IDAHO TRANSPORTATION DEPARTMENT

RESEARCH REPORT

Integration of Weed-Suppressive Bacteria with Herbicides to Reduce Exotic Annual Grasses and Wildfire Problems on ITD Right-of-Ways

RP 284

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16. Abstract

Invasion by exotic-annual grasses such as cheatgrass is impacting semiarid rangelands and especially transportation corridors, where it causes increased wildfire and many other environmental issues. Methods of reducing exotic annual grasses and restoring native perennials are needed, particularly testing of their intended target or unintended, non-target effects. In a series of experiments arrayed across different site and plant-community conditions on Idaho Transportation Department right-of-ways, the effects of chemical or biological herbicides, site preparation and co-treatments such as raking, and/or seeding were evaluated over 3 years. Strains of the soil bacterium *Pseudomonas fluorescens* that are supposedly weed-suppressive were generally ineffective, and resulted in relatively weak effects at a small proportion of plots and only at one site, but also resulted in highly undesirable non-target effects at another site. The chemical herbicides imazapic and especially indaziflam (Rejuvra) tended to have more consistent and stronger effects, and indaziflam furthermore provided a longer period of control, although additional years of observation would be required to assess its endurance. Seeding effects were weak, and preparation of seed beds through raking was not effective. In conclusion, indaziflam appeared to be the most effective tool for reducing cheatgrass, but techniques for increasing perennials after its application are needed.

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Data Availability

The data supporting the conclusions in this report is available on ScienceBase:
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Technical Advisory Committee

Each research project is overseen by a Technical Advisory Committee (TAC), which is led by an ITD project sponsor and project manager. The TAC is responsible for monitoring project progress, reviewing deliverables, ensuring that study objectives are met, and facilitating implementation of research recommendations, as appropriate. ITD's Research Program Manager appreciates the work of the following TAC members in guiding this research study.

- Project Sponsor: Steve Spoor
- Project Manager: Cathy Ford
- TAC Members: Alissa Salmore, Melinda Lowe, Wendy Terlizzi
- FHWA-Idaho Advisor: Brent Inghram

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List of Abbreviations and Acronyms

ALS	Acetolactate synthase
Cfu's	colony-forming units
cm	centimeters
DIMA	Database for Inventory, Monitoring, and Assessment
EAG	Exotic annual grass
EPA	Environmental Protection Agency
F	Fahrenheit
hrs	hours
HSB	Higher Elevation Sagebrush
ITD	Idaho Transportation Department
lb	pound
LBG	Lower Bunch Grass
LPI	Line Point Intercept
m	meters
mL	milliliters
MP	Milepost
oz	Ounce
TRT	treatment
USGS	U.S. Geological Survey
UTV	Utility Task Vehicle
WSB	Weed-suppressive bacteria

Executive Summary

Proliferation of cheatgrass and other exotic annual grasses such as medusahead and ventenata are a major environmental concern and operational problem for roadsides in Idaho. These annual grasses are highly flammable and they shorten fire-return intervals. Flammable vegetation is particularly hazardous in roadsides because of proximity to a ready source of ignition, and fires that start on roadsides can spread into adjacent public lands and urban communities with sprawling home development, causing extensive and expensive damage and degradation to wildlife habitat, rangelands, private or public property, utilities, etc. Thus, the Idaho Transportation Department (ITD) has a strong interest in preventing roadside vegetation from becoming invaded by exotic annual grasses and in improving vegetation on roadsides that are currently invaded, replacing exotic annual grasses with perennial bunchgrasses and forbs that support pollinators.

In this report, we describe roadside tests of various tools designed to reduce exotic annual grasses on the landscape, as well as techniques designed to improve the efficacy of these tools. These include the ACK55 strain of the bacterium *Pseudomonas fluorescens*, which was isolated to target cheatgrass, medusahead and jointed goatgrass (aka “weed-suppressive bacteria”, hereafter “WSB”). The scientific literature and previous ITD reports on this tool indicate that success in the field has been mixed. We also tested a relatively new pre-emergent herbicide, indaziflam (Rejuvra®) thought to remain active in soil for years after application. We tested these against and in combination with the “workhorse” pre-emergent herbicide most frequently sprayed by land managers for exotic annual grass control, Imazapic (Plateau®).

There was considerable variability in treatment effects among sites, particularly for WSB treatments. The lack of consistency in WSB effects in this study and in previous studies suggests that WSB are not yet ready to be used at an operational scale for exotic annual grass control, but that further experimentation may be warranted.

Imazapic and indaziflam both generally reduced exotic annual grass cover, with indaziflam having delayed but more extended effects on exotic annual grasses but also inhibiting a native forb seeded in the year after spraying.

1. Background and Objectives

The need for exotic annual grass control on Idaho's roadsides – the cheatgrass fire cycle

Upland landscapes such as the shrub and grasslands in sagebrush steppe of Idaho and much of the surrounding western United States are increasingly imperiled by invasion of exotic annual grasses (EAGs) such as *Bromus tectorum* L. (cheatgrass) and medusahead (*Taeniatherum caput-medusae*). These invasive grasses produce continuous mats of fine fuels that are green for a much shorter interval in early spring than the native perennials they displace. The annual grass fuels are more easily ignited, are more combustible, and they pass fire more readily across the landscape than perennial vegetation (D'Antonio and Vitousek 1992, Germino et al. 2016). The increases in wildfire often exceed the adaptive capacity of native or naturalized perennials, and invasion obliterates ecosystem structure and functionality to the detriment of nearly all human, livestock, recreation, or wildlife uses including pollinators (Germino et al. 2016).

Transportation corridors have facilitated the spread of cheatgrass and other invaders for over a century (Bangert and Huntly 2010), and in turn, the invasions and the wildfire disturbance caused by the invaders is a problem for roadway safety and operations. Fires that start in right-of-ways can also quickly spread to adjacent lands, causing extensive and expensive damage to wildlife habitat, rangelands, private or public property, utilities, etc. Thus, ITD seeks to prevent invasion where it has not yet occurred and to reduce exotic annual grasses and increase forbs that support pollinators and perennial bunchgrasses invaded sites.

Pre-emergent herbicides

The most compelling way to reduce annual invaders where native vegetation is perennial is through use of herbicides, especially those that are considered "pre-emergent" because they inhibit germinating seeds or emerging seedlings. Species that are annual must have regular germination every year or nearly so, for populations to persist, depending on seed longevity, which is generally perceived to be 2-3 years for cheatgrass. Pre-emergent herbicides are applied with the hope that reducing competition from exotic annual grasses will better allow for the expansion natives that are already present or have been seeded or planted, though timing seeding relative to pre-emergent herbicide application needs to be done thoughtfully. Moreover, growth of perennials towards a size and vigor that can sustain their presence and possible competition with exotic annual grasses can take several years to a decade, depending on weather and especially precipitation following treatment. The persistence and duration of herbicide effects is a key consideration in treatment plans.

Imazapic, the active ingredient in products such as Plateau® and Panoramic®, is the most used pre-emergent herbicide in sagebrush steppe ecosystems. Its mode of action is inhibition of the acetolactate synthase (ALS) enzyme, which is needed to produce certain branched-chain amino acids necessary for plant growth. Imazapic is thought to be non-toxic to non-target organisms that do not have ALS (i.e., mammals and insects, Tu and others 2001, but see Bourdineaud 2022). In addition to preventing seedling growth after germination, imazapic also has some post-emergent action, damaging or killing newly germinated exotic annual grasses (Mangold et al. 2013, Applestein et al. 2018).

Many studies have shown no effects of imazapic on native perennial grasses (e.g., Monaco et al. 2005, Elseroad and Rudd 2011, Kyser et al. 2013, Davis 2017, Applestein et al. 2018, Davies and Hammerlynck 2019). Some studies have shown increases in bunchgrasses after successful reduction of exotic annuals (i.e., releases, Davies and Sheley 2011, Monaco et al. 2017), and a couple of studies have shown damage to native perennial grasses (Shinn and Thill 2004, Pyke et al. 2014), though one of these was a spring application with an adjuvant to enhance imazapic uptake that was applied where perennials were relatively young (fall applications are much more common). Longevity of imazapic effectiveness against exotic annual grasses varies among studies and has been reported as three years (Pyke et al., 2014), 2–3 years (Kyser et al., 2013) or 1–2 years (Morris et al., 2009), though we have observed effects for as long as 4-6 years after treatment for imazapic applied after fire with a high carrier volume (100 gallons/acre, Lazarus and Germino 2022).

Indaziflam, the active ingredient in the relatively new herbicide Rejuvra®, is a true pre-emergent, having relatively little documented post-emergence action (Brosnan et al. 2012). Its mode of action is the inhibition of cellulose biosynthesis (Brabham et al. 2014), which inhibits cell wall formation, cell division, and cell elongation (Kaapro et al. 2011). Mature plants with fully developed leaves, tissues, and organs have already completed cell wall formation and are thus not affected or only slightly affected relative to germinating seeds and very young rapidly growing plants according to one source (Kaapro et al. 2011). However, a study in sagebrush steppe at Yellowstone National Park found indaziflam reduced both native annual and perennial forbs while not affecting perennial graminoids (Meyer-Morey et al. 2021). Another Wyoming study showed indaziflam reduced native seed bank richness and density, though not sagebrush-grassland plant diversity (Courkamp et al. 2022b). Native grass biomass increased 3-4 fold with indaziflam in a community with remnant native vegetation (Clark et al. 2020). Indaziflam is believed to be active for more years after application to soil than imazapic, but because it is newer, fewer longer-term tests in a variety of plant communities have been completed (see Donaldson and Germino 2022 and Clark et al. 2020 for 3-year evaluations and Courkamp et al. 2022a for a 5-year evaluation). More information is also needed about potential non-target effects of indaziflam, and because it is relatively expensive (~5x more than imazapic), comparison of repeat spraying of imazapic is also needed.

Weed-suppressive bacteria

Bioherbicides or weed-suppressive bacteria are another proposed management tool for reducing exotic annual grasses and have received attention because they were expected to provide a non-chemical and enduring soil agent to selectively inhibit annual grasses. Strains of the ubiquitous and diverse soil bacterium *Pseudomonas fluorescens* were isolated with the intent of applying them in field settings to selectively inhibit growth of target exotic annual grasses without harming desirable perennial grasses or other biota (Kennedy et al., 1991; Kennedy, 2018). Strain ACK55 was one of > 7000 bacterial strains isolated from soils sampled in early spring from areas where grass growth appeared stunted. Isolates that inhibited root growth of cheatgrass, medusahead, and jointed goatgrass, but not winter wheat or the native perennial bluebunch wheatgrass (*Pseudoroegneria spicata*) in petri plate agar bioassays were further tested in growth chamber and field settings (Kennedy 2018). No research has been undertaken on active ingredients or mechanisms of action for ACK55. Instead, the patent application and the Environmental Protection Agency (EPA) approval application have relied on the research conducted with the previously isolated strain D7, which produced a complex of chromopeptides and fatty acid esters in a lipopolysaccharide matrix (Gurusiddaiah et al., 1994) that inhibited root growth of *Bromus tectorum* via disruption of lipid synthesis and membrane integrity (Tranel et al., 1993). Possible selectivity of WSB for root damage in annual grasses over perennial grasses could result from annual grasses' weakly developed Casparian strip (Harris 1977), a waxy suberin cell layer within roots that limits the movement of molecules and microbes from soil into roots.

Three *P. fluorescens* strains, including ACK55, steadily reduced populations of the target exotic annual grasses in invaded pastures and grassland ecosystems to near 0% of control after 5 years and maintained that control through 7 years after application when desirable plants were present (Kennedy, 2018). However, Kennedy's 2018 results have not been reproduced in other field studies, where WSB treatments produced inconsistent exotic annual grass control (Lazarus et al., 2020) or no exotic annual grass control whatsoever (Reinhart et al., 2020; Germino and Lazarus, 2020; Pyke et al., 2020; Tekiela, 2020). Kennedy's 2018 laboratory agar bioassay results with respect to selectivity of ACK55 for target exotic annual grasses were also not reproducible using the methods described in that publication, though partial selectivity with earlier methods (Kennedy et al. 2001) has been reproduced (Lazarus et al. 2021).

In September 2020, the EPA approved strain ACK55 for use as a bioherbicide, and it was exclusively licensed and is being produced and sold by BioWest Ag Solutions as "Battalion Pro®".

General project objectives

In the projects described here, we applied emerging tools for exotic annual grass control – weed-suppressive bacteria and the pre-emergent herbicide indaziflam (Rejuvra®) – combined with or compared to imazapic (Plateau®). We did this at roadside sites that varied in climate and soils (i.e., Snake River Plain and Northern Basin and Range ecoregions). The sites also varied in plant communities, partly due to location but also to level of disturbance; some roadside sites such as freeway interchanges had undergone major re-shaping, while other right-of-way sections were relatively undisturbed or had burned in the 2015 Soda Wildfire.

Our objective was to test the efficacy of these tools on target exotic annual grasses, quantify the longevity of effects, and document any non-target effects. In a heavily disturbed site that was severely invaded and had a thick layer of exotic annual grass thatch and few native grasses and shrubs, we tested litter removal by raking after mowing as a method of getting spray treatments into better contact with soil. In an effort to increase the native plant component of the site, including forbs for pollinators, we drill seeded one year after spraying and asked whether spray treatments 1) allowed seeded plants to grow better by eliminating competition or 2) prevented seeded plants from germinating.

We opportunistically added additional plots in 2020 when given the opportunity to use Battalion Pro®, BioWest's most recent WSB product that contains the ACK55 strain. With those additional plots, our objectives were to observe the overall efficacy of Battalion Pro® alone and in combination with imazapic and to test the hypothesis that serial applications of weed-suppressive bacteria better control exotic annual grasses than single applications.

A map including all treatment locations is shown in Figure 1.1.

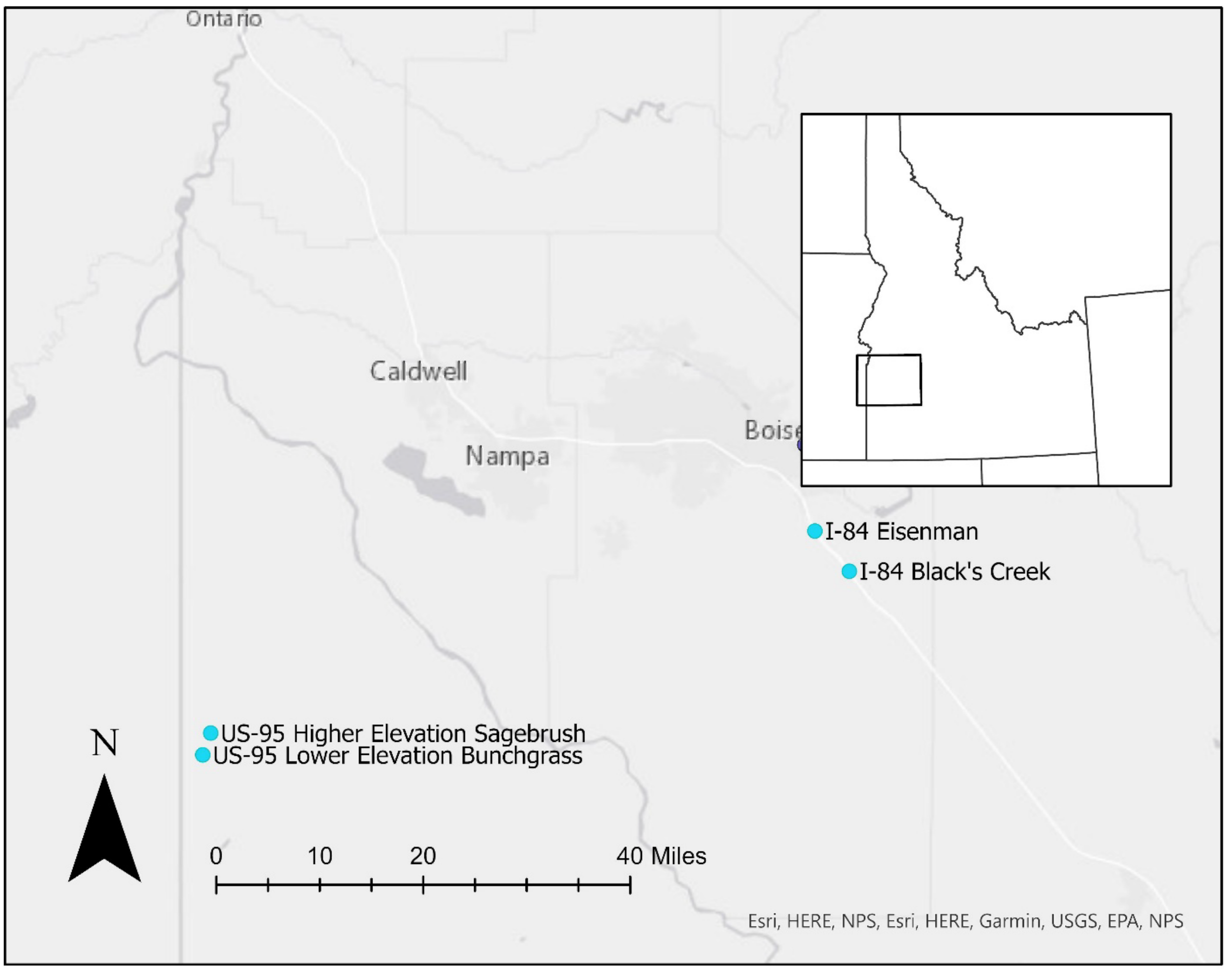

Figure 1.1 Map of all sites.

