use rates and recommended at 73 and 102 g AI ha^{-1} for residual winter annual grass control in open spaces and natural areas.

Although indaziflam is classified as a CBI, there is very little known about the actual mechanism of action.[9,17] Cellulose is a composite polymer of glucan chains, synthesized at the plasma membrane by large cellulose synthase (CESA) complexes that directly release the developing cellulose polymers into the cell wall.[18,19] The cellulose synthase complex (CSC), arranged in a rosette pattern, has recently been shown to consist of 18–24 catalytic CESA proteins; however, the number of different CESA gene products required for the assembly of a functioning CSC remains to be clarified.[20] Interestingly, all of these proteins (in addition to accessory proteins) are potential sites of action for CBI herbicides such as indaziflam.[9,17]

CBI herbicides, including indaziflam, isoxaben and dichlobenil, are a diverse group of compounds with different sites of action directly affecting cellulose synthesis.[7,21] Herbicides in the alkylazine class, such as indaziflam, are unique, resulting in inhibitory activity three orders of magnitude lower than benzonitriles (dichlobenil) or benzamides (isoxaben). Indaziflam, isoxaben and dichlobenil specific mechanisms of action have been compared. Isoxaben treatments resulted in the depletion of CESA proteins from the plasma membrane and accumulation in cytosolic vesicles,[19,22,23] while dichlobenil treatments resulted in immobilization of CESA proteins and hyperaccumulation in the plasma membrane.[24] Indaziflam, however, has been shown to increase the density of CESA particles at the plasma membrane, but also to reduce CESA particle velocity by approximately 65%, inhibiting polymerization.[9] This increase in density has also been shown to reduce the colocalization between the microtubules and the CESA in the region near the root apical hook.[9] Although these studies confirm that indaziflam has a unique interaction with the complex cellulose biosynthesis pathway, there is limited research attempting to explain indaziflam's phytotoxicity on both monocotyledonous (monocots) and dicotyledonous (dicots) plants, which is unusual as other CBI herbicides are more active on dicots.[7]

Indaziflam is unique in that is has been shown to provide long-term selective control of the most prevalent invasive winter annual grass in the United States, downy brome (*Bromus tectorum* L.).[14,25,26] Currently, there has been one downy brome biotype identified that is highly resistant to acetolactate synthase (ALS) (imazamox, primisulfuron, propoxycarbazone, sulfosulfuron) and photosystem II inhibitors (PSII) (atrazine, metribuzin) and moderately resistant to acetyl CoA carboxylase inhibitors (ACCase) (clethodim, fluazifop).[27,28] Imazapic and glyphosate are currently the two most commonly recommended herbicides for invasive winter annual grass control; however, these herbicides provide inconsistent control,[29–32] and represent two modes of action that are prone to resistance development.[1,3,33] New modes of action for resistance management, such as CBIs, will be increasingly important for controlling downy brome and other invasive winter annual grasses in non-crop areas.

Indaziflam has also been shown to control other monocot weeds, including feral rye, Japanese brome (*Bromus japonicus* Thunb. or *Bromus arvensis* L.), jointed goatgrass (*Aegilops cylindrica* L.), medusahead [*Taeniatherum caput-medusae* (L.) Nevski] and ventenata [*Ventenata dubia* (Leers) Coss].[34] Invasive winter annual grass invasions are increasing at an alarming rate, displacing native vegetation that is critical habitat for wildlife and livestock and increasing fire frequency and intensity owing to the dense accumulation of fine fuel.[35–40] Although land managers have been attempting for decades to recover these sites dominated by invasive winter annual grasses, few have been consistently successful.[29] As these natural ecosystems continue to shift from perennial grass domination to invasive winter annual grass domination,[41] the necessity for new management tools continues to increase.[40]

Better understanding of the mode of action and selectivity of new herbicides such as indaziflam for non-crop weed management will minimize potential non-target effects and provide insight into the potential large-scale application of this herbicide in open spaces and natural areas. The objectives of this study were (1) to evaluate the differential response of indaziflam on two monocots [downy brome (*Bromus tectorum* L.) and feral rye (*Secale cereale* L.)] and two dicots [Arabidopsis (*Arabidopsis thaliana*) and kochia (*Kochia scoparia* L.)] using root and greenhouse dose–response bioassays, (2) to investigate the inhibitory effect of indaziflam on cellulose biosynthesis using fluorescence microscopy and (3) to compare indaziflam with imazapic (currently the most commonly recommended herbicide) in terms of both invasive winter annual grass control and response of the native plant communities (co-occurring species). Based on previous field research, we hypothesized that indaziflam's relative potency would be higher with monocots than with dicots, and subsequent microscopy could be a tool used to visualize this differential response. This work also expands on past field research comparing indaziflam and imazapic for invasive winter annual grass control by comparing additional species and application timings and further evaluating non-target impacts.

2 MATERIALS AND METHODS

2.1 Chemicals

For the root bioassay and microscopy, we used indaziflam analytic standard provided by Bayer CropScience (Research Triangle Park, NC). Calcofluor white (Fluorescent Brightener 28; MP Biomedicals, Solon, OH) was used for cellulose fluorescence. For the greenhouse dose–response and field experiments, we used commercial herbicide formulations of indaziflam (Esplanade™; Bayer CropScience), imazapic (Plateau®; BASF, Research Triangle Park, NC) and glyphosate (Accord® XRT II; Dow AgroSciences, Indianapolis, IN).

2.2 Indaziflam root bioassay

2.2.1 Experimental design

For *in vitro* dose–response experiments, we used a series of 1.5% agarose plates that contained 0, 50, 100, 200, 400, 800, 1200, 1600 and 3200 pM of indaziflam. A series of plates were generated for each species (downy brome, feral rye, Arabidopsis, kochia) and repeated in triplicate. Before planting, seeds were sterilized using a 70% ethanol solution. Seeds (12 Arabidopsis and kochia seeds and eight feral rye and downy brome seeds) were placed in a line along one edge of the plates (~1 cm from the top edge). The plates were arranged vertically with the line of seeds on the uppermost edge of the plate and placed in a growth chamber under continuous dark conditions and allowed to germinate.

2.2.2 Data analysis

Photographs of each plate were taken at a constant distance (25 cm) using a Nikon D3X camera, every 12 h, up to 84 h after the seeds were planted. Root expansion (length) measurements were conducted using ImageJ.[42] Total root expansion for each treatment was converted to a percentage of the non-treated control 84 h after treatment. The means of the three replicates ($n = 8$ or 12 seeds per plate) were plotted and used for generating the dose–response curves. Graphpad Prism 6 software for Windows (La Jolla, CA, www.graphpad.com) was used to determine the indaziflam rates required to reduce root expansion by 50% (GR_{50}) for downy brome, feral rye, Arabidopsis and kochia. The four-parameter log-logistic regression equation regressing root length (as a percentage of the non-treated) with herbicide concentration is as follows:

$$Y = C + \frac{D - C}{1 + 10^{(\log GR_{50} - X)\cdot b}} \quad (1)$$

where C is the lower limit of the response, D is the upper limit of the response, b is the slope and GR_{50} is the herbicide rate resulting in 50% root length reduction. Means were separated for each species to determine significant differences in GR_{50} values using Fisher's protected LSD test at a 5% level of probability.

Additionally, the average root length for each species, time point, replicate and concentration were plotted in an X,Y scatterplot, and a line of best fit was calculated for each growth curve. The slope of this line was calculated and was representative of the average rate of root growth from 0 to 72 h after planting (distance/time). The average growth rate for three replicates of each species were calculated and then plotted against increasing indaziflam concentrations. The same four-parameter log-logistic regression equation shown above was used for regressing the average rate of root growth as a percentage of the non-treated, with herbicide concentration.

2.3 Root fluorescence microscopy

Roots from treated and control plants (Section 2.2) were stained for 1 min in 1% Calcofluor white (Fluorescent Brightener 28, MP Biomedicals), followed by 1 min destaining in deionized water.[43–45] Roots were mounted in water and imaged using a Leica 5500 microscope (Leica Microsystems, Wetzlar, Germany) running IPLab v.4 software (BD Biosciences, San Jose, CA, USA) with a C4742-95 camera (Hamamatsu Photonics, Hamamatsu, Japan). Fluorescence was observed with a DAPI filter cube (Leica Microsystems). Bright-field images were made concurrently with the fluorescence images. Images were composited from multiple focal planes to get a single, uniformly in-focus image for each root using Adobe Photoshop (http://www.photoshop.com/) and Image Composite Editor (http://research.microsoft.com/en-us/um/redmond/groups/ivm/ice/).