Precipitation during the study period

Precipitation patterns at the Snake River Plain sites (I-84 Blacks Creek and Eisenman) showed similar seasonality to but overall greater precipitation than the Northern Basin and Range (US-95) sites (Figures 1.2-3).

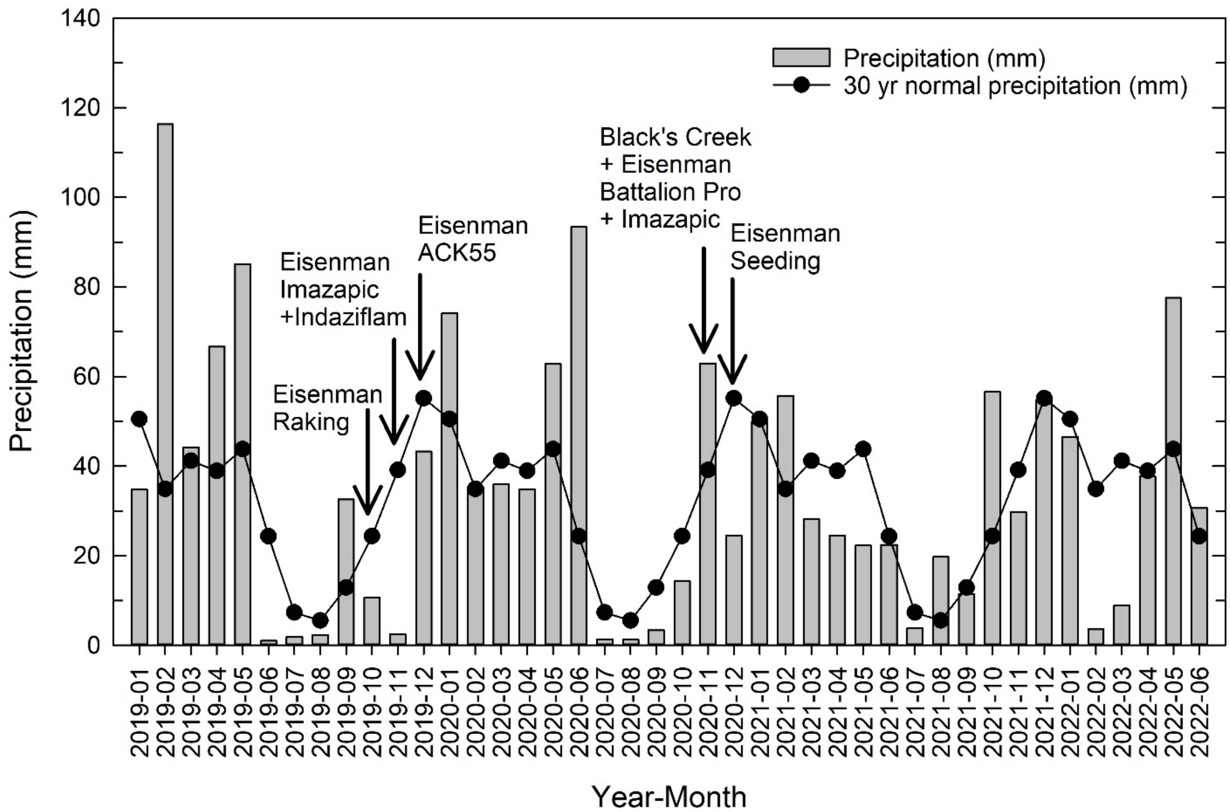

Figure 1.2 Precipitation at I-84 Black's Creek and Eisenman during the study period compared with 30-year (1981-2010) normal precipitation. Data are from 4km resolution gridded images (PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, accessed 2 Dec 2022). Timing of treatments is indicated with arrows. Treatments are described in sections 2 and 4.

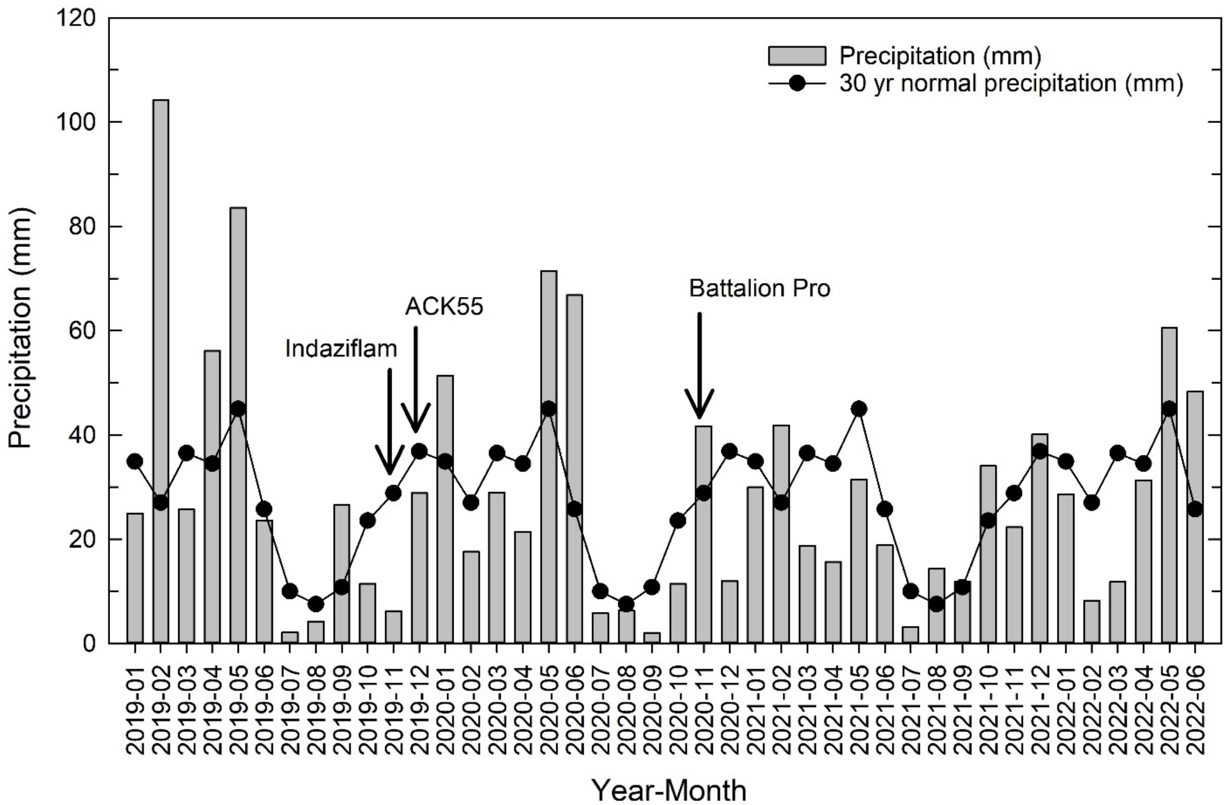

Figure 1.3 Precipitation at US 95 during the study period compared with 30-year (1981-2010) normal precipitation. Data are from 4km resolution gridded images (PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, accessed 2 Dec 2022). Timing of treatments is indicated with arrows. Treatments are described in sections 3 and 4.

2. Interstate-84 (I-84), MP59 Eisenman 2019 – few natives, high level of intervention

Site description, specific project objectives

Located on the eastern edge of Boise in the Snake River Plain (Figure 1.1), the I-84, Milepost (MP) 59 Eisenman interchange is a highly-disturbed and engineered site with a substantial cheatgrass and exotic forb component as well as a planted non-native perennial bunchgrass component that consists of crested wheatgrass and sheep fescue. Prior to treatments, bunchgrass density averaged 1.825 ± 0.22 individuals/m^2 but was somewhat patchy, and native forbs and shrubs were rare to absent.

At this site, we tested ACK55, indaziflam, and imazapic. We also experimented with combinations of treatments – imazapic+indaziflam, and ACK55+imazapic+indaziflam. Because this site had a thick layer of exotic annual grass thatch, few native grasses, and no shrubs, we tested litter removal by raking after mowing as a method of placing spray treatments into better contact with soil. In an effort to increase the native plant component of the site, including forbs for pollinators, we drill seeded one year after spraying and asked whether spray treatments 1) allowed seeded plants to germinate and grow by eliminating competition or 2) prevented seeded plants from germinating.

Project installation/treatment application

The location of treatment blocks within the site is shown in Figure 2.1, and the spatial patterns in which treatments were applied is depicted in Figure 2.2, with spray treatments running east-west and raking and seeding treatments running north-south such that every possible combination of raking, spraying, and seeding occurred once in each of three replicate blocks. Plots were 30’x30’, the width of a typical truck spray boom.

The entire study area was mowed to approximately 6-8 inches in September 2019 by ITD. In October of 2019, USGS staff raked away litter and thatch (a combination of cuttings from mowing and what occurred naturally) from half of each treatment block, exposing the soil surface (Figure 2.3). On 11/1/2019, Ada County Weed and Pest sprayed imazapic (Plateau®) at a rate of 6 oz/acre with the adjuvant methylated seed oil (.25% v/v) and indaziflam (Rejurvra®) at a rate of 5 oz/acre with no adjuvant. Carrier volume was 30 gallons/acre for both chemicals. They returned on 12/6/2019 to spray ACK55 at a rate of 8.0×10^8 cfu/m^2. Weather was partly sunny with light wind temperature of 38°F. Rain fell that evening and more rain fell the following day. ACK55 was raised by United States Geological Survey (USGS) staff in a laboratory at Boise State University (see Appendix A).

The study area was mowed by a private contractor on 11/20/2020 in preparation for seeding. Drill seeding was completed 12/7/2020 with a Land Pride overseeder with drill blades set 2 inches apart and a depth of 0.5 inches (Figure 2.4). The seed mix included native perennial grasses and forbs (Table 2.1).

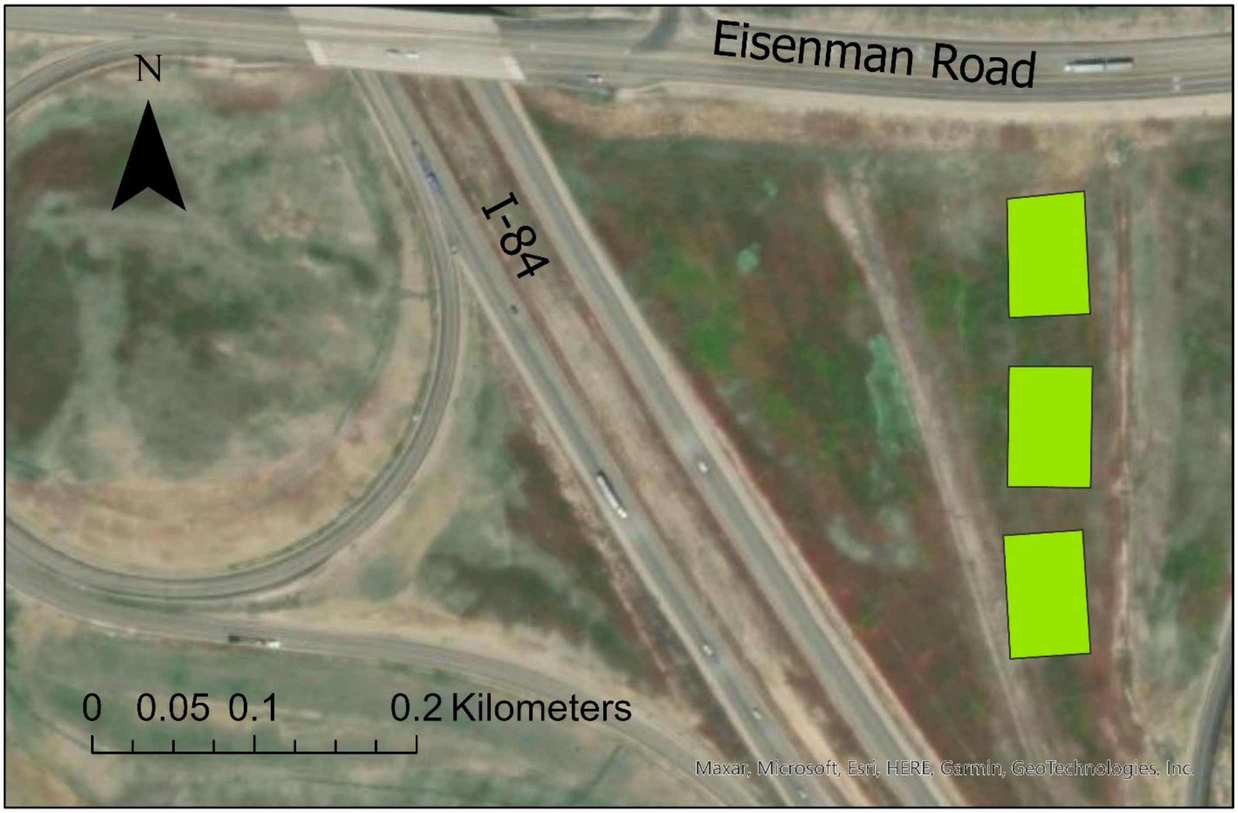

Figure 2.1 Aerial Map of the I-84 Eisenman interchange showing the locations of approximately 1/2 acre treatment blocks. See Figure 2.2 for treatment patterns within the blocks.

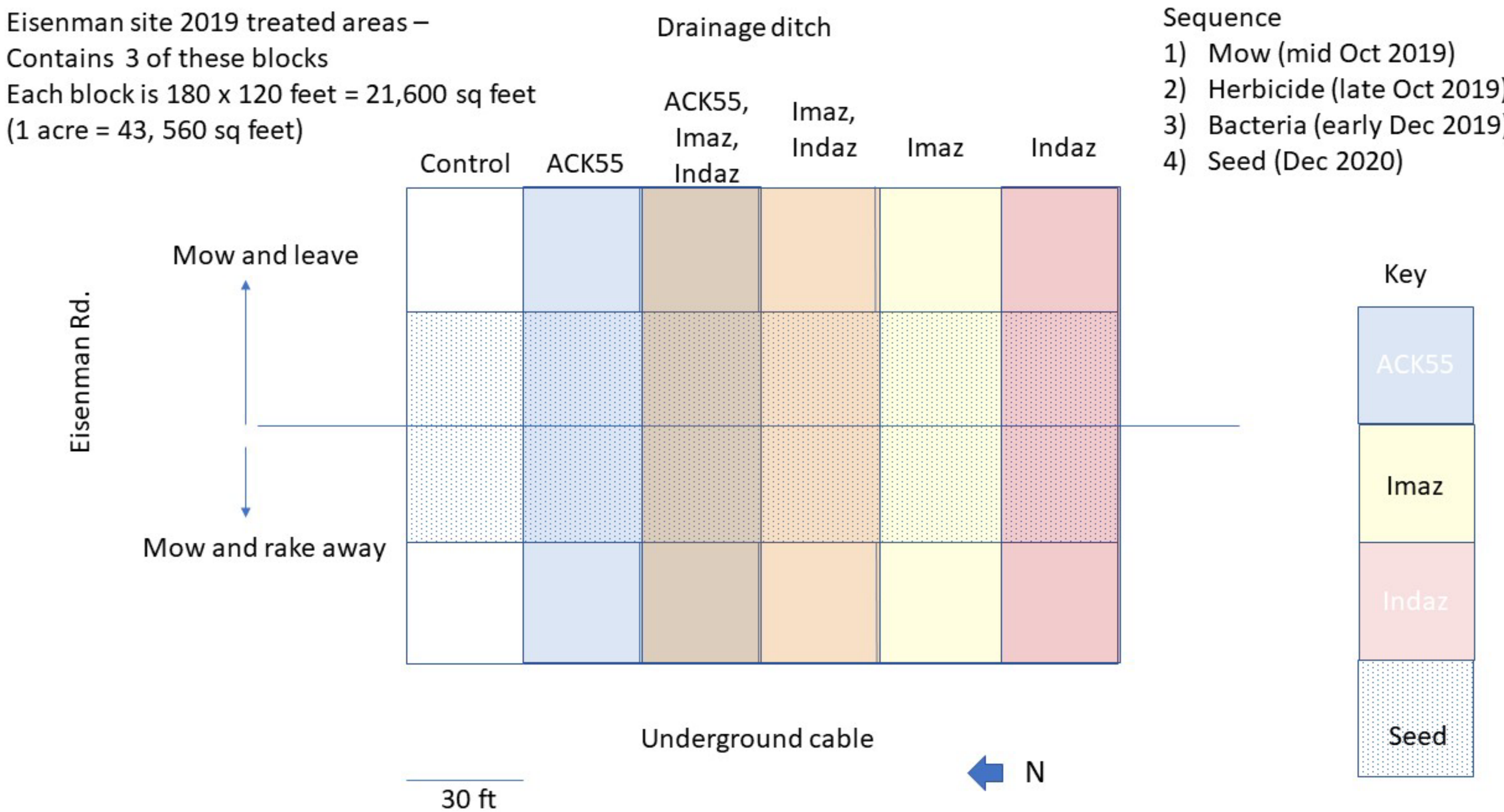

Figure 2.2 I-84 Eisenman 2019 treatment configuration. Imaz = imazapic. Indaz=indaziflam.

Figure 2.3 I-84 Eisenman site looking south just after the raking treatment had been completed. The area to the right of the pink marking whisker was raked, and the area to the left was not raked.

Figure 2.4 I-84 Eisenman site looking south shortly after the seeding treatment was completed.

Table 2.1 Eisenman seed mix, applied in fall of 2020 at a rate of 31.65 pounds live seed/acre

Species/Provenance	**Supplier**	**Pounds**
Elymus elymoides (squirreltail) Roaring Springs	BFI	4
Festuca ovina (sheep fescue) VNS	Comstock	3
Poa secunda (Sandberg bluegrass) Snake River Plain	BFI	5.5
Psuedoroegneria spicata (Bluebunch wheatgrass), Sheepshead Mtn.	BFI	7.5
Achillea millefolium (yarrow) Eagle	BFI	0.25
Aseclepias speciosa (milkweed) Snake River Seeds	Snake River Seeds	0.5
Machaeranthera canescens (Hoary tansyaster), Simco Rd./Warm Springs pollinator garden	Collected by USGS staff	0.29
Sanguisorba minor (small burnet) Delar	Comstock	2
Sphaeralcea munroana (Munro's globe mallow), Red Mountain	BFI	0.5
Grindelia squarrosa (Curlycup gumweed), Simplot soccer complex	Collected by USGS staff	0.20
Rice Hulls (2 lbs)	BFI	
Total		**23.74**

Data collection and analysis

Prior to treatments, we counted the number of bunchgrasses in a 13 x 0.5 m belt transect running diagonally across the entirety of each plot to detect any pre-treatment plant community differences among plots. Treatment responses included measurements of plant cover, density, and other traits. Plant cover was measured at the time of peak biomass in spring (May) of 2020, 2021, and 2022 with the Line Point Intercept (LPI) technique. This entailed recording the plant cover by species at 0.25 m intervals along two 13-m diagonal transects per plot, using a thin metal pin positioned vertically at each interval to ensure precision. More than one species could be recorded at a point. Data were collected directly into a DIMA database (Database for Inventory, Monitoring, and Assessment; jornada.nmsu.edu/monit-assess/dima). We also measured density (individuals/m^2) of newly germinated exotic annual grasses, annual forbs, and perennial forbs in the fall of 2020 and 2021 in two 1x1 m quadrats per plot.

To test effects of spray treatment, raking, and seeding on plant cover values, we used generalized linear mixed models with a beta distribution and logit link (glmmTMB, R, Brooks et al. 2017). Fixed effects were a full factorial of spray treatment x raking x seeding x year, and random effects were plot (because we measured the same plots in multiple years) and block. For variables where there was a significant correlation between cover and pre-treatment perennial bunchgrass density (exotic annual grass cover, perennial grass cover), we included pre-treatment perennial bunchgrass density as a co-variate, which we first scaled by subtracting the mean and dividing by the standard deviation. Because seeding had not yet occurred when we collected our first cover data in spring 2020, we first ran this model without the 2020 data and we discovered that seeding had no effect on cover in 2021 and 2022 (indeed, few seeded species emerged). Thus, we report the results of a simplified model that does not include seeding but therefore can include all three years of cover data (i.e., those collected both before and after seeding).

For density of newly emerged exotic annual grass seedlings (collected in fall 2020 and fall 2021) we used separate models for each year because seeding had not yet occurred in fall 2020. For each year individually, we used a generalized linear model with a negative binomial distribution and log link. For 2020, fixed effects were spray treatment, raking, and spray treatment x raking. For 2021, fixed effects were a full factorial of spray treatment x raking x seeding. For both years, treatment block (see Figure 2.1) was included as a random effect, and we also included the scaled pre-treatment perennial bunchgrass density as a covariate.

Results

Cover

Exotic annual grasses (EAG) cover was strongly affected by spray treatment, though spray treatment effects varied from year to year. Effects of raking on EAG cover varied with spray treatment. Pre-treatment perennial bunch grass density was also a strong predictor of EAG cover (Appendix B, Table 1). More specifically, EAG cover was not affected by ACK55 alone. Imazapic reduced EAG cover in the first year after treatment only. Treatments containing indaziflam reduced EAG cover more in the second and third years after treatment than in the first year (Figure 2.5), likely because indaziflam has no post-emergence effectiveness and cheatgrass had already begun to germinate when we sprayed in November of 2019.

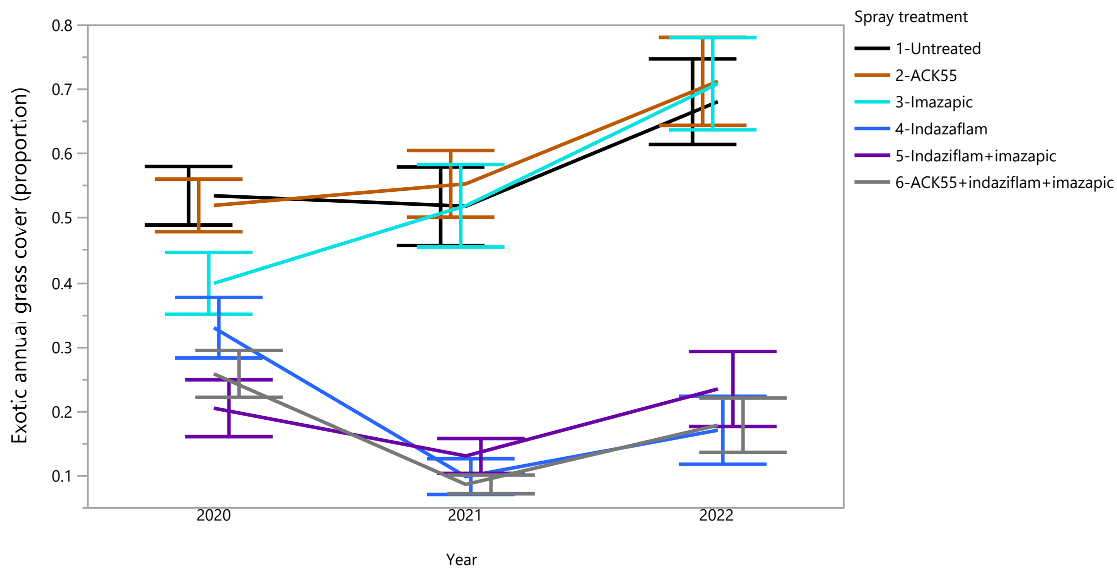

Figure 2.5 Mean (±SE) exotic annual grass cover for six treatments across three post-treatment years at the Eisenman site.

The intention of raking was to increase the contact of the herbicides and bacteria sprayed with soil surfaces and thus potentially improve the performance of the spray treatments. However, raking increased exotic annual grass cover, particularly in plots where the spray treatment did not contain imazapic (Figure 2.6). One possible explanation for this observation is that cancellation effects occurred between possible raking benefits (better contact between spray treatment and soil) and possible raking drawbacks (micro tillage that promotes cheatgrass germination) in plots where imazapic was present. Imazapic has both pre- and post-emergence action, while indaziflam has only pre-emergent action. Thus, increased cheatgrass germination likely caused by raking may have been countered somewhat by the post-emergent action of imazapic, while treatments without such post-emergent action were not able to counter the increased cheatgrass germination and thus show an increase in EAG cover with raking.

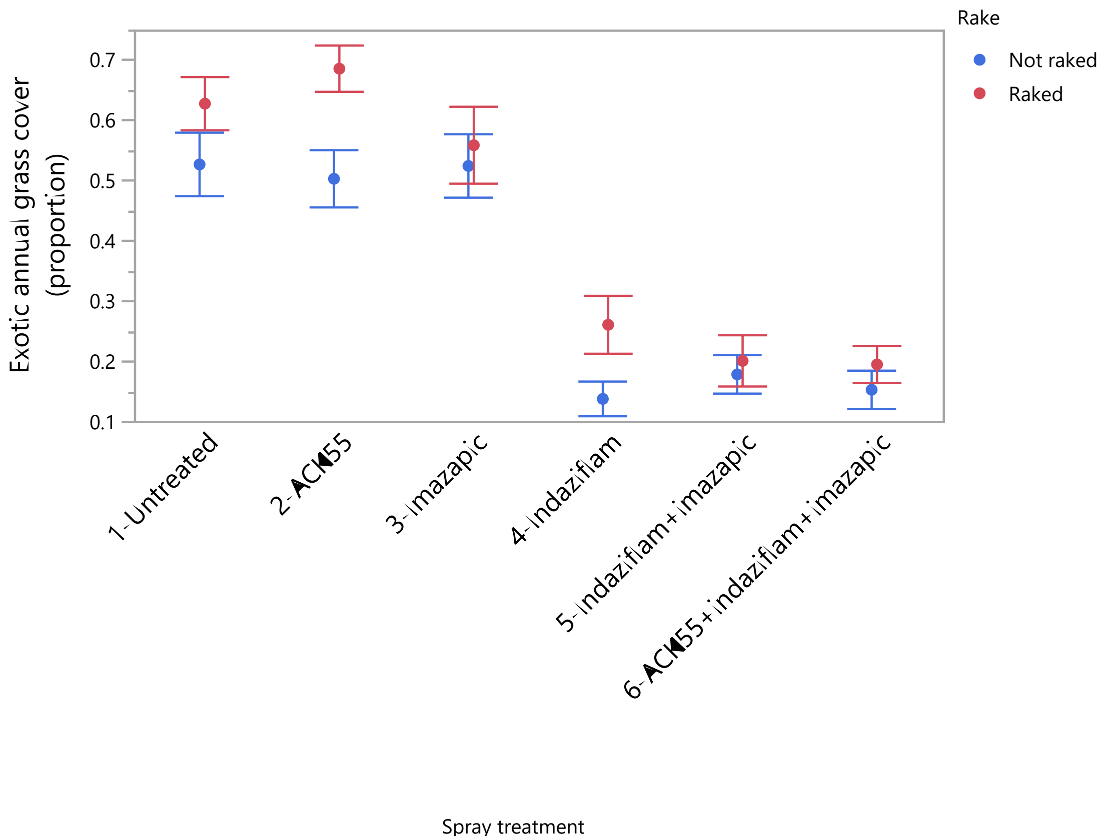

Figure 2.6 Mean (±SE) exotic annual grass cover by raking and spray treatment (years are averaged for this plot).

Fall green-up density

In 2020, density of new exotic annual grass seedlings was lower in treatments that included indaziflam and otherwise unresponsive to any other treatment (Figure 2.7, Appendix B, Table 2). In fall 2021, the year after seeding, density of exotic annual grass seedlings was greatest of all sampling years but was reduced where spray treatments included indaziflam, and higher in plots that were raked and seeded (Figure 2.8, Appendix B, Table 3).

Of all the species seeded, only Hoary tansyaster (*Machaeranthera canescens*) was observed during fall 2021 germination counts (though the individuals observed had likely germinated the previous spring). Hoary tansyaster was detected almost exclusively in seeded plots and was rare or absent in plots sprayed with indaziflam, indicating that indaziflam likely prevented germination or growth of this species at this site (Figure 2.9).

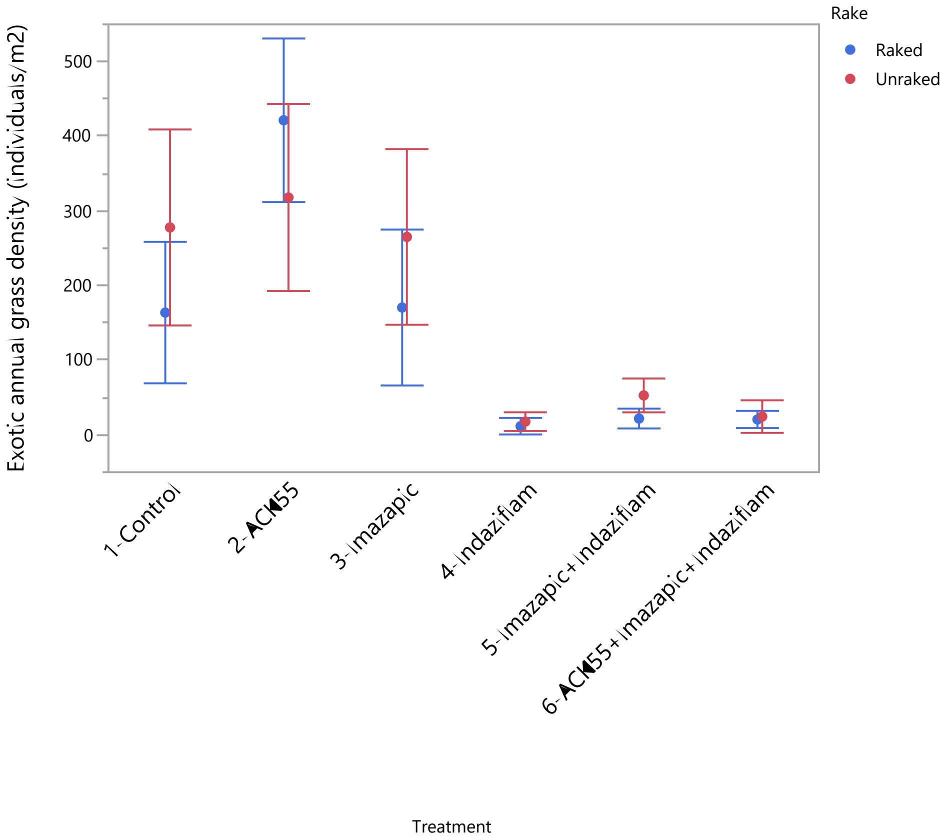

Figure 2.7 Mean (±SE) of exotic annual grass germinant density across spray and raking treatments, collected in fall 2020.

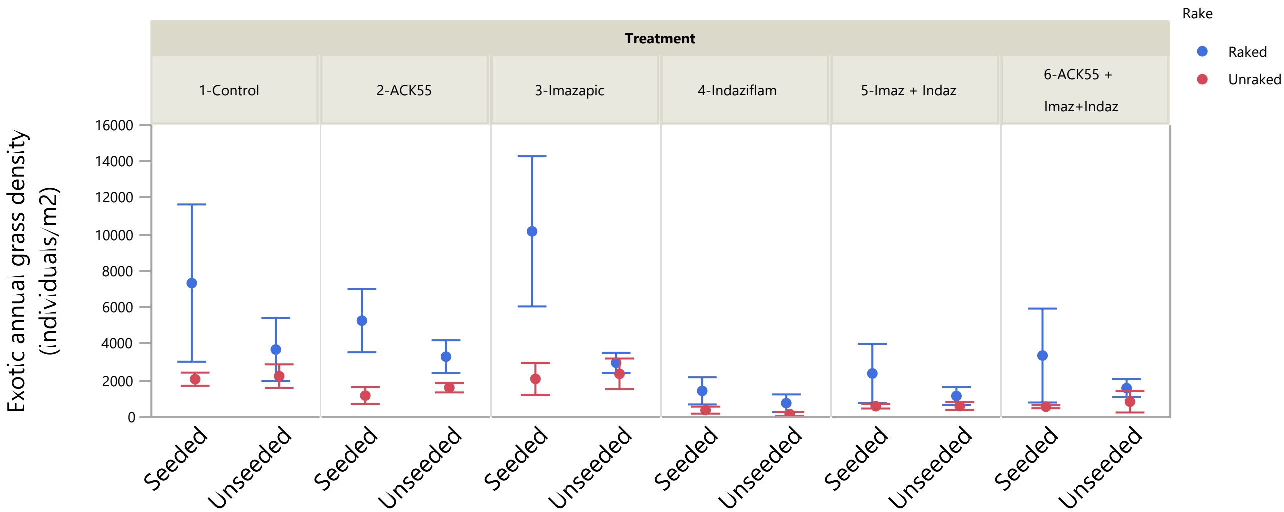

Figure 2.8 Mean (±SE) of exotic annual grass germinant density across spray and raking, and seeding treatments, collected in fall 2021. Note considerably higher y axis values in this plot than in the previous plot. Imaz = imazapic, Indaz = indaziflam.