2.4 Indaziflam greenhouse dose–response

A greenhouse dose–response experiment was conducted to confirm the results from Section 2.2 and to evaluate further the relative sensitivity of the monocot (downy brome, feral rye) and dicot (Arabidopsis, kochia) species to indaziflam in field soil. Arabidopsis did not germinate consistently under these experimental conditions and was omitted from further analysis.

2.4.1 Experimental design

The study used seven herbicide concentrations and a non-treated control arranged in a completely randomized design with four replications. The study was performed on 29 December 2015 and repeated on 19 January 2016. Based on the results from a preliminary experiment, the indaziflam concentrations used for the kochia dose–response were 0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9 and 11.7 g AI ha^{-1}. The indaziflam concentrations used for all other species were 0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9 and 5.9 g AI ha^{-1}.

Seeds were planted in square plastic containers ($12 \times 12 \times 6$ cm) in an Otero sandy clay loam field soil (coarse-loamy, mixed, mesic Aridic Ustorthents) with 3.9% organic matter and pH 7.7. All species were planted at a depth of 0.5 cm, with the exception of Arabidopsis which was planted at the soil surface. Seeding densities were adjusted on the basis of germinability to reach a target density of 30 plants pot^{-1}. Indaziflam was applied using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle calibrated to deliver 187 L ha^{-1} at 172 kPa. Treated pots were transferred immediately to a greenhouse with a 15 h photoperiod and 25/20 °C day/night temperature regime. Natural light was supplemented with high-intensity discharge lamps when light was below 25 mW cm^{-2}. Plants were misted daily to reduce soil crusting and subirrigated as needed. Above-ground plant biomass was harvested at the soil surface 3 weeks after treatment and dried for 3 days at 60 °C before recording dry weights.

2.4.2 Data analysis

Total dry weights for each treatment were converted to a percentage of the biomass of the non-treated control and analyzed in Graphpad Prism 6 (Section 2.1.3). Data from repeated studies were combined after the null hypothesis of equal variance was not rejected. The same four-parameter log-logistic regression equation from Section 2.1.3 was used to construct the species-specific dose–response curves and to determine the indaziflam concentrations required to reduce dry biomass by 50% (GR_{50}). Significant differences in GR_{50} values were evaluated using Fisher's protected LSD test at a 5% level of probability.

2.5 Invasive winter annual grass field efficacy studies

2.5.1 Site description

In 2014, field experiments were conducted to expand on previous literature comparing the effectiveness of indaziflam and imazapic for long-term invasive winter annual grass control, and to evaluate the response of the native plant communities. The experiments were established at two sites on the Colorado Front Range dominated by invasive winter annual grasses. Site 1 (latitude 40° 15′ 2″ N, longitude 105° 12′ 56″ W) was infested with equal amounts of downy brome and Japanese brome, and site 2 (latitude 40° 43′ 23″ N, longitude 104° 55′ 58″ W) was infested with feral rye. Sites were approximately 58 km apart. Site 1 was located on Rabbit Mountain Open Space (Boulder County), and site 2 was located on a Colorado Parks and Wildlife Area (Larimer County). Before herbicide application (July 2014), we made visual estimates across the entire study area of percentage of living canopy cover for all species present at both sites. Site 1 was characterized by ∼80–100% downy brome and Japanese brome canopy cover with a dense fine fuel layer (2–5 cm) and a scattered stand of co-occurring species (∼0–10% canopy cover) (Table 1). Site 2 had >95% canopy cover of actively growing feral rye, a fine fuel layer of 2–5 cm and <5% canopy cover of western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Love] and sand dropseed [*Sporobolus cryptandrus* (Torr.) A. Gray].

The soil at site 1 was Baller sandy loam (loamy-skeletal, mixed, mesic Lithic Haplustolls), with 1.5% organic matter in the top 20 cm.[46] The average elevation was 1737 m (5700 ft). The soil at site

Table 1. List of co-occurring species at site 1

Common name	Scientific name
Blue grama	*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths
Western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Love
Western ragweed	*Ambrosia psilostachya* DC.
Tarragon	*Artemisia dracunculus* L.
Fringed sagebrush	*Artemisia frigida* Willd.
Prairie sage	*Artemisia ludoviciana* Nutt.
Winged buckwheat	*Eriogonum alatum* Torr.
Blanketflower	*Gaillardia aristata* Pursh
Parry's geranium	*Geranium caespitosum* James var. parryi (Engelm.) W.A. Weber
Dotted gayfeather	*Liatris punctata* Hook.
Pricklypear cactus	*Opuntia polyacantha* Haw.
Slender-flowered scurfpea	*Psoralidium tenuiflorum* (Pursh) Rydb.
Prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.
Woods' rose	*Rosa woodsii* Lindl.
Scarlet globemallow	*Sphaeralcea coccinea* (Nutt.) Rydb.
Porter's aster	*Symphyotrichum porteri* (A. Gray) G.L. Nesom
Yellow salsify	*Tragopogon dubius* Scop.

2 was Terry sandy loam (coarse-loamy, mixed, superactive, mesic Ustollic Haplargids), with 1.3% organic matter in the top 20 cm.[46] The average elevation was 1646 m (5400 ft). At sites 1 and 2, mean annual precipitation based on the 30 year average (1981–2010) was 379 and 363 mm, and the mean annual temperatures were 9.1 and 8.6 °C respectively.[47] Precipitation was close to the 30 year average in 2014; however, in 2015, both sites received an additional 199 and 212 mm above the 30 year averages, respectively.[48] A drought occurred in 2016, with an annual precipitation of 235 and 290 mm at sites 1 and 2 respectively.

2.5.2 Experimental design

Herbicides were applied at five application timings to evaluate variations in invasive winter annual grass control, potential non-target impacts and the potential release of co-occurring species after herbicide treatment. Herbicides were applied both before (PRE) and after (POST) winter annual grass emergence. Timings were designated as early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015) and late POST (LPOST, April 2015). We had four treatments at each application timing: indaziflam at three concentrations (44, 73 and 102 g AI ha^{-1}) and imazapic at 123 g AI ha^{-1}. Imazapic and indaziflam have limited to no POST activity; therefore, all POST treatments included 420 g ae ha^{-1} of glyphosate as the burndown herbicide. The 21 herbicide treatments (including a non-treated control) were applied to 3 by 9 m plots arranged in a randomized complete block design with four replications. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat-fan nozzles at 187 L ha^{-1} at 207 kPa. All treatments included 1% (v/v) methylated seed oil.

2.5.3 Treatment evaluation and data analysis

Biomass harvests and species richness evaluations were conducted in August (2015 and 2016) to evaluate invasive winter annual grass control and response of co-occurring species. Above-ground biomass of the winter annual grasses, perennial grasses and forbs were harvested from randomly placed 1 m^2 quadrats; quadrats were not taken from the same location in consecutive years. Site 1 had an equal distribution of downy brome and Japanese brome (Section 2.5.1); therefore, biomasses of both species were combined for analysis. Directly following harvest, the material was dried at 60 °C for 5 days to calculate dry biomass. Additionally, at site 1, species richness was calculated for each treatment as a simple estimate of biological diversity.[49] Species richness was defined as the total number of unique species (grasses and forbs) occurring per unit area (e.g. 27 m^2 plot size). These count data were assumed to follow a Poisson distribution.

Invasive winter annual grass biomass was converted to a percentage of the non-treated control, and data were combined across sites after the null hypothesis of equal variance was not rejected. However, owing to unequal variances across sites for perennial grass biomass ($P < 0.0001$), data from sites 1 and 2 were analyzed separately. Because site 2 only had two desirable grass species and no forbs, forb biomass data and richness are only presented for site 1. All response variables (invasive winter annual grass biomass, perennial grass biomass, forb biomass and species richness) were first evaluated for significant main effects and interactions by performing an ANOVA using the PROC MIXED method in SAS 9.3.[50] Factors included in the model statement were treatment, site, year after treatment and all interactions, with year after treatment defined as the repeated measure. The random factor was site nested within replication, and a Tukey–Kramer adjustment was performed. To meet ANOVA assumptions of normality, we used an arcsine square root transformation for invasive winter annual grass biomass (% of non-treated) and a square root transformation for perennial grass and forb biomass; however, no transformations were required for forb richness. To evaluate the significant treatment-by-year interaction for all response variables ($P < 0.0001$), an ANOVA was conducted using the PROC GLIMMIX method and the LINES statement. This provided comparisons between all pairs of least-squares means across years ($P < 0.05$). All means presented in the figures are non-transformed data.

3 RESULTS AND DISCUSSION

3.1 Differential response of monocotyledons and dicotyledons to indaziflam

Currently, there is limited research attempting to further explain the unique phytoxicity of indaziflam on both monocots and dicots. Because CBI herbicides involve a complex mechanism of action and it appears as though different CBIs inhibit different proteins within the cellulose synthase complex, most of the published literature has been constrained to studies of a model organism. These model organisms, such as Arabidopsis, have a fully sequenced genome that provides the opportunity to identify unique genes in a pathway of interest such as cellulose synthesis. In these studies we expand on previous research with Arabidopsis and quantify the differential response of indaziflam-treated monocot and dicot weeds. Previous research has used CBIs as a tool to better understand cellulose biosynthesis, whereas the focus of these data was to better understand indaziflam's mode of action for practical use in non-crop weed management.

3.1.1 Root bioassay and microscopy

Downy brome, feral rye, Arabidopsis and kochia were susceptible to indaziflam, and their growth was inhibited in a dose-dependent manner (Fig. 1). The indaziflam concentrations resulting in 50%

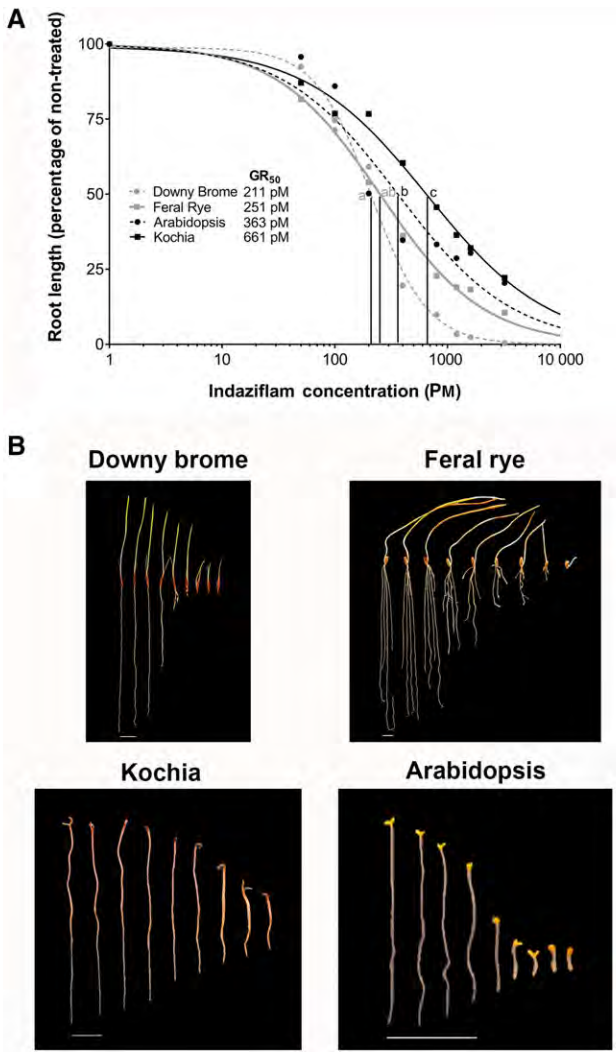

Figure 1. (A) Response of root length to increasing herbicide concentrations 84 h after planting, represented as a percentage of the non-treated control. Dose–response curves were fitted using four-parameter log-logistic regression. Mean values of the three replicates (plates) are plotted ($n = 8$ or 12 seeds per plate) at each indaziflam concentration. Vertical lines represent the indaziflam concentration resulting in 50% reduction in root length (GR_{50}) for each species, and letters signify differences in GR_{50} values using Fisher's protected LSD test at a 5% level of probability. (B) Representative images of the indaziflam root bioassay with seven-day-old seedlings. Indaziflam concentrations used from left to right were 0, 50, 100, 200, 400, 800, 1200, 1600 and 3200 pM. Scale bars = 1 cm.

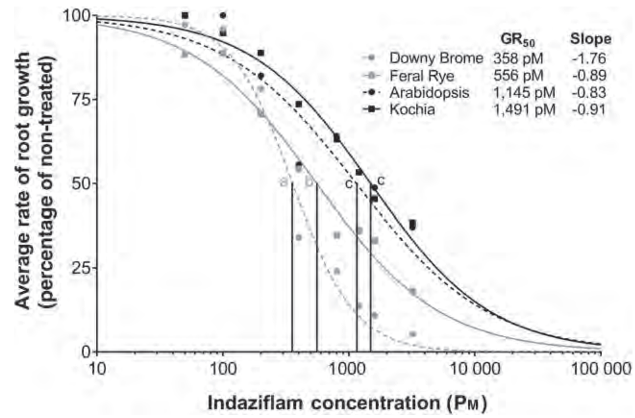

Figure 2. Effect of indaziflam on the average rate of root growth from 0 to 72 h (12 h increments) after planting. Dose–response curves were fitted using four-parameter log-logistic regression. Mean values of three replicates (plates) are plotted ($n = 8$ or 12 seeds per plate). Vertical lines represent the indaziflam concentration resulting in 50% reduction in root growth rate (GR_{50}) for each species. Letters signify differences in GR_{50} values using Fisher's protected LSD test at a 5% level of probability.

reduction in root length (GR_{50}) compared with the non-treated control for downy brome, feral rye, Arabidopsis and kochia were 211, 251, 363 and 661 pM respectively. There were significant differences between GR_{50} values for monocots (downy brome, feral rye) and dicots (Arabidopsis, kochia). Downy brome was most susceptible to indaziflam, with a GR_{50} value approximately 3 times lower than kochia's GR_{50} ($P < 0.0001$). Indaziflam GR_{50} values for feral rye ($P = 0.0069$) and Arabidopsis ($P = 0.0016$) were also significantly lower than the kochia GR_{50} value. Indaziflam-treated seedlings exhibited common CBI symptomology, including radial expansion and inhibition of root and hypocotyl elongation[7,51] (Fig. 1).

Evaluating changes in the average growth rate of indaziflam-treated roots (0–72 h) revealed a differential response for monocots and dicots (Fig. 2). The herbicide concentration resulting in 50% reduction in root growth rate was on average 2.9 times lower for monocots than for dicots. This finding is consistent with the root bioassay (Fig. 1), providing additional evidence that, while indaziflam inhibits root expansion and elongation, the speed at which this inhibition occurs is faster for monocots than for dicots (Fig. 2).

Using treated roots from the root bioassay, qualitative fluorescence microscopy using Calcofluor white to visualize cell walls by cellulose fluorescence revealed similar and also unique symptomologies from other published indaziflam research. Treated roots were wider and their cells were larger than in non-treated roots, as has been previously reported.[7,9] Cell walls in monocot roots showed a strikingly different response compared with dicot roots (Fig. 3). Treated roots of downy brome and feral rye exhibited large areas without fluorescence, presumably owing to cellulose deficiency. This is a more severe symptomology than the previously reported gapped cell walls[45,51,52] (Figs 3A and B). A previous study showed somewhat similar results with *prc1* (CesA6 mutation), or dichlobenil/isoxaben-treated wild-type seedlings.[45] In that case, incomplete cell walls were observed, but shown to be connected by a membranous structure that was not stainable by Calcofluor white.[45] However, in our study, these incomplete, non-staining areas spanned large areas of the root, and in some cases the root appeared to be split open (downy brome, 1200 pM; feral rye, 800 and 1200 pM) (Figs 3A and B). Areas where the root was split were also missing in the bright-field view, indicating that cells were totally absent rather than being present, but lacking cellulosic cell walls (Figs 3A and B). The bright-field view also revealed that in the gapped areas there is subtending tissue that is only faintly stained in the fluorescence images. This deeper tissue is likely part of a different tissue system (i.e. parenchyma). Further study will be needed to determine the effect of indaziflam on different root tissues.