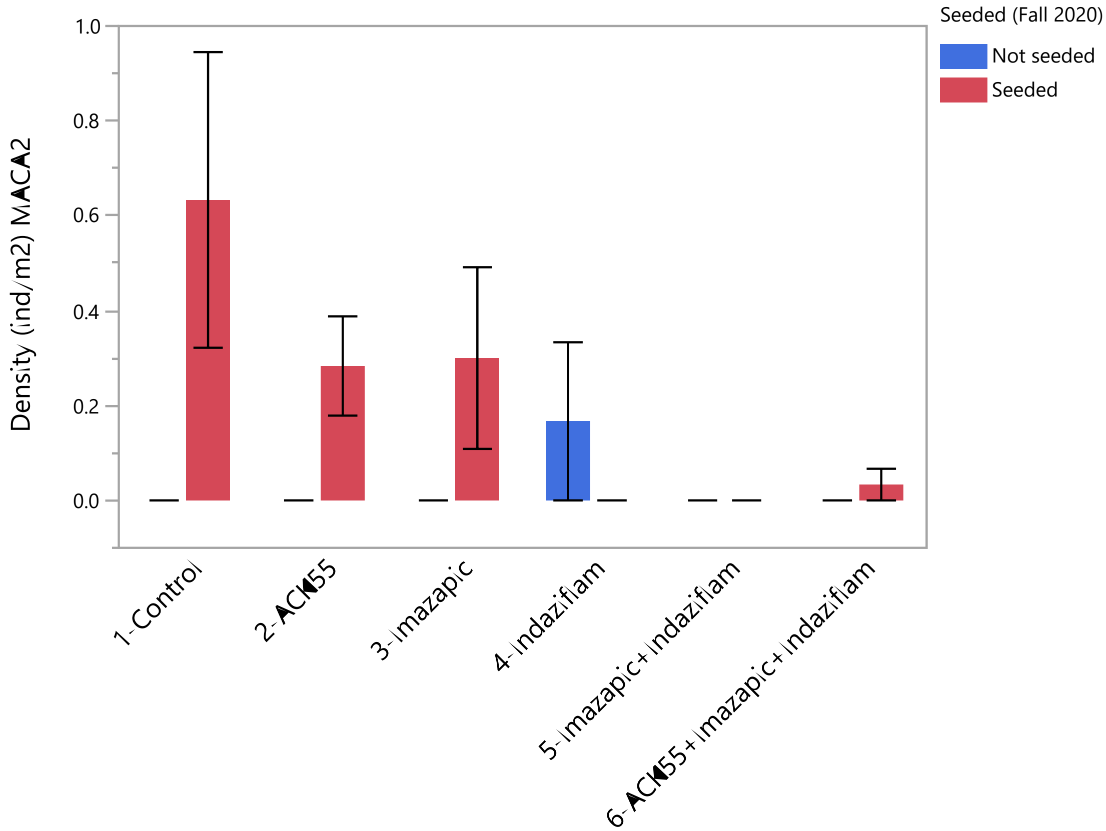

Figure 2.9 Mean (±SE) of Hoary tansyaster (*Machaeranthera canescens*) density across spray and seeding treatments, collected in fall 2021. This is the only seeded species we have observed to this point.

3. US-95 2019 – more natives (incipient invasion), lower level of intervention

Site description, specific project objectives

These sites are located on right-of-ways along US-95 on near the Oregon border (Figure 1.1). Although both sites are inside the footprint of the 2015 Soda wildfire, the "Higher elevation sagebrush" site is a remnant unburned mature sagebrush stand with a native perennial bunchgrass and forb component as well as bare soil/crust and only a minor exotic annual grass component (~7±1% cover, incipient invasion, Figure 3.1). The "Lower elevation bunchgrass" site burned in the Soda fire but is in relatively good ecological condition – it is dominated by native perennial bunchgrasses (mostly bluebunch wheatgrass) but also includes young sagebrush, native forbs, and an exotic annual grass component that is greater than that of the higher elevation sagebrush site (17±1.2%, Figure 3.2). Neither site received any post-fire seeding, but both are close enough to the spray treatment boundary (~6 m) to have potentially received some herbicide drift in fall 2016 (6 oz/acre imazapic applied by helicopter), though this would likely have worn off by fall 2019. Because these sites already included a substantial native perennial component, we did not attempt to augment it by seeding or planting, and we did not think the disturbance of mowing or raking was appropriate. Instead, at these sites, we tested the idea that a "spray and release" treatment could improve site conditions, reducing exotic annual grass cover and thereby "releasing" native perennials.

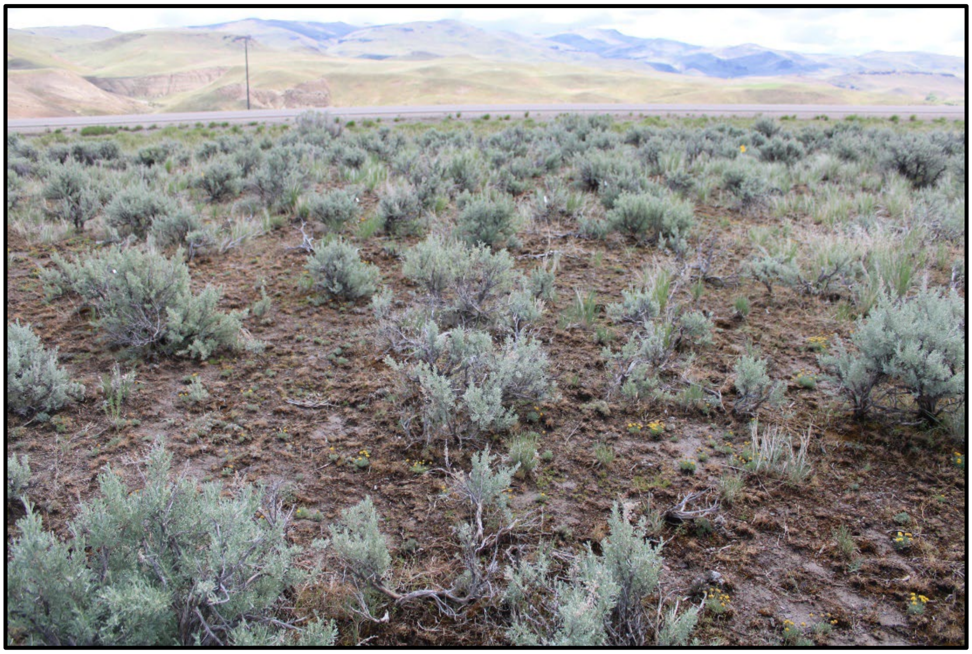

Figure 3.1 US-95 "Higher elevation sagebrush" site.

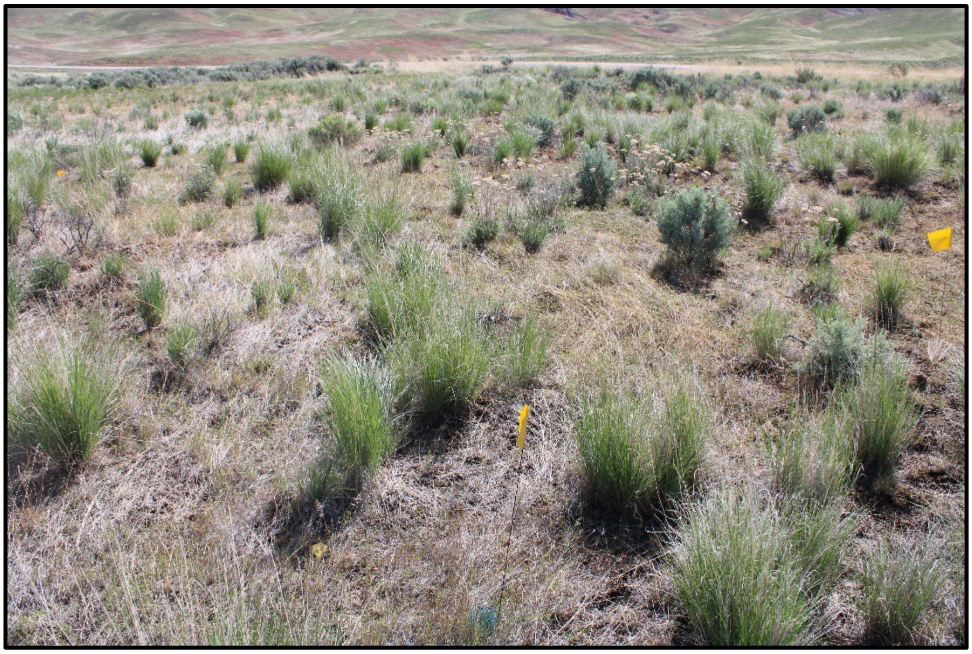

Figure 3.2 US-95 Lower elevation bunchgrass site.

Project installation/treatment application

The spatial configuration in which treatments were applied is depicted in Figure 3.3. Plots were 10 m x 10 m. On 10/25/2019, a private contractor sprayed indaziflam (Rejurvra®) at a rate of 5 oz/acre with no adjuvant using a UTV-mounted sprayer (Figure 3.4). Carrier volume was 60 gallons/acre. They returned on 12/5/2019 to spray ACK55 at a rate of 2.45×10^9 cfu/m^2 (note that this was approximately 5x the target rate of 5×10^8 cfu/m^2 due to shifts in concentration during storage, see discussion) using a pressurized hose attached to a tank and spraying at 130 gallons/acre carrier volume (Figure 3.5). Weather was partly cloudy with no wind and a temperature of approximately 36°C. Both elevations had 0.25-0.5 inches of snow on the ground in a discontinuous cover (approximately 50% of plot). Rain and/or snow fell on the evening of December 6 and December 7. ACK55 was raised by USGS staff in a laboratory at Boise State University (see Appendix A).

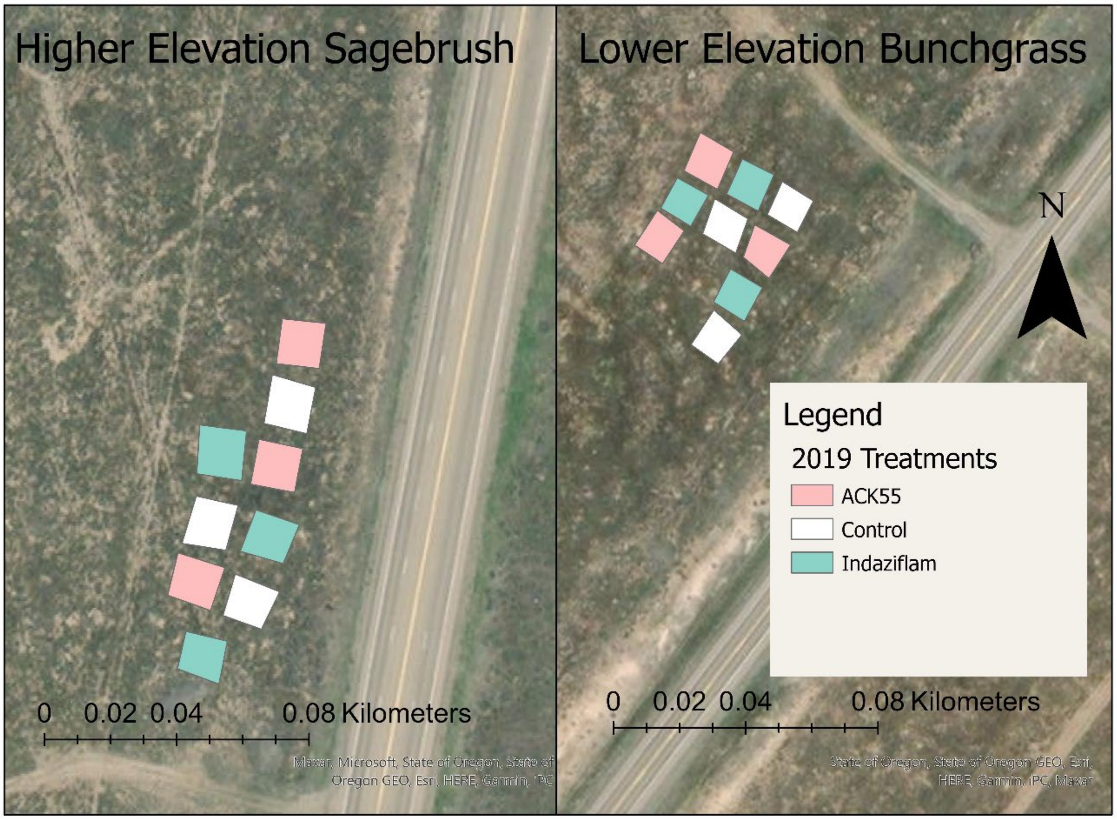

Figure 3.3 Treatment configuration for US-95 research plots.

Figure 3.4 Private contractor spraying indaziflam in October 2019 with UTV-mounted sprayer.

Figure 3.5 Private contractor spraying ACK55 in December 2018 with pressurized hose. Blue spray dye is visible on a sprayed plot.

Data collection and analysis

Prior to treatments we performed a rapid vegetation monitoring effort. We recorded the number of times a pin dropped every half meter along a 14 meter transect tape stretched diagonally from the northwest to the southeast corner of each plot touched an exotic annual grass (i.e., like the LPI technique but recording only presence/absence of exotic annual grasses rather than all species). At the higher elevation sagebrush site, we counted the number of perennial bunchgrass and sagebrush individuals that touched this tape. At the lower elevation bunchgrass site, we counted the number of perennial bunchgrass individuals that touched this tape and the total number of sagebrush individuals in each plot (because there were not enough sagebrush individuals at this site to get a good estimate using the method from the higher elevation site)

We measured post-treatment plant cover at peak biomass in spring (June) of 2020, 2021, and 2022 with the LPI technique. This involved dropping a straight pin at 0.25 m intervals along two 14-m diagonal transects per plot and recording each species that touched the pin. Data was collected and directly entered into a DIMA database. We also measured density (individuals/m^2) of the native perennial forb yarrow that was greening up in the fall of 2020 and 2021 in five 1 m^2 quadrats per plot. In addition, we selected 10 big sagebrush (*Artemisia tridentata)* individuals in a spatially-dispersed random fashion in each plot and measured length of new growth on 5-randomly-selected branches in June 2020 and in August 2021 and 2022.

To test effects of spray treatment on plant cover values, we used generalized linear models with a beta distribution and logit link. Fixed effects were a full factorial of site, year, and treatment, and we included plot as a random effect (because we measured the same plots in multiple years). Where we observed significant treatment effects, we ran pre-planned linear comparisons -- ACK55 vs. control and indaziflam vs. control – for each site and year. Because this was 12 comparisons, we used a Holm-Bonferroni correction to reduce the possibility of a type 1 error (i.e., a false positive). We ran the same model for sagebrush growth but log-transformed the data and used a Gaussian distribution and identity link. For fall green-up density, we used the same models except that we used a negative binomial distribution and log link.

Results

Cover

Exotic annual grass cover was reduced by ACK55 at both sites in all years except for year 3 at the lower elevation site (Figure 3.6). Reductions in EAG with indaziflam were not observed until the 3rd post-treatment year, and these were only marginally significant (Figure 3.6). Native perennial grass cover was reduced with ACK55 in all sites and years and was not affected by indaziflam at any site or in any year (Figure 3.7). Native annual forb cover varied considerably from year to year in control plots, but at times and places where native annual forbs were abundant in control plots, we observed reductions for at least 2 years after treatment with indaziflam and also reductions with ACK55 except in 2022 at the lower elevation bunchgrass site, where native annual forb cover was greater in the ACK55 plots (Figure 3.8).

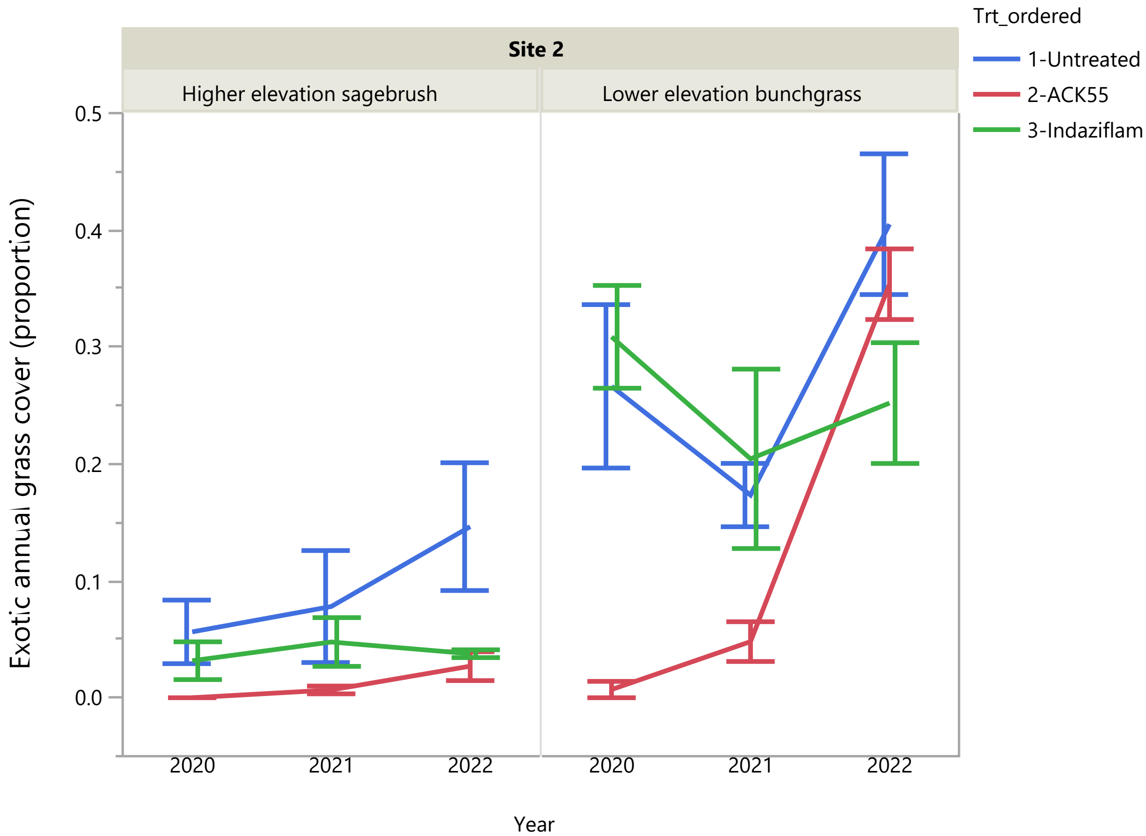

Figure 3.6 Mean (±SE) exotic annual grass cover for all treatments (TRT) in three post-treatment years.

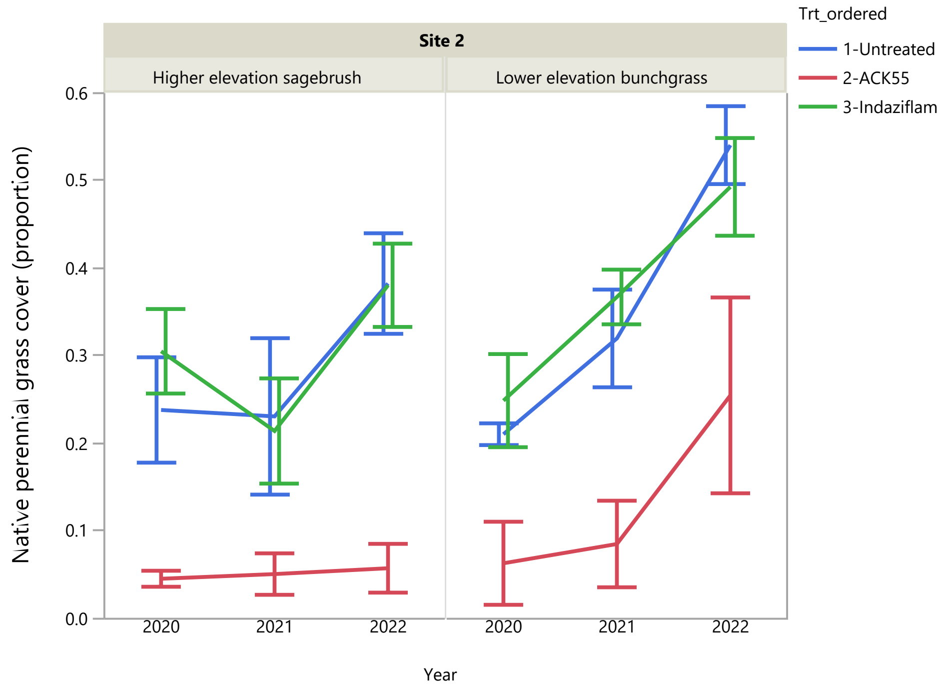

Figure 3.7 Mean (±SE) native perennial grass cover for all treatments (TRT) in three post-treatment years.

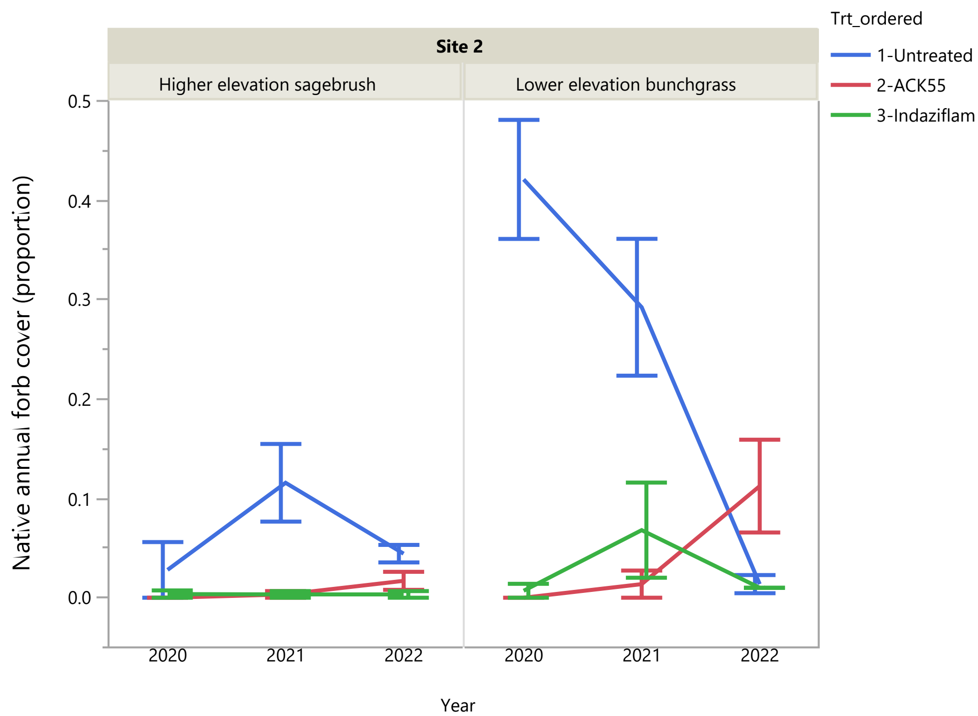

Figure 3.8 Mean (±SE) native perennial forb cover for all treatments (TRT) in three post-treatment years.

Sagebrush growth

Sagebrush growth was considerably lower in 2020 than in 2021 and 2022 (Figure 3.9), but this is likely an artifact of measuring growth much earlier in the year in 2020 (June) and later in 2021 and 2022 (August). Sagebrush growth was reduced with ACK55 treatments in 2020 (Figure 3.10) but increased relative to control at the lower elevation site in 2021 and at the higher elevation site in 2022. Sagebrush growth was not significantly affected by indaziflam treatments.

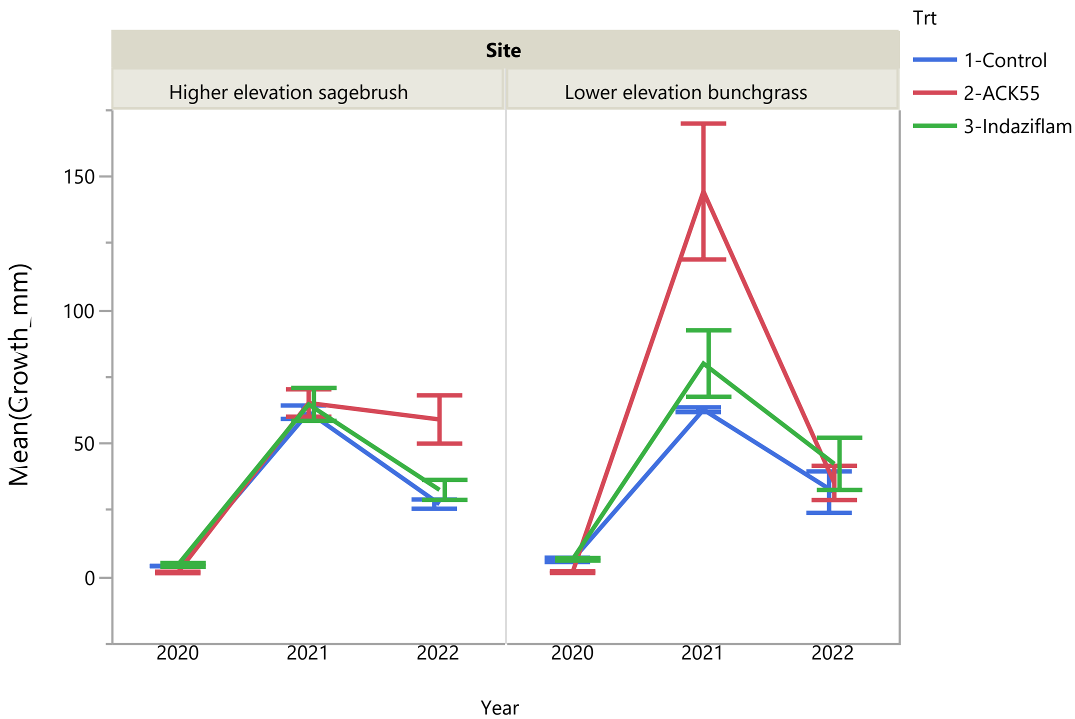

Figure 3.9 Mean (±SE) sagebrush annual growth for all treatments (TRT) in three post-treatment years. Measurements were made in June of 2020 and in August of 2021 and 2022. Measurements for 2020 only are shown in Figure 3.10.

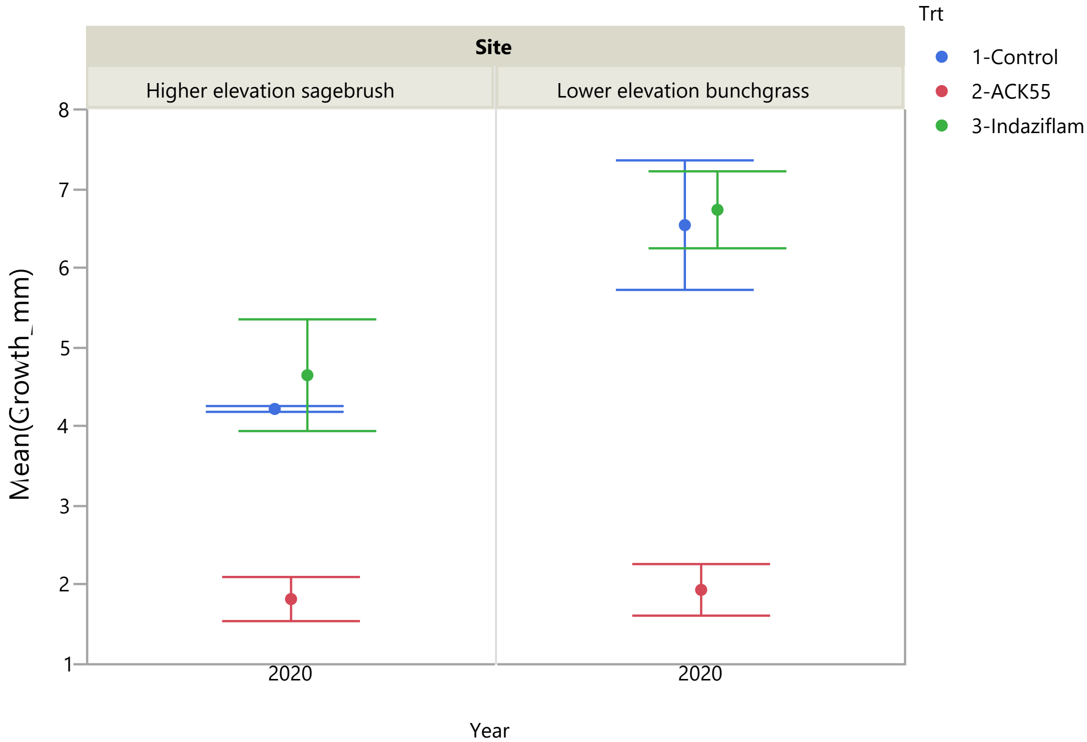

Figure 3.10 Mean (±SE) sagebrush annual growth for all treatments (TRT) in 2020 only.

Fall green-up density of yarrow

Density of yarrow (*Achillea millefolium*) that greened up in fall varied considerably between years. ACK55 reduced the yarrow density in 2020 and considerably in 2021 (Figure 3.11). Indaziflam did not affect yarrow density.

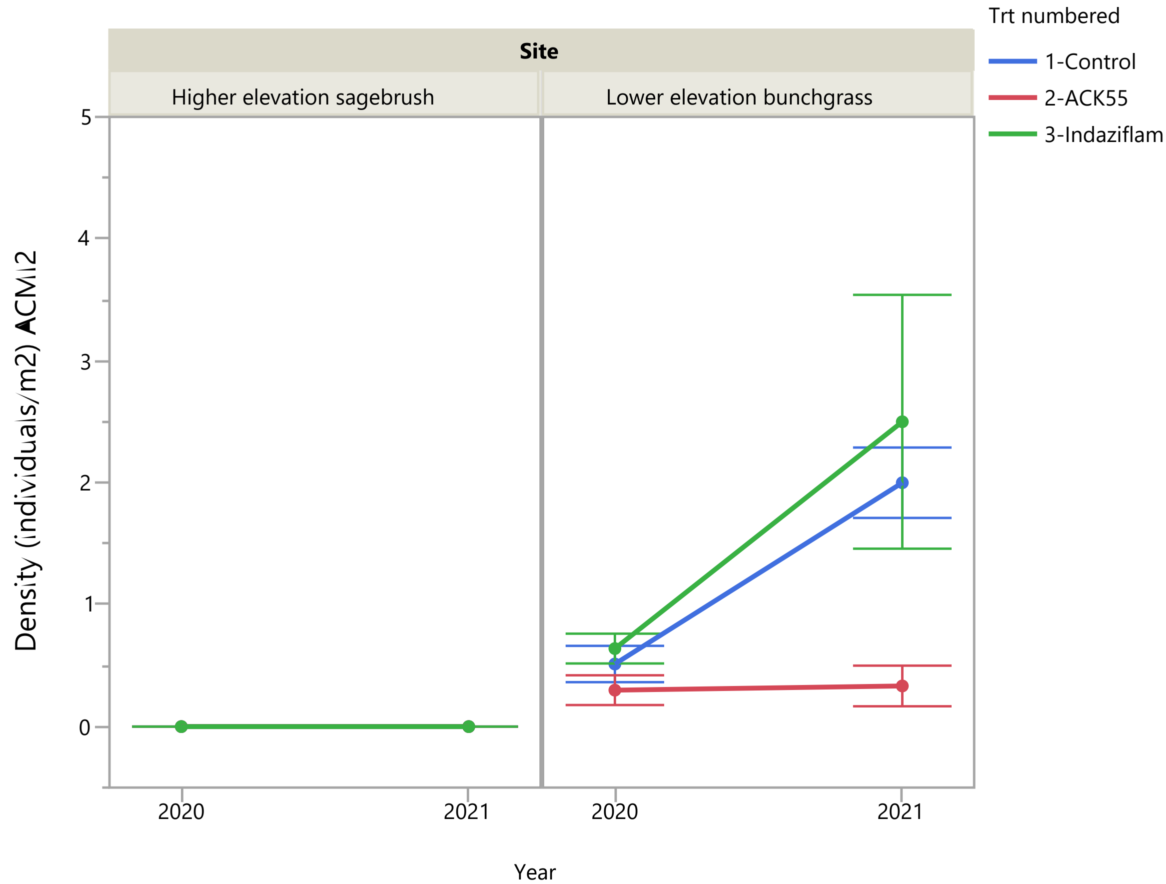

Figure 3.11 Mean (±SE) density of yarrow (*Achillea millefolium*) greening up in fall for all treatments (TRT) in two years after spraying. Yarrow was relatively common in the lower elevation bunchgrass site but scarce in the higher elevation sagebrush site.

4. Battalion Pro® 2020 (I-84 Black's Creek and Eisenman) – additional opportunity to test newest formulation of ACK55

In September 2020, the EPA approved strain ACK55 for use as a bioherbicide. In late fall 2020, BioWest Ag Solutions provided USGS with their newly released ACK55 formulation, Battalion Pro®, and ITD approved an extension of this project to allow USGS to test it on Idaho roadsides.

Specific project objectives, site description

Through the Battalion Pro® applications we made in 2020, we sought to answer three questions:

1) Are repeated applications of WSB more successful at controlling exotic annual grasses than one-time applications?
2) What are the effects on plant communities of ACK55 (specifically, the Battalion Pro® formulation) alone vs. tank mixed with the pre-emergent herbicide imazapic?
3) Does application of Battalion Pro® prior to hydroseeding prevent exotic annual grass growth?

We addressed question 1 by spraying Battalion Pro® on sites that we had previously sprayed with ACK55 in 2019, I-84 Eisenman and US-95 (described in sections 2 and 3). Configurations of these applications are shown in Figures 4.1 and 4.2.

We addressed question 2 by establishing new plots with the following treatments:

1) Battalion Pro® only
2) Imazapic, only
3) Battalion Pro® plus imazapic, tank mixed
4) Unsprayed control

We established three replicate plots of each of these treatments at two different southwest Idaho sites: the I-84 Eisenman interchange, south of the 2019 treatment blocks (Figure 4.2) and the I-84 Black's Creek Interchange (Figure 4.3). I-84 Black's Creek had recently undergone reconstruction. Nevertheless, a substantial portion of the site was not affected by the reconstruction and contained an east-facing slope with a plant community that included native perennial bunchgrasses (Bluebunch wheatgrass, Bottlebrush squirreltail) and forbs (Arrowleaf balsamroot) but also a substantial cheatgrass component (Figure 4.4).