Although we observed gaps in the root structure of monocots, indaziflam-treated dicot roots had differing phenotypes. In Arabidopsis, an overabundance of root hairs was observed, so that it was difficult to discern the underlying root, while in kochia, some cells acquired a nearly circular shape, but only at doses of at least 1200 pM. Although monocot cells also appeared swollen and misshapen, they did not quite reach the circularity of kochia

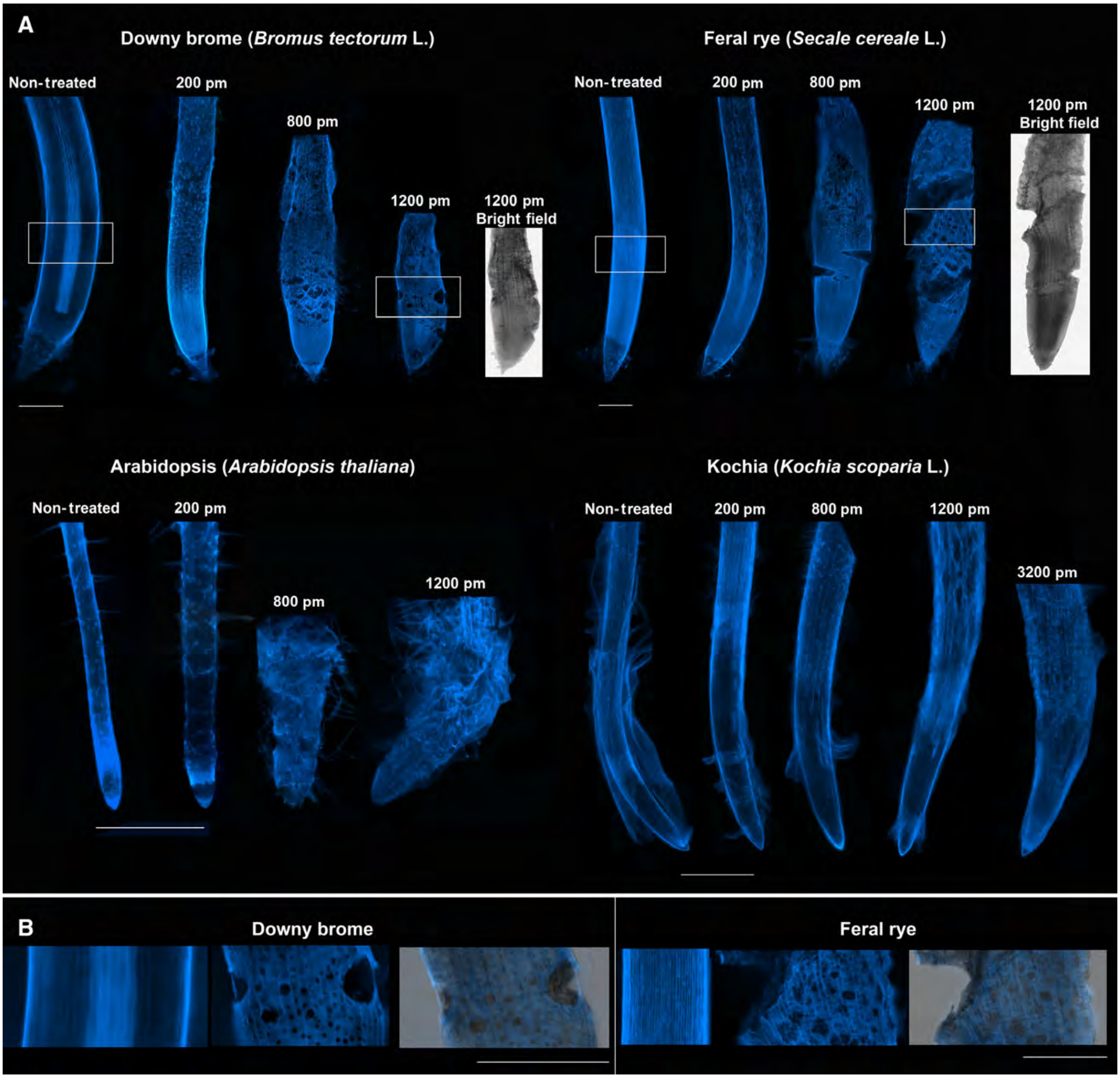

Figure 3. (A) Cellulose fluorescence of indaziflam-treated monocot (downy brome, feral rye; scale bars = 250 μm) and dicot (Arabidopsis, kochia; scale bars = 500 μm) seedlings examined using a Leica 5500 microscope (DAPI filter cube) and Calcofluor white stain or bright field, as indicated. White boxes indicate the regions shown in part B. (B) Indaziflam symptomology of downy brome (non-treated, 1200 pM) and feral rye (non-treated, 1200 pM). The rightmost image for each is an overlay of the fluorescence and bright-field images. Non-treated roots (left) show uniform cellulose synthesis. Indaziflam-treated seedlings exhibited radial swelling, malformed circular cells (dicots), large structural gaps and split roots (monocots). Scale bars = 250 μm.

root cells. Perhaps the swollen cells in time lead to the gapped areas observed in the monocots; a time-course of roots growing in indaziflam-treated plates could be useful to reveal how these symptoms arise.

In all species, few cellular deformities (other than enlarged cells) were observed in the zone of division. Symptoms appeared concurrently with root hairs in the elongation zone and persisted and grew more dramatically through the zone of elongation. Misshapen cells were also present in the root caps, most prominently in the monocot species. As the root cap is also composed of mature cells arising from the zone of division, this suggests that indaziflam acts during the cell elongation and maturation process.

3.1.2 *Indaziflam greenhouse dose–response*

Arabidopsis has a very small seed size, and growth is affected by many environmental factors;[53] therefore, it was not surprising that it was difficult to generate dose–response curves. Similar results were observed between the root and greenhouse bioassays in terms of the differential response of monocots and dicots to indaziflam (Figs 1 and 4). The indaziflam concentrations resulting in 50% reduction in root length (GR_{50}) compared with the non-treated control for downy brome, feral rye and kochia were 0.25, 0.51 and 0.87 g AI ha^{-1} respectively (Fig. 4). It is not unusual for herbicides to be more active in the greenhouse under ideal environmental conditions, so it was not surprising to us that GR_{50} values were much lower than recommended field concentrations (73 and

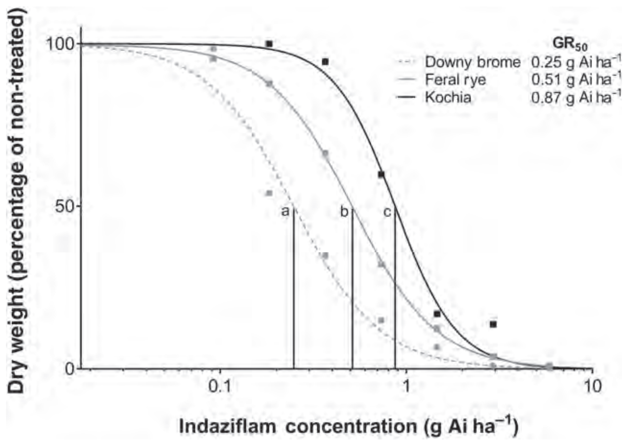

Figure 4. Greenhouse dose–response evaluating the reduction in dry weight represented as a percentage of the non-treated control. Herbicide concentrations used for kochia were 0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9 and 11.7 g AI ha^{-1} and 0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9 and 5.9 g AI ha^{-1} for downy brome and feral rye. Dose–response curves were fitted using four-parameter log-logistic regression. Mean values of four replications are plotted. Vertical lines represent the indaziflam concentration resulting in 50% reduction in dry weight (GR$_{50}$) for each species. Letters signify differences in GR$_{50}$ values using Fisher's protected LSD test at a 5% level of probability.

102 g AI ha^{-1}). The indaziflam concentration needed to reduce kochia dry biomass by 50% was approximately 2 and 4 times the concentration required for feral rye ($P < 0.0001$) and downy brome ($P < 0.0001$) respectively (Fig. 4).

Indaziflam has a unique mode of action compared with other CBI herbicides because it can control both monocots and dicots; however, our results suggest that the relative potency of indaziflam varies across these two plant classes. Increased monocot inhibition at lower use rates as compared with dicots has been confirmed with mitotic disrupter heribicides such as dinitroanilines (i.e. trifulralin, oryzalin, pendimethalin),[54] but this is not the case for CBI herbicides.[7,55] In particular, isoxaben activity is specific to dicots and primarily used for PRE control of broadleaf weeds.[7] Because the mechanism of action of these chemically diverse CBI herbicides is very complex and poorly understood, these data provide useful information that could be utilized for further exploration of indaziflam's unique cellulose biosynthesis inhibiting mechanism.

3.2 Invasive winter annual grass field efficacy study

3.2.1 Invasive winter annual grass control

The significant treatment-by-year ($P < 0.0001$) interaction on invasive winter annual grass control was evaluated. The combined data from sites 1 and 2 showed a similar level of invasive winter annual grass control (downy brome, feral rye, Japanese brome) 1 year after treatment (YAT), except for imazapic at the EPRE timing (~41% control) (Fig. 5). Across all five application timings, indaziflam at 73 and 102 g AI ha^{-1} provided >99% control 1 YAT (2015). These data suggest that 1 YAT, imazapic treatments at the POST timings provided superior control to imazapic applied PRE. This difference in efficacy could be explained by the addition of the glyphosate burndown at the POST timings, or the later application timings had less microbial degradation and therefore an increased concentration of imazapic in the soil during peak growth (summer 2015).

Indaziflam treatments across all application timings (except indaziflam applied at the lowest rate of 44 g AI ha^{-1}, EPRE and PRE), provided superior invasive winter annual grass control 2 YAT (2016) compared with imazapic (Fig. 5). Indaziflam applied at 102 g AI ha^{-1} controlled 97–99 ± 0.5% (mean ± SE) of downy brome, feral rye and Japanese brome, while imazapic provided only 32–35 ± 1.5% control, 2 YAT (Fig. 5). An additional observation of this study was the impact of herbicide treatments on fine fuel accumulation. Before herbicide treatments were initiated (2014), both sites had accumulated fine fuel layers of ~2–5 cm. At both sites, indaziflam treatments eliminated further residue inputs via residual control 2 YAT, resulting in the complete decomposition of these fine fuel layers (~9–12 months after treatment).

Invasive winter annual grass control responded to indaziflam treatments in a dose-dependent manner. The 102 g AI ha^{-1} concentration is highly effective and should be strongly considered for management of invasive winter annual grasses with a short seed viability (~3–5 years).[56,57] To achieve or increase the success of long-term invasive winter annual grass control, it is imperative to limit the seed rain during this 3–5 year period and choose management options that provide close to 100% control. If the soil seed bank is able to regenerate, the invasive winter annual grass is likely to re-establish. This has often been the case for herbicides with limited soil residual activity beyond the initial year of application, such as imazapic.[29] These data support previous downy brome research;[14] however, we also provide evidence that indaziflam can provide residual control of multiple invasive winter annual grasses that may coexist at a site (Fig. 5).

3.2.2 Perennial grass response

The significant treatment-by-year interaction ($P < 0.0001$) was evaluated separately at sites 1 and 2. The increased level of invasive winter annual grass control (Fig. 6) 2 YAT, for indaziflam, was evident in the re-establishment of co-occurring species compared with imazapic (Fig. 6). By providing residual control of the invasive winter annual grasses, this likely made available a surplus of moisture and nutrients, resulting in the positive response of co-occurring perennial grasses. Across application timings at sites 1 and 2, indaziflam at the highest concentration (102 g AI ha^{-1}) provided the greatest increase in perennial grass biomass 2 YAT, while biomass in imazapic-treated plots was no different than the non-treated control ($\alpha = 0.05$) (Fig. 6). Averaged across both sites, indaziflam applied EPRE, PRE, EPOST, POST or LPOST resulted in a 38-, 35-, 39-, 28- and 42-fold increase in perennial grass biomass compared with the non-treated control (Fig. 6). Across site 2, the decrease in overall perennial grass biomass in 2016 was a result of the drought conditions during that growing season, and was not a result of herbicide treatments. At both sites, indaziflam treatments provided greater residual control of invasive winter annual grasses 2 YAT compared with imazapic, allowing for significant increases in biomass and re-establishment of co-occurring species, 1 and 2 YAT (Fig. 6).

At site 1 there was no difference in perennial grass dry biomass for all POST and LPOST treatments compared with the non-treated check 1 YAT (2015) ($\alpha = 0.05$) (Fig. 6). At site 1, western wheatgrass and other cool-season grasses were not dormant at these late-spring POST and LPOST timings; therefore, reduced perennial grass biomass at these timings (compared with EPRE, PRE, EPOST) was attributed to glyphosate injury. In year 2, biomass significantly increased for all indaziflam treatments applied POST, and for the LPOST indaziflam 102 g AI ha^{-1} treatment. At site 1, indaziflam treatments POST and LPOST resulted in a 14–20-fold and 10–32-fold biomass increase compared with the non-treated control 2 YAT, respectively. Imazapic treatments at the POST and LPOST application timings resulted in a seven- and threefold increase in

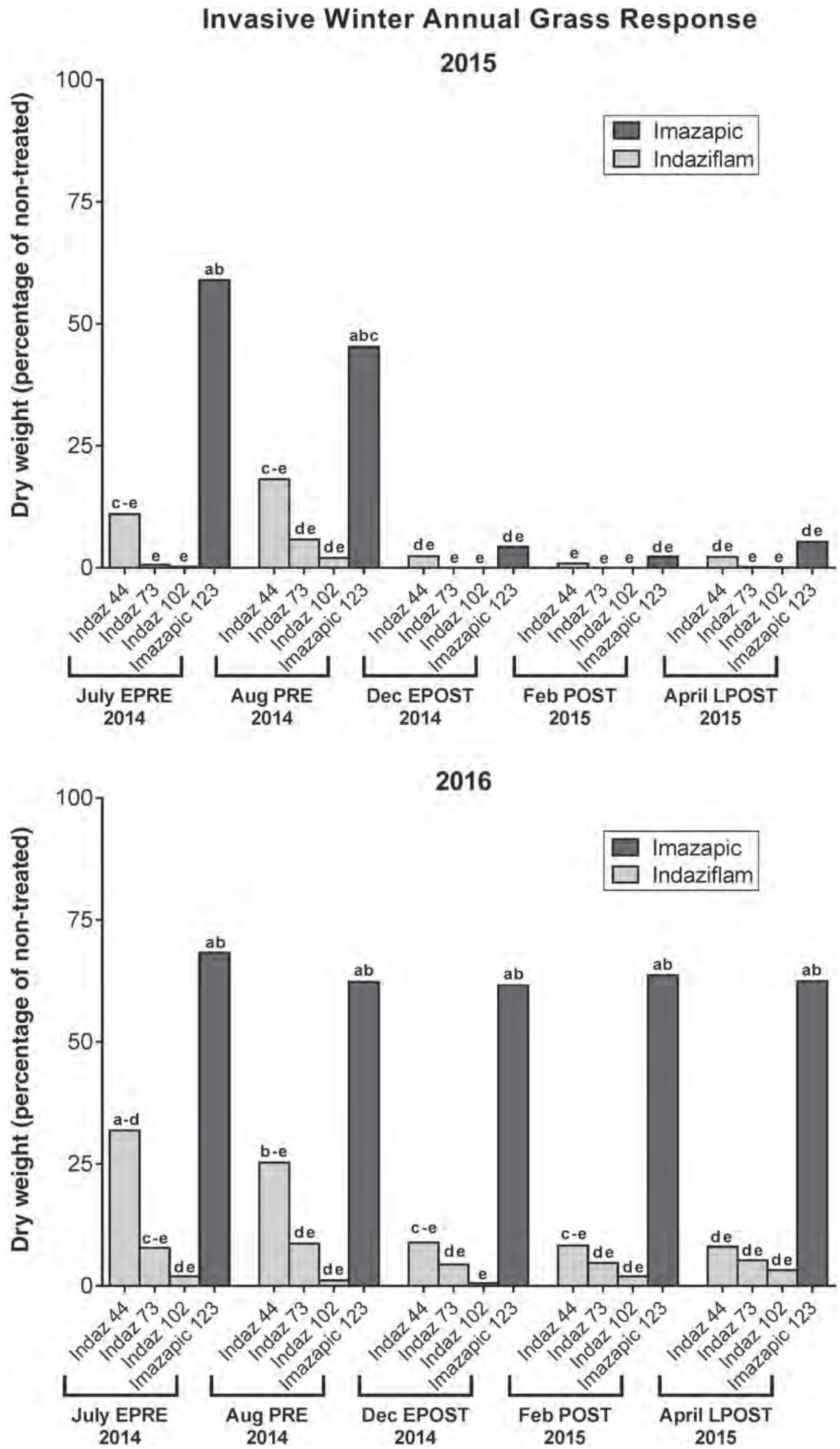

Figure 5. Site 1 and 2 percentage invasive winter annual grass control (downy brome, feral rye, Japanese brome) compared with the non-treated 1 YAT (2015) and 2 YAT (2016). Five application timings were evaluated, including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015) and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least-squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73 and 102 g AI ha^{-1} and imazapic at 123 g AI ha^{-1}. All POST treatments included 420 g ae ha^{-1} of glyphosate as the burndown.

perennial grass biomass 2 YAT, respectively; however, this was not statistically different from the non-treated control (Fig. 6). Summarizing these data across years, indaziflam treatments applied EPRE, PRE or EPOST resulted in the greatest increase in perennial grass biomass across sites, although recovery of co-occurring species was also seen in the POST and LPOST timings, 2 YAT.