We addressed question 3 by spraying Battalion Pro® on four 20.25 m^2 plots (and marking four comparable unsprayed plots) on newly graded soil on the I-84 Black's Creek interchange prior to hydroseeding there (Figure 4.4).

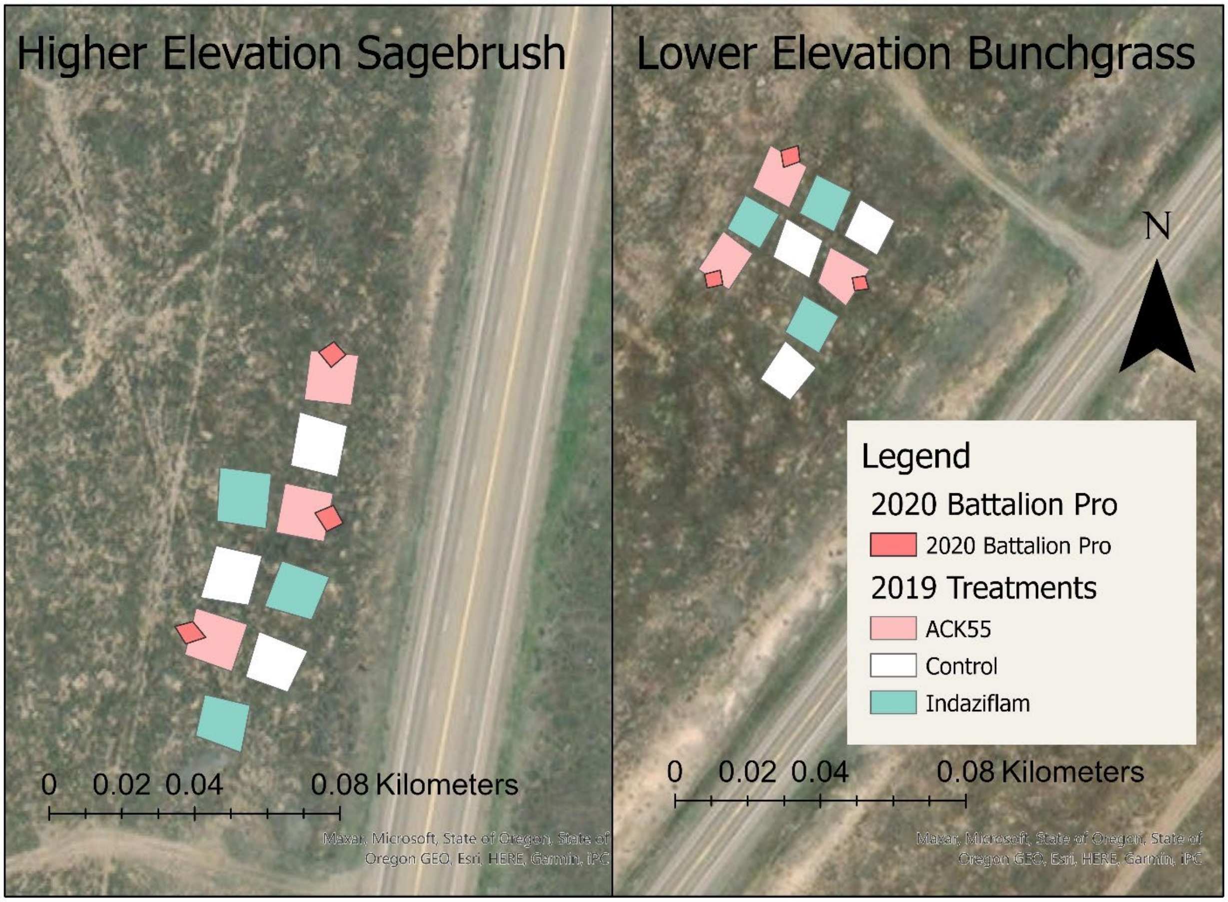

Figure 4.1 Configuration of 2020 Battalion Pro® applications at US-95 sites.

Figure 4.2 Configuration of 2020 Battalion Pro® applications at the I-84 Eisenman Interchange. Details of 2019 treatments are shown in Figure 2.2.

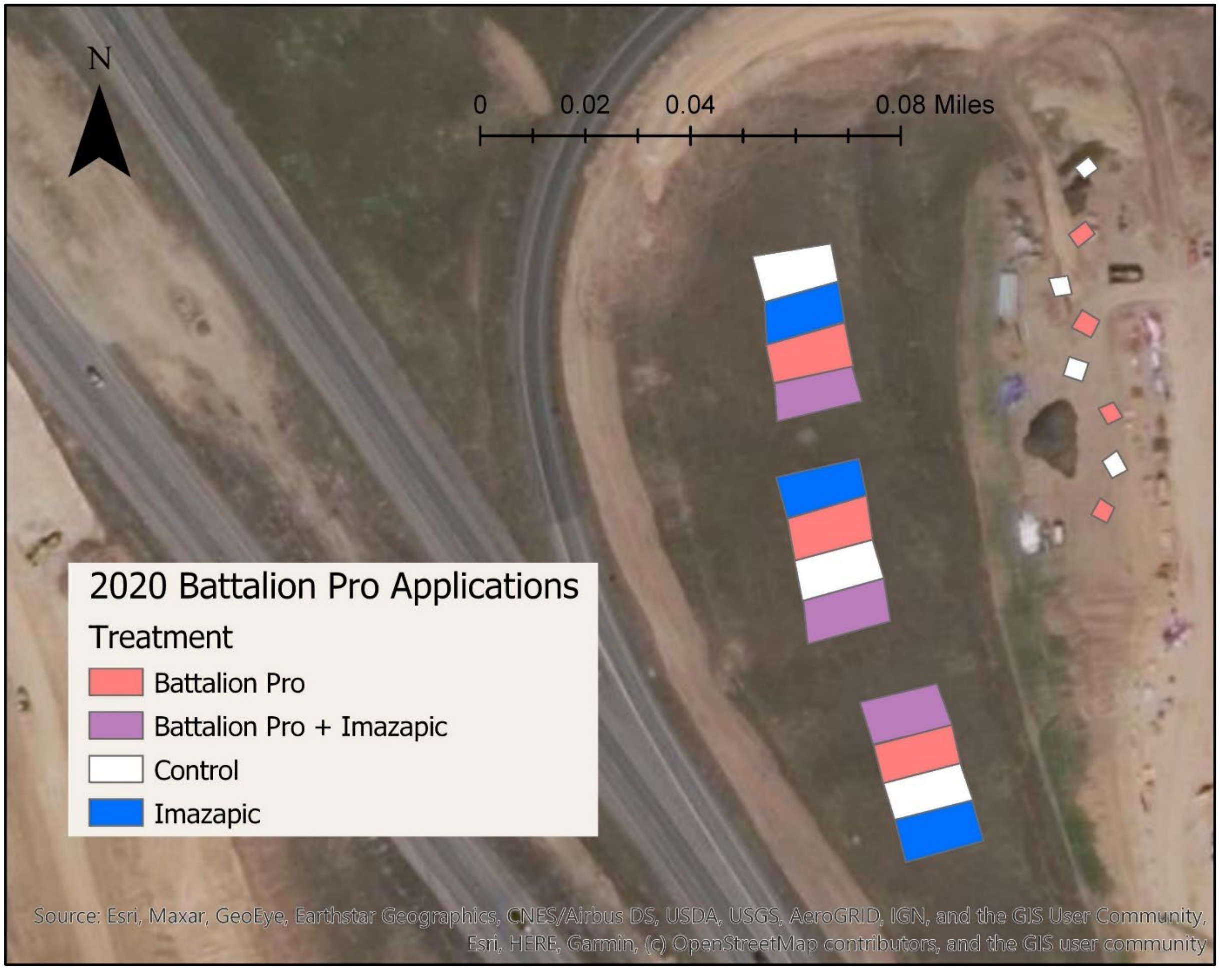

Figure 4.3 Configuration of 2020 Battalion Pro® applications at the I-84 Black's Creek Interchange. Note that the aerial photo is outdated – construction was finished, and the site had been fully contoured by the time the hydroseeded plots (right side of image) were treated.

Figure 4.4 Plant community in the undisturbed portion of I-84 Black's Creek Interchange.

Project installation/treatment application

Re-spray plots at I-84 Eisenman and US-95 and hydroseed plots (Figure 4.5) at I-84 Blacks Creek were 4.5x4.5 m (20.25 m^2) and were sprayed by USGS staff with a backpack sprayer using a carrier volume of 38 gallons/acre in late November 2020 (11/23 for I-84 Eisenman and Black's Creek, 11/25 for US-95). Battalion Pro® was provided by BioWest and applied at a rate of 1 gallon/acre. Bacterial counts showed that application rate was $2x10^7$ cfu/mL for I-84 Eisenman re-spray and Black's Creek hydroseeding and $7.46x10^6$ cfu/mL for US-95 re-spray (see Appendix A Raising and counting ACK55). Weather for the I-84 Eisenman/Black's Creek spraying was a mix of clouds and sun with a high of 39°F. Rain and snow fell two days later, and there was a very wet fog three days later. Weather for the US-95 re-spray was foggy, then sunny with temperatures of 31°F and 34°F. Approximately 2.5 inches of snow lay on the ground at the time of spraying, and melted away gradually over the next week.

New Battalion Pro® and imazapic plots at I-84 Eisenman and Black's Creek were 30'x60' and were sprayed by Ada County Weed and Pest on 11/24/20 using a truck with a tank and spray boom spraying 25 gallons/acre carrier volume. One truck sprayed Battalion Pro® only, one truck sprayed imazapic only, and one truck sprayed a mix of the two. Imazapic was applied as 6 oz/acre Plateau without adjuvant.

Battalion Pro® was applied at a rate of 1 gallon/acre. Bacterial counts showed that application rates were $4.4x10^6$-$1.01x10^7$ cfu/m^2. Weather was sunny with a higher of 39°F. Rain and snow fell on the following day.

Hydroseeding at I-84 Black's Creek was scheduled for November 2020 but was delayed and was completed by a contractor in May of 2021.

Figure 4.5 Battalion Pro® applied to I-84 Black's Creek area slated for hydroseeding.

Data collection and analysis

We monitored vegetation cover at all sites in spring 2021 and spring 2022 using the LPI method. This involved identifying all species intercepted by a straight pin dropped at intervals along a transect or within a frame (for re-spray plots). The interval varied with plot size (minimum interval = 10 cm, maximum interval = 0.5 m) such that a minimum of 50 points per plot was obtained, resulting in a minimum of 2% resolution. For re-sprayed plots, we monitored separately inside and outside the 2019 plots (i.e., we monitored the triangular area that was re-sprayed separately from the triangular area that was sprayed only in 2020).

To determine whether re-application of WSB reduced cover of EAGs relative to plots sprayed only once, we used linear mixed models with beta distribution and logit link (glmmTMB, R) with WSB treatment (not treated, treated once, treated twice) as the independent variable and (where applicable) block as a random effect. In the case of US-95, where we had two sites in close proximity, we ran data from both sites together and included Site, WSB treatment and their interaction as independent variables. If there was a significant treatment effect, we used Tukey-Kramer pairwise comparisons to compare all treatments. We ran separate models for each year, as year x treatment interactions were of little interest.

To determine whether EAG cover was reduced by Battalion Pro®, imazapic, or a tank mix of the two, we ran linear mixed models with beta distribution and logit link with treatment (no treatment, Battalion Pro® only, imazapic only, Battalion Pro® + Imazapic) as the independent variable and block as a random effect. If a significant treatment effect was detected, we did pre-planned linear comparisons (1- Imazapic vs. no imazapic, 2- Battalion Pro® vs. no Battalion Pro®, 3 – Interaction (1*2). If the interaction was significant, we performed separate contrasts for Battalion Pro® vs. no Battalion Pro® with and without imazapic. We ran separate models for I-84 Black's Creek and Eisenman.

To determine whether EAG cover in hydroseeded plots at I-84 Black's Creek was reduced by applying Battalion Pro® prior to hydroseeding, we used a linear model with beta distribution and logit link and treatment as the independent variable (note that we collected cover data only in 2022, as hydroseeding did not occur until May 2021 and thus there was not plant cover at the time of 2021 data collection at Black's Creek).

Results

Reapplication

I-84 Eisenman – EAG cover was not reduced in 2021 or 2022 for plots that were re-sprayed with WSB relative to plots that were sprayed only once with WSB only (ACK55 only in 2019, Battalion Pro® only in 2020, Figure 4.6). However, EAG cover in 2022 (year 2 after re-spray) was 63% less in plots that were sprayed with WSB+imaz+indaz in 2019 and then re-sprayed with WSB in 2020 relative to plots that were sprayed with WSB+imaz+indaz in 2019 but not sprayed with Battalion Pro® in 2020 (Figure 4.7). Note that these plots already had very low EAG cover due to the combined imazapic + indaziflam treatment. This effect did not occur in 2021 (year 1 after re-spray)

US-95 – EAG cover was not reduced in 2021 or 2022 for plots that were re-sprayed with WSB relative to plots that were sprayed only once with WSB (ACK55 only in 2019, Battalion Pro® only in 2020, Figure 4.8). EAG cover was lower in plots sprayed with WSB in 2019 than in control plots (as reported in section 3 – WSB sprayed in 2019 reduced EAG cover but also damaged native perennials).

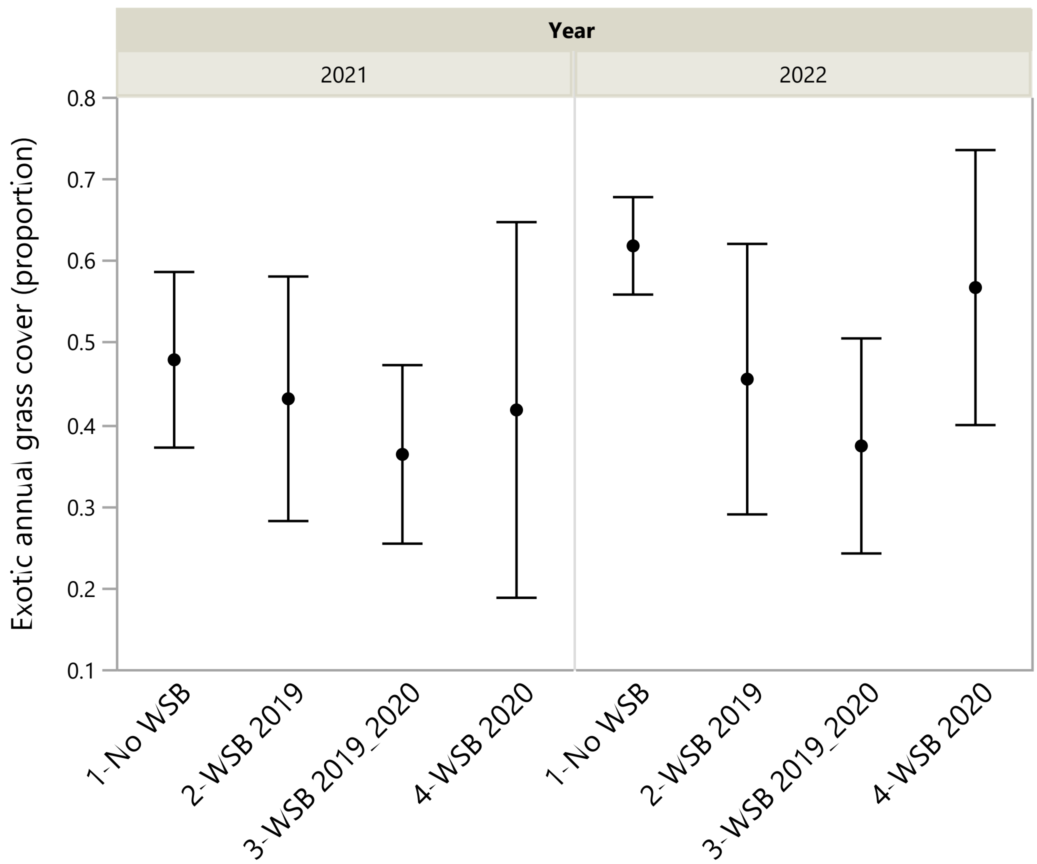

Figure 4.6 Mean (± SE) exotic annual grass cover for I-84 Eisenman plots sprayed with WSB (ACK55) in 2019 and re-sprayed (or not) with WSB (Battalion Pro®) in 2020. Data are for the two years after re-spraying (2021 and 2022). Plots sprayed with WSB (Battalion Pro®) in 2020 only are included for visual comparison but were not included in the statistical model.