3.2.3 *Forb response and species richness*

There was a similar response of forb biomass compared with perennial grass biomass. Treatments at the EPOST and POST timings resulted in the greatest increase in forb biomass, 1 YAT (Fig. 7). With the exception of imazapic PRE, no treatments 1 YAT resulted in a reduction in forb biomass. All imazapic treatments 2 YAT had

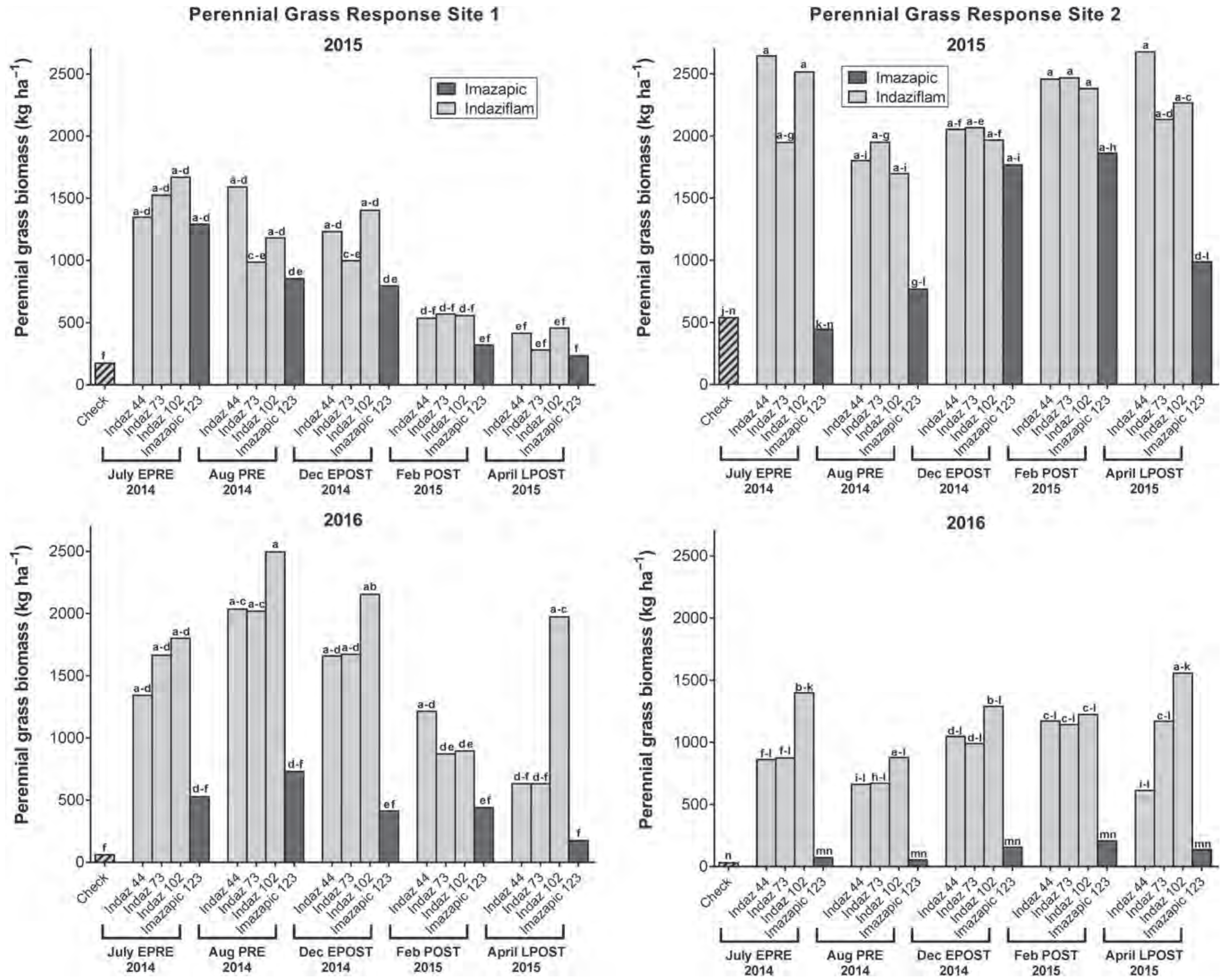

Figure 6. Site 1 and 2 perennial grass biomass response to herbicide treatments, 1 YAT (2015) and 2 YAT (2016). Five application timings were evaluated, including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015) and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least-squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73 and 102 g AI ha^{-1} and imazapic at 123 g AI ha^{-1}. All POST treatments included 420 g ae ha^{-1} of glyphosate as the burndown.

similar levels of forb biomass compared with the non-treated control plots (Fig. 7). A significant increase in the re-establishment of forbs in indaziflam-treated plots was not seen until 2 YAT (2016). With the exception of the indaziflam 44 g AI ha^{-1} EPRE treatment, all other indaziflam treatments resulted in a significant increase in forb biomass compared with the non-treated control plots. Averaged across timings, indaziflam treatments at 44, 73 and 102 g AI ha^{-1} resulted in a three-, five- and fivefold increase in forb biomass, respectively, compared with the non-treated control plots (Fig. 7).

The forb biomass data can be used as an estimate of the quantity of forbs in a plot; however, species richness evaluations allowed us to evaluate further the effect of herbicide treatments on species diversity. The list of co-occurring species present at site 1 can be seen in Table 1. Species richness increased 1 YAT for all species, but this increase was not significantly greater compared with the non-treated control (Fig. 8). Species richness further increased 2 years after indaziflam treatments, whereas species richness after imazapic treatments remained fairly constant between 1 YAT (6.0 ± 0.3 species plot^{-1}) and 2 YAT (6.4 ± 0.4 species plot^{-1}). All treatments with indaziflam, regardless of application rate, increased species richness compared with the non-treated control, from 4.3 ± 0.6 species plot^{-1} 1 YAT in the control plot to an average of 7.9 species plot^{-1} 2 YAT in the treated plots (Fig. 8). These data provide strong evidence for the selectivity of indaziflam on perennial co-occurring species, allowing for an increase in establishment as early as 1 YAT (Fig. 9). The increase in forb biomass, species composition and diversity over time is evidence that indaziflam treatments have positive impacts on the perennial native plant communities (Figs 9 and 10).

4 CONCLUSION

Indaziflam represents a new weed management opportunity in non-crop areas with a unique mode of action that currently has no reported cases of herbicide resistance. In this study, we expand on previous work with Arabidopsis,[9] providing practical implications for how indaziflam could be used to increase weed management success in open spaces and natural areas.

Monocots and dicots diverged appoximately 200 million years ago,[58] resulting in significant variations in cellulose synthesis

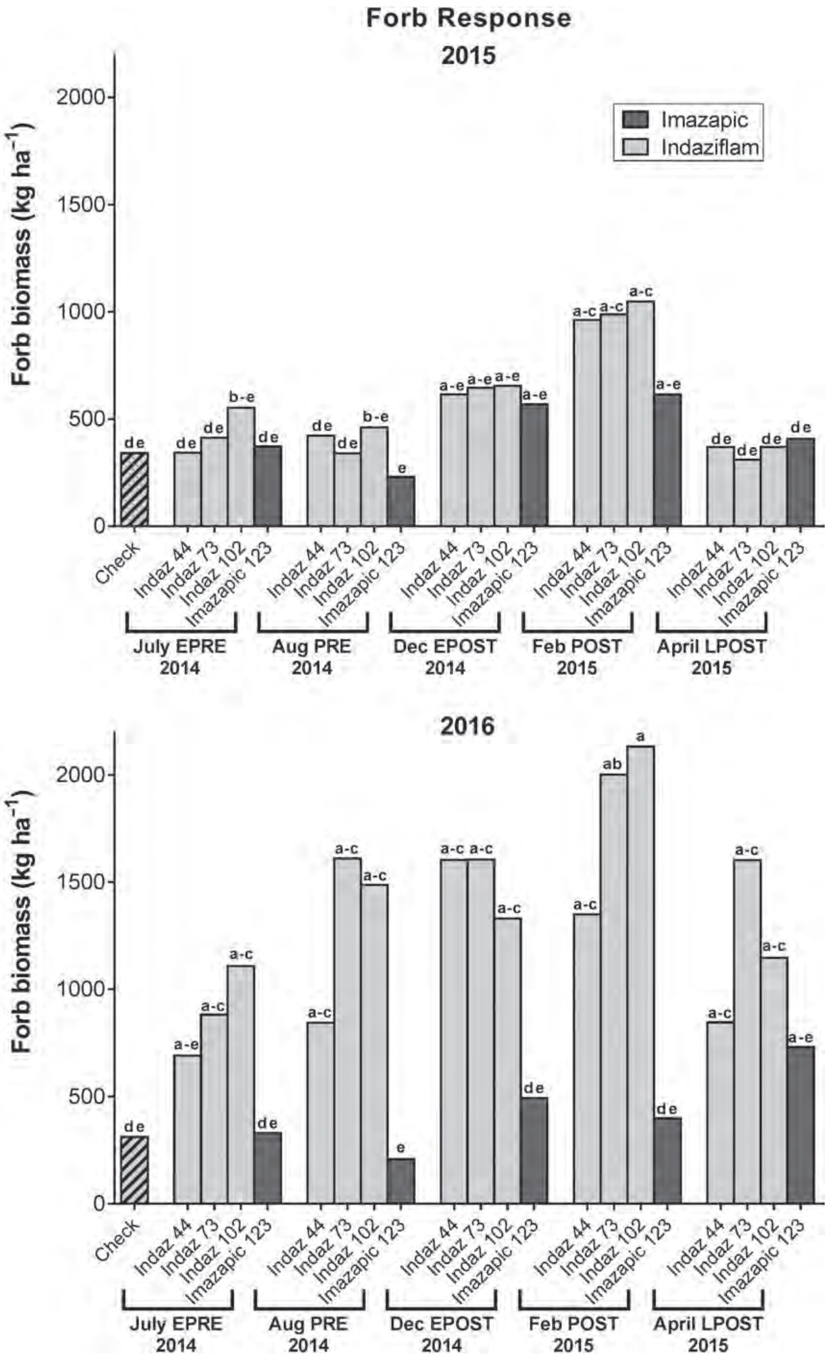

Figure 7. At site 1, forb biomass response to herbicide treatments 1 YAT (2015) and 2 YAT (2016). Five application timings were evaluated, including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015) and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least-squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73 and 102 g AI ha^{-1} and imazapic at 123 g AI ha^{-1}. All POST treatments included 420 g ae ha^{-1} glyphosate as the burndown.

and cell wall architecture between these plant classes. One explanation for the differences in relative potency of indaziflam on monocots and dicots could be the unique cell wall structure between dicots/liliaceous monocots (type 1 cell walls) and Poales/commelinid monocots (type 2 cell walls).[17] In this study, Arabidopsis and kochia, both dicots, have type 1 cell walls while downy brome and feral rye, both commelinid monocots, have type 2 cell walls. Factors within the two plant classes that could also influence relative indaziflam potency are seed size, metabolism, herbicide sequestration, herbicide absorption or genetic differences.[17] Because cellulose synthesis is such a complex process, there are likely many contributing factors involved in indaziflam's ability to control both monocots and dicots. We can conclude from the root bioassay, greenhouse dose–response and fluorescence microscopy that indaziflam does in fact inhibit monocot root elongation and provide control at lower rates compared with dicots. The methods from our study were highly consistent with the dark-grown bioassay used by Brabham *et al.*;[9] however, they found the dose needed for inhibition of dicots was lower than monocots. This difference in phytotoxicity compared with

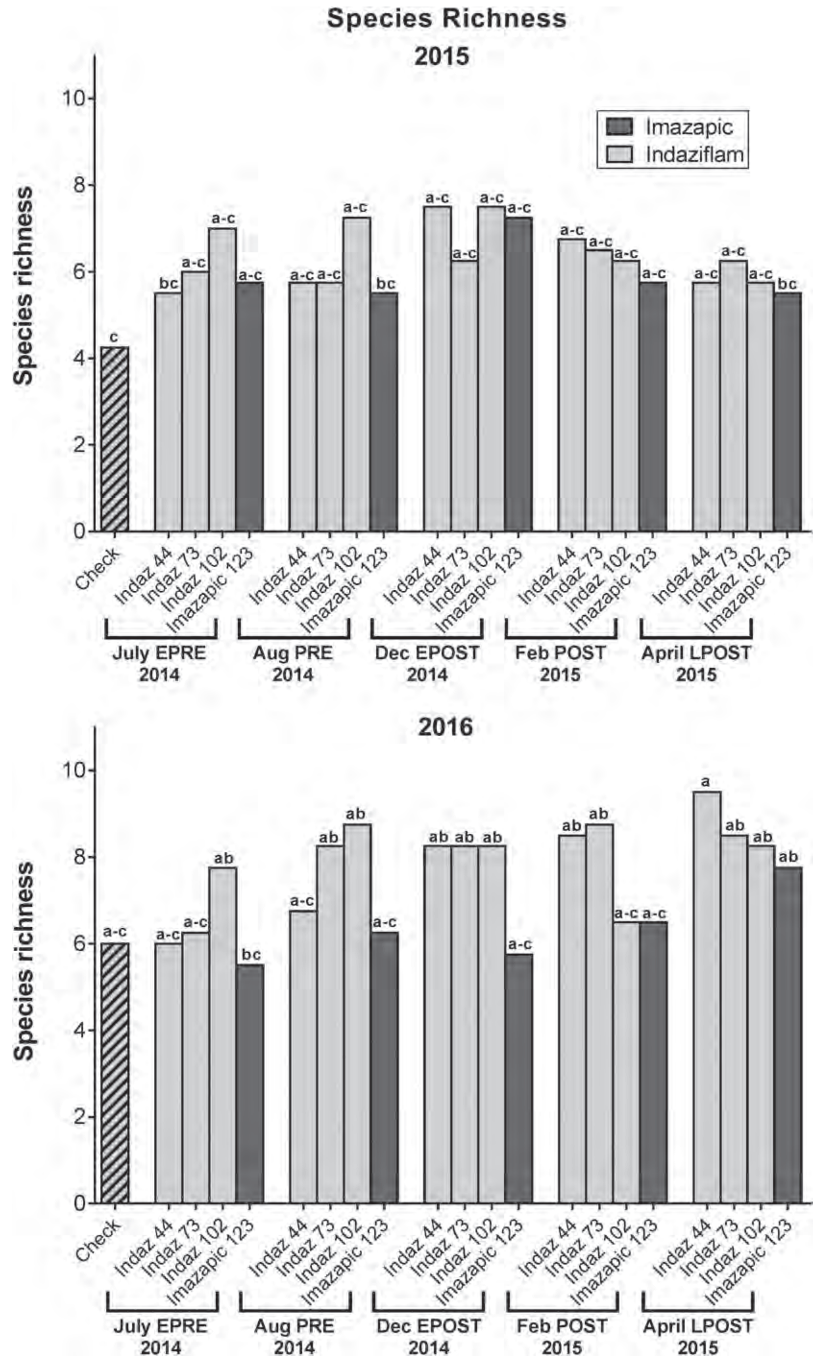

Figure 8. At site 1, species richness defined as the total number of unique co-occurring species (grasses and forbs) occurring per unit area (27 m^2 plot size), 1 YAT (2015) and 2 YAT (2016). Five application timings were evaluated, including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015) and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least-squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73 and 102 g AI ha^{-1} and imazapic at 123 g AI ha^{-1}. All POST treatments included 420 g ae ha^{-1} of glyphosate as the burndown.

our study is likely an example of differences in relative potency across species, and for the species we tested, monocots displayed a greater response compared with dicots. We also observed more severe cellular symptoms in monocot species than in dicot species treated with the same herbicide concentration. Understanding the difference between the monocot and dicot response to indaziflam treatment will require further studies to identify the target protein of indaziflam, such as forward and reverse genetic screens in Arabidopsis (a model dicot) and *Brachypodium distachyon* or rice (both model monocot species). Indaziflam may also prove useful in basic research into the still unresolved complexities of cellulose synthesis.