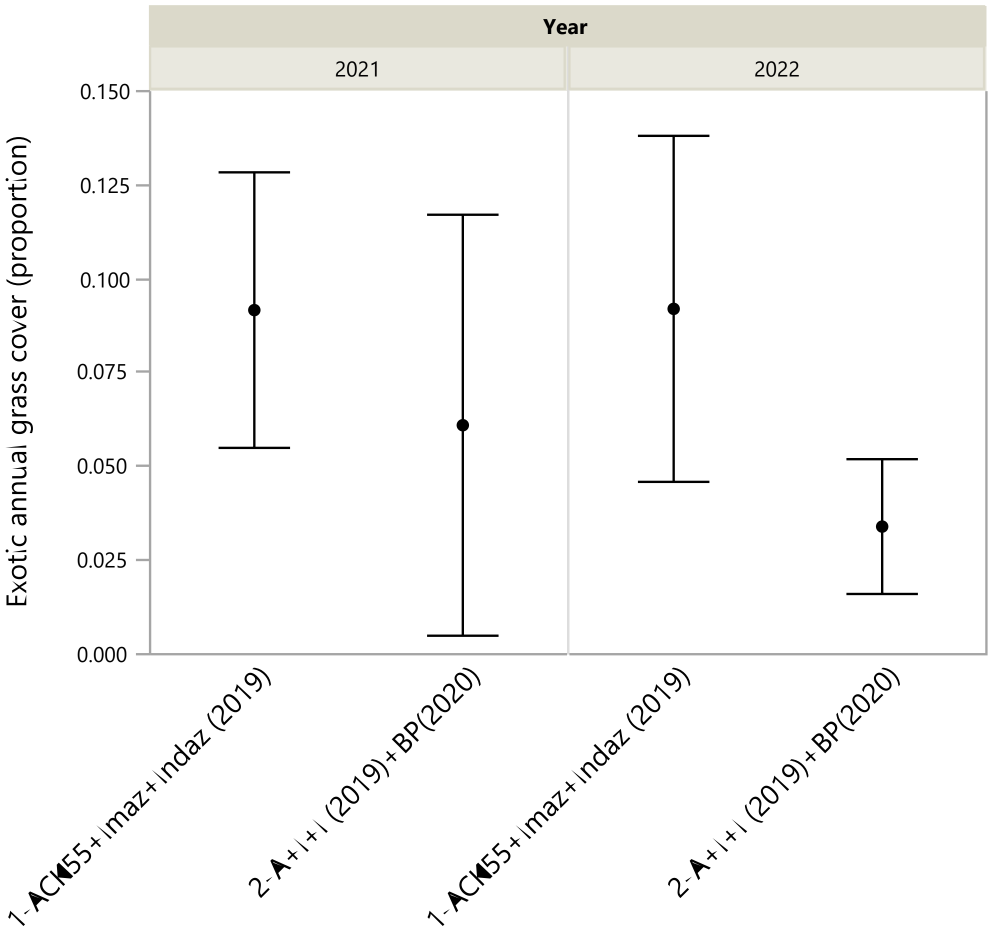

Figure 4.7 Mean (± SE) EAG cover for I-84 Eisenman plots sprayed in 2019 with a combination of ACK55+imazapic+indaziflam and then resprayed (or not) with Battalion Pro® in 2020. Data are for the two years after re-spraying (2021 and 2022). Note very low EAG cover due to the combined effects of imazapic and indaziflam.

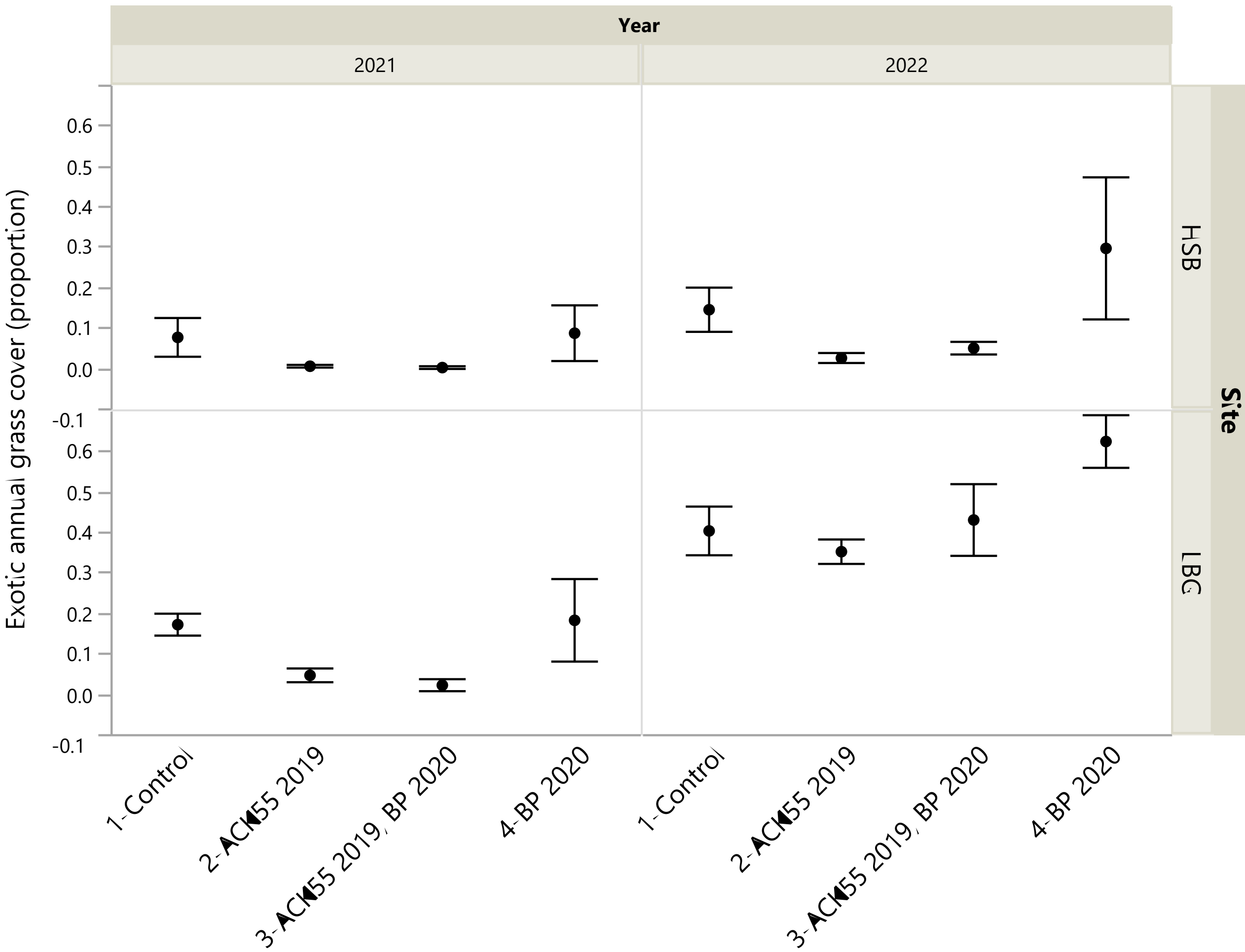

Figure 4.8 Mean (± SE) EAG cover for US-95 plots sprayed in 2019 with ACK55 and then resprayed (or not) with Battalion Pro® in 2020 or sprayed only with Battalion Pro® in 2020. Data are for the two years after re-spraying (2021 and 2022). HSB=Higher Elevation Sagebrush site, LBG = Lower Elevation Bunchgrass site. Recall that ACK55 sprayed in 2019 not only reduced EAG cover but also damaged native perennials (see section 3). Plots sprayed with Battalion Pro® in 2020 only are included for visual comparison but were not included in the statistical model.

Battalion Pro® x Imazapic

At I-84 Black's Creek, EAG cover varied by year and treatment. EAG cover was 88% greater in control plots in 2022 than in 2021 (Figure 4.9). Imazapic reduced EAG cover by 94% in 2021 but only by 51% in 2022. EAG cover was reduced 32% relative to control with Battalion Pro® in 2021 but only 14% in 2022.

At I-84 Eisenman, EAG cover varied by year but not by treatment, with EAG cover 31% greater in control plots in 2022 than in 2021 (Figure 4.9).

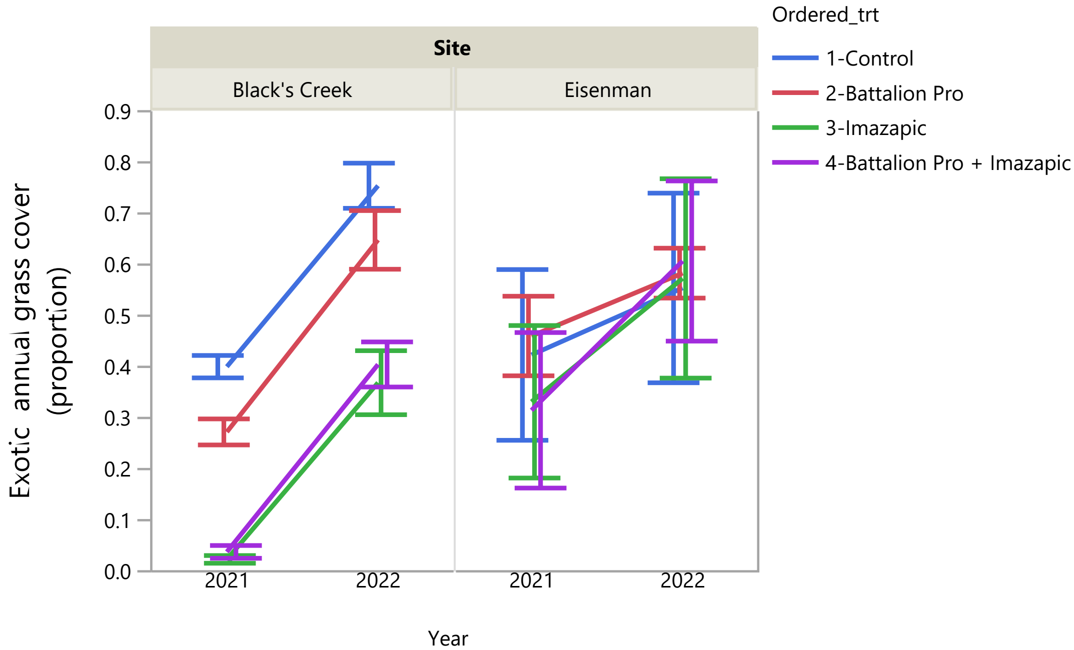

Figure 4.9 Mean (±SE) EAG cover for all treatments at two sites.

Hydroseeding

EAG cover in hydroseeded plots was not affected by spraying with Battalion Pro® prior to hydroseeding in the first growing season after hydroseeding (2022, Figure 4.10)

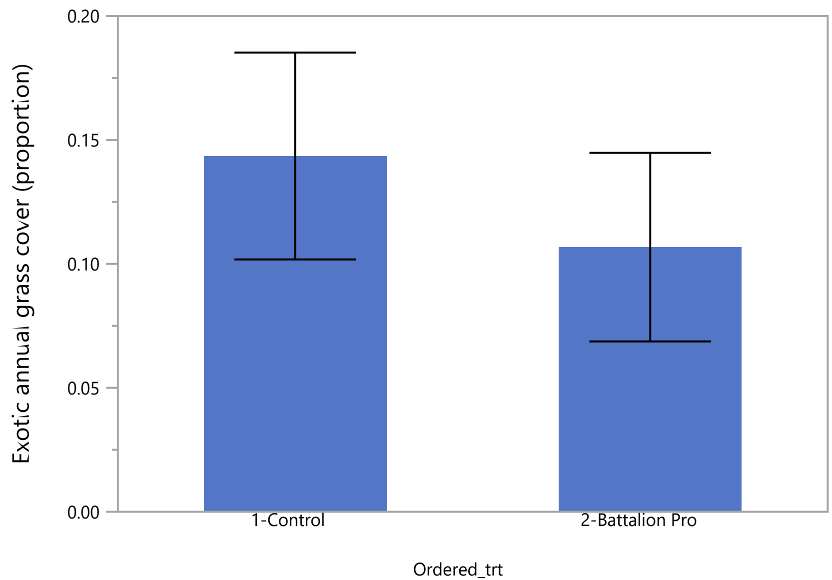

Figure 4.10 Mean (±SE) EAG cover measured at Black's Creek in 2022 for plots treated (or not) with Battalion Pro® in November 2020 prior to hydroseeding in May 2021.

5. Discussion

There was considerable variability in treatment effects among sites, particularly for WSB, which was ineffective in two different applications at I-84 Eisenman (Figures 2.5, 4.9), effective but also damaging to native perennials when over-applied at US-95 (Figures 3.6-11), and moderately effective at I-84 Black's Creek (Figure 4.9). The literature to date is also mixed in its reports of WSB success. In one study, WSB (strain ACK55 and two others that have not been commercialized) gradually and steadily reduced EAG cover over a period of seven years in six locations in eastern Washington (Kennedy 2018), but this has not been repeated in any other study. Another study in which many ITD right-of-ways were treated with ACK55 reported varying reductions in exotic annual grass cover among sites, though not all were statistically significant in the final year (Kennedy 2017). We revisited the sites with the greatest reported reductions in exotic annual grass cover 4-5 years after application and did not detect qualitative (visual) or quantitative differences in exotic annual grass cover between treated and untreated areas (Appendix C). WSB were moderately effective in year 2 (but not years 1 and 3) after treatment at some sites in one study (Lazarus et al. 2020) and completely ineffective in several other studies (Reinhart et al. 2020, Germino and Lazarus 2020, Pyke et al. 2020, Tekiela 2020). A logical explanation for the variation in effectiveness of WSB would be variation in survival of bacteria applied in the field. Unfortunately, *P. fluorescens* is ubiquitous in soils, and tools do not yet exist that allow for detection of the particular strain applied (aside from genetic transformation of the strain to make it resistant to an antibiotic – see Stubbs et al. 2014, Kennedy 2018), thus making it difficult to determine whether bacteria applied in any given situation perished or survived and multiplied in soils. We did collect soils from treated and untreated plots, and these are stored in a -80°C freezer so that future analyses could be completed if such a tool is developed.

The non-target damage to native perennials with over-application (approximately 5x) of WSB that we observed at the US-95 site (Figures 3.7-11) was concerning, and so we performed an exhaustive follow-up inquiry on sources of application error to identify possible causes of the unusual outcome. The tanks and equipment used to apply the herbicides by the licensed and reputed spray contractor were clean and in fact had been used to spray water prior to our application. The tanks were loaded with bacteria reared from the vats adjacent to and similar to the other trials reported here, and other than the high concentrations that we verified and which resulted from the innate lack of control of bacterial growth while the product is in transit, there was nothing unusual about the growth of cultures from the bacteria applied. We have anecdotally observed a similar effect in only one other plot where WSB was over-applied (and thus removed from the study) in a community of mixed natives and exotics and resulted in nearly bare soil. However, even greater WSB application rates (approximately 200x) in a highly invaded site without native perennials showed no response to treatments in a previous unpublished study (Lazarus and Germino, unpublished). Taken together, these results suggest the need for further fine-scale experimentation with various application rates in a variety of plant communities.

Imazapic was generally effective for 1-2 years after treatment in our observations, which is consistent with much of the existing literature (e.g., Morris et al. 2009, Pyke et al. 2014, Munson et al. 2015), though one study showed EAG reductions continued for 4-6 years after a post-fire imazapic application (Lazarus and Germino 2022).

Indaziflam generally had a delayed but sustained effect on EAG cover (Figures 2.5, 3.6). This delay is likely caused by the lack of post-emergence action of indaziflam on newly germinated cheatgrass seedlings that had begun to emerge when spraying occurred in November of 2019. These seedlings escaped indaziflam control in the first year. No delay in effectiveness of indaziflam occurred when it was applied with imazapic (Figure 2.5), likely due to the post-emergent action of imazapic on newly germinated cheatgrass seedlings. While the combination of imazapic and indaziflam was more effective in the first year than indaziflam alone, indaziflam alone was as effective as treatments including both imazapic and indaziflam in the second and third years after treatment. This contrasts with work by Donaldson and Germino (2022), where the combined herbicides continued to provide a greater and more sustained reduction of EAG cover than indaziflam only. A similar pattern has been observed for indaziflam vs. imazapic + indaziflam treatments at the Minidoka Wildlife Refuge (Germino, Mathews et al., unpublished data).

A drawback to the sustained effectiveness of indaziflam is that intentionally seeded species may also be prevented from germinating, as we showed here with hoary tansyaster seeded one year after indaziflam treatment at I-84 Eisenman (Figure 2.9). Indaziflam reduced germination and growth of bluebunch wheatgrass planted shortly after spraying by more than 90% (Terry et al. 2021b) but grasses and forbs seeded 8 months after indaziflam treatment were able to establish (Clarke et al. 2020). One possible solution to negative effects of indaziflam on seeded species is to plant plugs instead of seeding where feasible. Investigations into seed coating and using furrows to physically separate seed from herbicide-treated soils are also showing promise (Clenet et al. 2019, Terry et al. 2021a).

6. Conclusions

The weight of available evidence suggests that weed-suppressive bacteria are not yet consistent enough in their effects to be used at an operational scale for exotic annual grass control, but further experimentation with this potential tool may be warranted.

Both imazapic and indaziflam consistently reduce exotic annual grass cover, indaziflam for a more extended period of time, but seeding can be compromised by pre-emergent herbicide use (particularly with indaziflam use) if the herbicide and seed are not sufficiently separated in time and/or space. Repeated herbicide treatments and seedings or plantings are likely needed in more degraded sites. If Indaziflam continues to suppress annual grass populations in our ITD plots, which could be verified with sustained observations, then it could be deemed the best available control agent for exotic annual grasses.

7. Cited works

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8. Appendix A: Raising and counting ACK55

Freeze-dried ACK55 was placed in Sands and Rovira broth (Sands and Rovira 1970), shaken overnight at room temperature (22°C), diluted in 0.9 M NaCl solution, plated on Sands and Rovira agar, and allowed to grow for three days at room temperature. We selected 10 colonies from the agar and transferred them with a sterile loop to 5 mL Sands and Rovira broth. After shaking overnight at room temperature, we again diluted and plated them on Sands and Rovira agar. Repeating this process ensured that we did not have contamination by any other organisms. After three days, we selected 10 colonies from the agar, placed them into Sands and Rovira broth, and allowed them to grow to early stationary phase (approximately 45 hrs) while shaking at room temperature (see Yates et al. 2016 for descriptions of bacterial growth phases). We autoclaved two 2 L flasks, each containing 1 L of King's B broth, inoculated each of them with a 10^{-6} mL/mL dilution of these early stationary phase colonies, and allowed them to come to early stationary phase (approximately 45 hrs) while shaking at room temperatures. Upon completion, we transferred the early stationary phase colonies to sterile 1 L Nalgene bottles and stored at 4°C. Approximately every three days, we serial diluted a sample from each colony, plated on King's B agar, and counted colonies to determine the current concentration of live cells (cfu/m^2).

A sample of each spray solution containing ACK55 was taken from each spray tank at the time of spraying, kept on ice in the field, and transported back to the laboratory where (on the same day) it was serial diluted in 0.9 M NaCl solution and plated on King's B agar. Plates were incubated for three days at room temperature and colonies were counted to determine the cfu content of the spray solution.

9. Appendix B: Tables of statistical results

Table 9.1 Fixed effects from generalized linear model for EAG cover at I-84 Eisenman across three years (2020-2022).

	Chisq	Df	Pr(>Chisq)	
(Intercept)	0.7726	1	0.379399	
Spray treatment	31.7227	5	6.74E-06	***
Rake	0.0905	1	0.763561	
Year	8.2304	2	0.016323	*
PBG_pretrt_scaled	161.7571	1	< 2.2e-16	***
Spray treatment:Rake	20.0851	5	0.001205	**
Spray treatment:Year	68.7418	10	7.75E-11	***
Rake:Year	0.1642	2	0.92119	
Spray treatment:Rake:Year	15.6781	10	0.109225	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 9.2 Fixed effects from generalized linear model of EAG density at I-84 Eisenman in 2020.

	Chisq	Df	Pr(>Chisq)	
(Intercept)	654.4981	1	< 2.2e-16	***
Spray treatment	27.5825	5	4.39E-05	***
Rake	1.8637	1	0.172198	
PBG_pretrt_scaled	12.6272	1	0.00038	***
Spray treatment:Rake	4.6605	5	0.458694	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 9.3 Fixed effects from generalized linear model of EAG density at I-84 Eisenman in 2021.

	Chisq	Df	Pr(>Chisq)	
(Intercept)	2281.658	1	< 2.2e-16	***
Spray treatment	50.6698	5	1.01E-09	***
Rake	8.2148	1	0.004155	**
Seed	4.6669	1	0.030749	*
PBG_pretrt_scaled	20.4546	1	6.11E-06	***
Spray treatment:Rake	3.6564	5	0.599866	
Spray treatment:Seed	3.9911	5	0.550697	
Rake:Seed	1.985	1	0.158866	
Spray treatment:Rake:Seed	6.8574	5	0.231463	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

10. Appendix C: Resampling historic Kennedy/ITD plots

IDAHO TRANSPORTATION DEPARTMENT

RESEARCH REPORT

INTERIM REPORT ON SUBTASK FOCUSED ON RESAMPLING HISTORIC KENNEDY/ITD PLOTS FOR RP-284 "Integration of Weed-Suppressive Bacteria With Herbicides to Reduce Exotic Annual Grasses and Wildfire Problems on ITD Right-of-Ways"

RP 284

By

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Idaho Transportation Department

ITD Research Program, Contracting Services

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Technical Report Documentation Page

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<tr><td colspan="6">16. Abstract
In October 2019, an ITD Cooperative Transportation Research Program award was made to BSU/USGS to investigate the use of weed-suppressive bacteria (Pseudomonas fluorescens strain ACK55) with pre-emergent herbicides (imazapic and indaziflam) to reduce exotic annual grasses (cheatgrass, medusahead) on ITD right-of-ways. The work proposed includes continued monitoring of right-of-ways previously treated with ACK55 by Ann Kennedy as well as establishment of new experimental plots that test both ACK55 and herbicides, alone and in combination. This report describes progress up to September 2020 on the re-sampling of the areas previously treated by Ann Kennedy.</td></tr>
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Technical Advisory Committee

Each research project is overseen by a Technical Advisory Committee (TAC), which is led by an ITD project sponsor and project manager. The TAC is responsible for monitoring project progress, reviewing deliverables, ensuring that study objectives are met, and facilitating implementation of research recommendations, as appropriate. ITD's Research Program Manager appreciates the work of the following TAC members in guiding this research study.

- Project Manager: Cathy Ford
- TAC Members: Alissa Salmore, Melinda Lowe, Wendy Terlizzi
- FHWA-Idaho Advisor: Brent Inghram

List of Abbreviations and Acronyms

USGS .... United States Geological Survey

Executive Summary

In October 2019, an Idaho Transportation Department (ITD) Cooperative Transportation Research Program award was made to Boise State University in partnership with the U.S. Geological Survey to investigate the use of weed-suppressive bacteria (*Pseudomonas fluorescens* strain ACK55) with pre-emergent herbicides (imazapic and indaziflam) to reduce exotic annual grasses (cheatgrass, medusahead) on ITD right-of-ways. The work includes a subtask in which ITD right-of-ways treated with ACK55 by Dr. Ann Kennedy 4-5 years previously (2017 report; ITD-RP-258) were resampled in summer 2020, focusing only on ACK55 and not the herbicides (which are tested separately and will be reported on in the future). The 2020 sampling protocol was similar but more intensive than the ITD-RP-258. There were no differences in annual grasses on areas sprayed with ACK55 and nearby untreated areas.

Appendix C-1 Tasks completed to date

The purpose of this study was to determine if weed-suppressive bacteria, specifically *Pseudomonas fluorescens* strain ACK55, exhibits negative, control-like effects on exotic annual grasses such as cheatgrass. We have completed a subtask in which plots on ITD lands sprayed with ACK55 by Dr. A Kennedy were resampled by us.

We used the maps provided in Ann Kennedy's ITD report (Kennedy 2017) to find and mark her five most successful ACK55 treatments (though one of these, Black's Creek, is now a construction site and cannot be safely visited). The sites differed in the type of plant community present before and thus after treatment. The sites at "I-84 Eisemann" (Figures C1.1- 2) were located at ~3000' elevation that appears to have burned repeatedly in recent decades and thus has no sagebrush or other woody species and also has relatively few perennial forbs remaining. The US-95 site (Figure C1.3) was located at ~4000' elevation and had a relatively greater proportion of perennial forbs and bunchgrasses. We followed a more rigorous version of Kennedy's method for determining plant cover, taking 8 pairs of downward-facing aerial photos in each sprayed area and another 8 pairs of aerial photos in comparable adjacent unsprayed areas, at each site, in late spring of 2020 (see maps in Figure C1.4). Each photo was captured with the camera held at 2 m height facing downward (nadir) towards the soil and vegetation, and the resulting images encompassed a 2 x 3 m area of ground and the camera was held a 2 m height, resulting in a resolution in which fine-textured grass blades could be observed. A list of all species present in the photographed areas was recorded along with notes on their apparent relative abundances, for the purpose of guiding the subsequent analysis of the photographs.

We cropped the outer area (approximately 0.5 m) in each image to include only the central area to minimize parallax distortion. We then used Sample Point software to place a grid of 50 points on each image (100 points per pair of images) and identified the species that occurred at each point using the species list from field notes to guide the photo interpretation. The I-84 Eisenman sites were treated in Fall 2014, and the US-95 site was treated in Fall 2015 (following the Soda Fire), and so our measurements represent year 6 for the I-84 Eisenman sites and year 5 for the US-95 site.

Analysis of variance was used to determine if mean differences in annual grasses existed between ACK55 sprayed and non-sprayed area, using a log transformation to meet assumptions about the distribution of data and equality of variances. The factors in the model were "site", "spray", and "site x spray", with 4 sites (see Figure C1.4) and two levels of spraying (sprayed, or not).

Figure C1.1. I-84 Eisenman 1 site, viewed from the north. Photo taken May 2019 by B. Lazarus.

Figure C1.2 I-84 Eisenman 2 Burned site, viewed from south end of site looking north. Photo taken May 2019 by B. Lazarus.

Figure C1.3 US-95, MP1 site. Photo taken May 2019 by B. Lazarus.

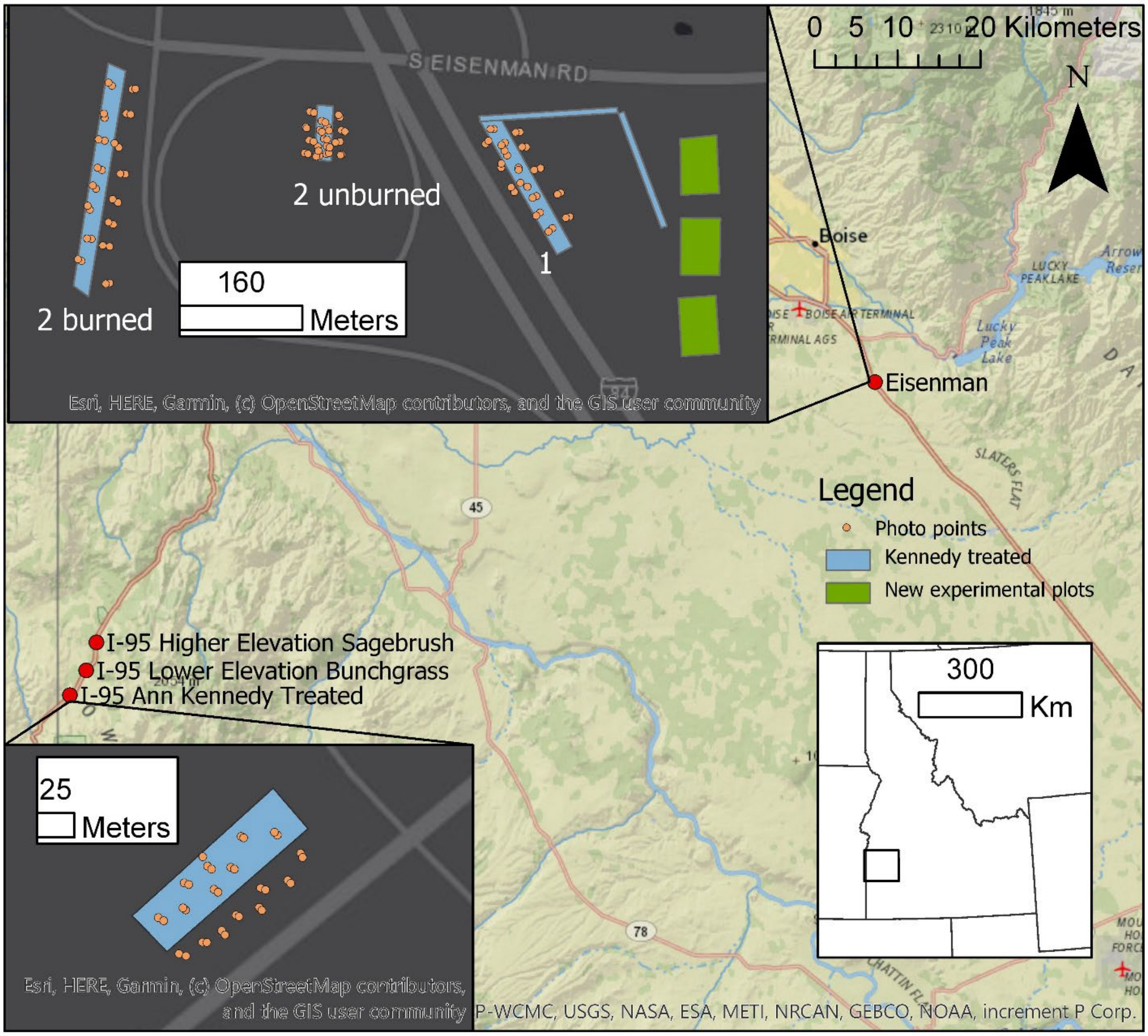

Figure C1.4. Map showing study site locations, including areas treated previously by Dr. A Kennedy which were resampled in 2020 and are reported on here (blue), in addition to new plots treated in 2019 that are not reported on here (green). Inset maps provide detail of photo plot areas and new experimental plots. Only one of the three US-95 sites were part of Kennedy's experiment and thus were resampled and reported here.

Appendix C-2 Findings

Mean cover of invasive annual grasses (cheatgrass and medusahead) and perennial grasses are plotted by site and spray treatment in Figure C2.1. The site naming conventions follow those used in Kennedy's 2017 report. There were no statistical differences in the abundance of exotic/invasive annual grasses between ACK55-sprayed and unsprayed areas, in contrast to Kennedy's report.

According to Kennedy, the effects of weed-suppressive bacteria should last 5-6 years in soil, but no effects are currently evident (in 2020). We visited the same sites in years 3-4 and 4-5 post treatment (in 2018 and 2019, prior to this project), and while we did not collect data on those trips, we also could not visually identify any treatment effects at those times, either.

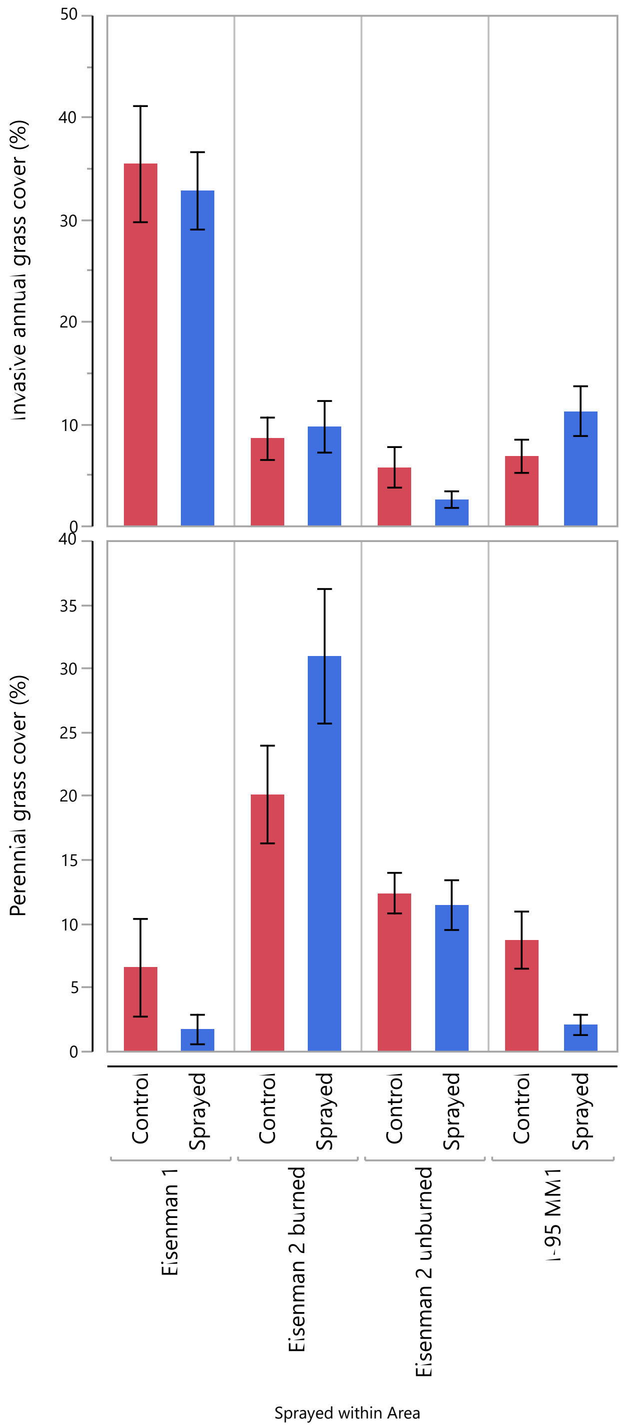

Figure C2.1 Mean (±SE) canopy cover of invasive annual grasses (cheatgrass and medusahead) and perennial grasses for areas sprayed with ACK55 and comparable controls at four sites.

Appendix C-3 Cited Works

Idaho Transportation Department. 2017. Weed-Suppressive Soil Bacteria to Reduce Cheatgrass and Improve Vegetation Diversity on ITD Rights-of-Way. Kennedy, Ann C. ITD-RP-258.