Root inhibition was noticeable at pM concentrations. This observable activity at extremely low concentrations could explain the increased residual weed control provided by indaziflam compared with other herbicides. Dichlobenil and isoxaben, two other CBI herbicides, are labeled at approximately 10 and 40 times greater herbicide concentrations than indaziflam (73 and 102 g AI

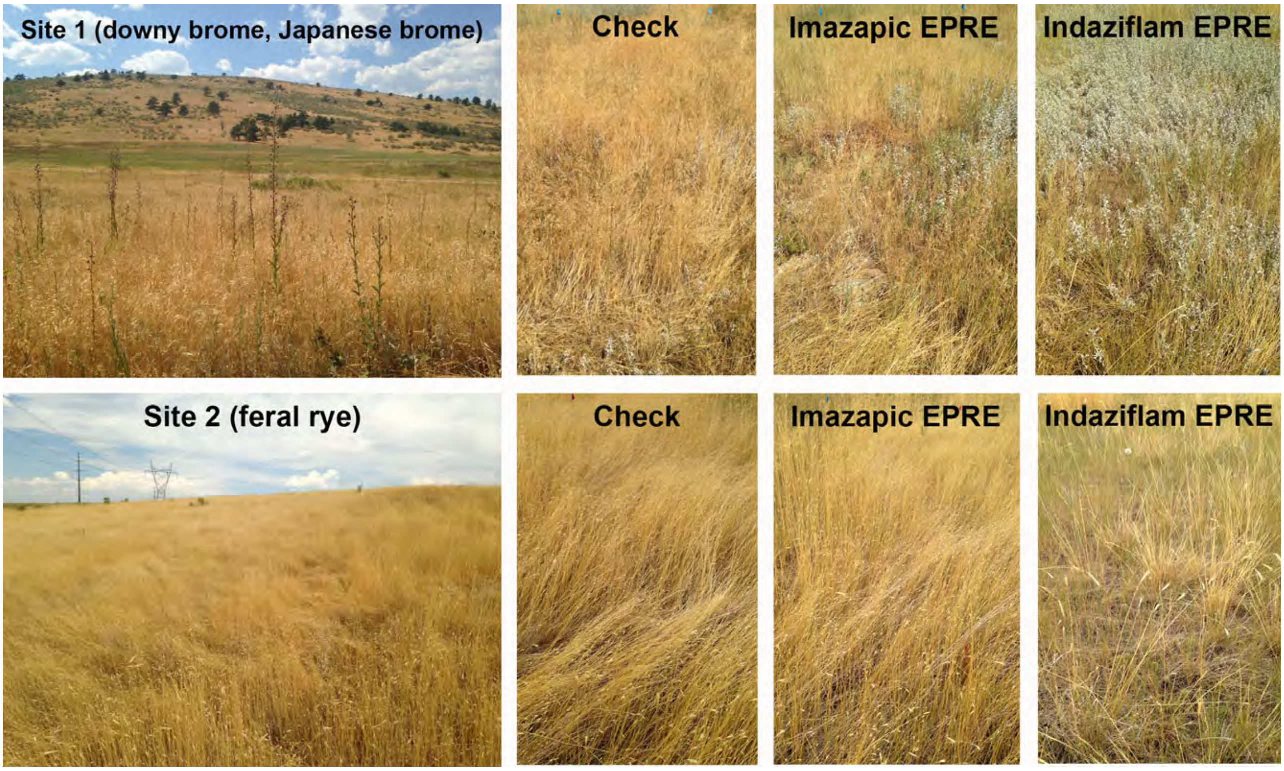

Figure 9. Photos of sites 1 and 2, taken in July 2016. Treatment photos include imazapic 144 g AI ha^{-1} and indaziflam 102 g AI ha^{-1} in July 2014, EPRE timing (2 YAT). Indaziflam treatments provided the long-term invasive winter annual grass control necessary for the re-establishment of co-occurring grasses and forbs.

ha^{-1}).[21,59] In addition, indaziflam has several other chemical properties that result in enhanced residual weed control: lipophilicity (log $K_{ow} = 2.8$), low water solubility (2.8 mg L^{-1}), no photodegredation and a moderate positive correlation between sorption and soil organic matter.[16,59] Therefore, lethal indaziflam concentrations are biologically available at the soil surface with sufficient moisture for plant uptake,[16] resulting in extended weed control. This response has been observed under several of indaziflam's labeled use patterns; however, there is limited supporting data in non-crop markets including indaziflam's new open space and natural areas label.

In this study, we provide the first field data showing that indaziflam can provide superior residual control of multiple invasive winter annual grasses (downy brome, feral rye, Japanese brome) compared with the currently recommended herbicide, imazapic. These data directly support the limited field[14,60] and greenhouse studies[34] that have been conducted evaluating the effectiveness of indaziflam to provide residual control of invasive winter annual grasses and other invasive biennial weeds[61] in open spaces and natural areas. Overall, indaziflam provided residual control 2 YAT, ultimately reducing the seed rain back into the soil seed bank. Because invasive winter annual grasses have seed viabilities of approximately 3–5 years,[56,62] land managers should consider applying a sequential indaziflam treatment 2 or 3 years after initial treatments potentially to exhaust the seed bank of these invasive grasses. The sequential treatments could provide the residual control necessary to reach the 3–5 year seed longevity period.[62] This management approach could reduce labor and herbicide costs compared with herbicides with limited residual control that require yearly applications (e.g. imazapic), while also minimizing the herbicide's environmental footprint.

An additional observation in this field study, associated with indaziflam's long-term residual control, was its utility as a tool for fine fuel reduction (Fig. 10). These fine fuel layers associated with invasive winter annual grasses have resulted in major changes in fire return intervals, dramatically increasing fire frequency and intensity,[38] particularly in sagebrush ecosystems of the Great Basin.[40,63] Additionally, many open spaces and natural areas infested with invasive winter annual grasses are bordered by houses or other structures and are at a high fire risk with these dense, highly flammable fine fuel layers. Additional research should be conducted to quantify fine fuel decomposition over time with other common invasive winter annual grasses found in the United States, including jointed goatgrass (*Aegilops cylindrica* L.), medusahead [*Taeniatherum caput-medusae* (L.) Nevski] and ventenata [*Ventenata dubia* (Leers) Coss]. Herbicide efficacy should also be compared between sites with no remaining fine fuel in recently burned areas (natural or prescribed) and non-burned sites.

This field study also provided much needed field tolerance data for the response of co-occurring grasses and forbs to herbicide treatments (Fig.10). Indaziflam promoted the re-establishement of the co-occurring plant community by increasing perennial grass and forb biomass and plant diversity (richness) over time. Imazapic at all application timings did not provide the necessary residual invasive winter annual grass control for re-establishment of co-occurring species, 2 YAT. Depleting the invasive winter annual grass soil seed bank and reducing fine fuel ultimately allowed the invaded sites to be converted from an annual-weed-dominated plant community to one that was primarily perennial dominated by natives. Across both sites evaluated in this study, indaziflam treatments promoted (released) the remnant perennial grass and

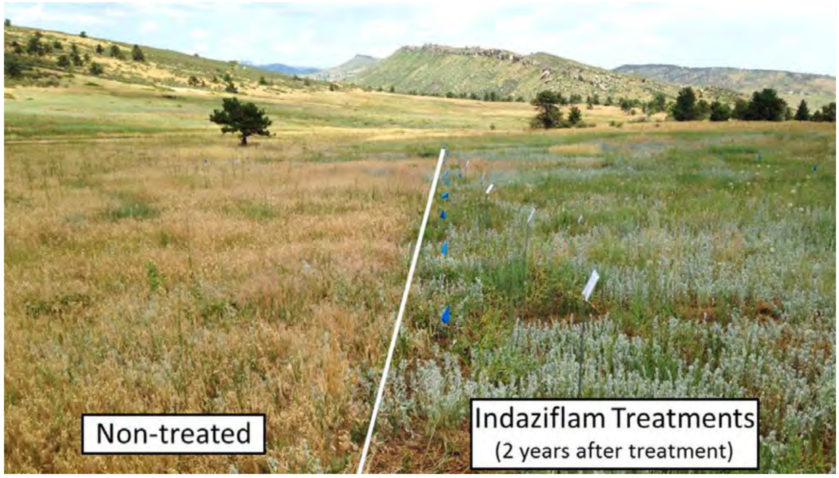

Figure 10. At Site 1, downy brome and Japanese brome non-treated (left) compared to indaziflam long-term selective control, release of desirable co-occurring species, and fine fuel reduction (right).

forb plant communities, and these sites are now more resistant and resilient to future invasions.[40]

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