TECHNICAL ARTICLE

Native seeds incorporated into activated carbon pods applied concurrently with indaziflam: a new strategy for restoring annual-invaded communities?

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Reestablishing native perennial vegetation in annual grass-invaded rangelands is critical to restoring ecosystems. Control of exotics, often achieved with preemergent herbicides, is essential for successful restoration of invaded rangelands. Unfortunately, desirable species cannot be seeded simultaneously with preemergent herbicide application due to nontarget damage. To avoid this, seeding is commonly delayed at least 1 year. Delaying seeding increases the likelihood that annual grasses will begin reestablishing and compete with seeded species. Activated carbon (AC) can provide preemergent herbicide protection for seeded species because it adsorbs and deactivates herbicides. Previous studies suggest that a cylindrical herbicide protection pod (HPP), containing AC and seeds, allows desired species to be seeded simultaneously with the application of the preemergent herbicide imazapic. Unfortunately, imazapic is only effective at controlling annual grasses for 1–2 years. Indaziflam is a new preemergent herbicide which exhibits longer soil activity, with which HPPs may be useful. To assess this possibility, we evaluated seeding two native species (Wyoming big sagebrush [*Artemisia tridentata* Nutt ssp. *wyomingensis*] and bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Á. Löve]), both incorporated into HPPs and as bare seed, at four application rates of indaziflam in a grow room study. HPPs protected seeded species at low, mid, and high rates of indaziflam. The abundance and size of plants was greater in HPPs compared to bare seed treatments. These results suggest that HPPs can be used to seed native grasses and shrubs simultaneously with indaziflam application.

Key words: herbicide protection pods, indaziflam, revegetation, sagebrush, seeding technologies

Implications for Practice

- Activated carbon herbicide protection pods (HPPs) can be used to seed native species simultaneously with indaziflam application to control exotic annual grasses.
- HPPs used with indaziflam increase the likelihood of successful restoration because indaziflam should reduce exotic annual grass competition for extended time frames.
- Shrubs, bunchgrasses, and likely other plant functional groups, can be seeded in HPPs when indaziflam is applied to control exotic annuals.
- HPPs will likely be effective when combined with other preemergent herbicides.
- Refinement in the formulation of HPPs tested in this study may be needed to improve establishment of small-seeded species.

Introduction

Invasive annual grasses have pervaded, and often negatively impacted, rangelands and other ecosystems around the world (D'Antonio & Vitousek 1992). In the United States, cheatgrass (*Bromus tectorum* L.) and medusahead (*Taeniatherum caput-medusae* (L.) Nevski) cause degradation of rangeland ecosystems by reducing biodiversity, decreasing native plant species density and cover, and altering important ecosystem functions such as nutrient cycling (Evans et al. 2001; Davies & Svejcar 2008; Davies 2011). Both grasses are highly competitive with native species because of high seed production, earlier spring emergence and use of soil water and nutrients, and physical characteristics such as dense litter, which restrict seed establishment of native species (Evans & Young 1970; Young 1992; Sperry et al. 2006). Most importantly, invasive annual grasses can decrease fire return intervals from 50 plus years to less than 10 years, decreasing the likelihood of native plant establishment and survival and creating a positive feedback cycle that encourages and maintains invasive grass monocultures (Whisenant 1990; D'Antonio & Vitousek 1992; Brooks et al. 2004).

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Restoration of invaded rangelands is imperative in order to support native fauna and regain agricultural and recreational services provided by sagebrush (*Artemesia* L.) steppe ecosystems (Masters et al. 1996; Davies et al. 2014*a*). Competition from invasive annual grasses limits restoration success (Young et al. 1999; Boyd & Davies 2012; Madsen et al. 2016*a*). Invasive annual grasses need to be controlled to decrease competition with seeded native perennial grass (Young et al. 1999; Sheley & Krueger-Mangold 2003; Huddleston & Young 2005) and this is often achieved with preemergent herbicides (Kyser et al. 2007; Sheley et al. 2007). However, the decrease in competition afforded by preemergent herbicides is difficult to take advantage of while the herbicide is active due to nontarget damage to seeded species (Sheley et al. 2007; Davies et al. 2014*b*). To avoid this, a multiple entry method is used wherein the herbicide is applied and a year or more passes before seeds are sown (Huddleston & Young 2005). While waiting a year to seed limits herbicide damage to seeded species, it also increases the likelihood that invasive species will begin to reestablish (Madsen et al. 2014). A single-entry method, where preemergent herbicide and seeds are applied concurrently, has been attempted, but very low herbicide application rates are required to limit damage to nontarget species and results in limited control of invasive species (Sheley 2007; Sheley et al. 2012).

An alternative single-entry approach is one that uses activated carbon (AC) to protect seeded species from preemergent herbicide damage (Davies et al. 2017). AC has very high surface area and can therefore adsorb and deactivate organic chemicals, including many herbicides (Coffey & Warren 1969). Recently, AC has been incorporated into an herbicide protection pod (HPP) (Madsen et al. 2014; Davies et al. 2017, 2018). Seeds incorporated within HPPs may be protected from preemergent herbicides. If they are sown concurrently with preemergent herbicide application, seeds within HPPs will be protected while undesirable species are controlled, and therefore have increased time to establish with limited competition (Davies et al. 2017). Research with the herbicide imazapic shows that HPPs provide herbicide protection for seeded grasses (Madsen et al. 2014; Davies et al. 2017; Davies 2018). However, imazapic is normally only effective at controlling invasive annual grasses for 1–2 years (Kyser et al. 2007; Sheley et al. 2012). It would be advantageous to test HPPs with preemergent herbicides that remain active longer and with functional groups other than perennial grasses.

Indaziflam is a new preemergent herbicide which has a longer soil residue time compared to other preemergent herbicides used on rangelands (Brabham et al. 2014; Sebastian et al. 2017*a*). Compared to imazapic, indaziflam has exhibited greater and longer lasting control of invasive species up to 3 years after treatment (Sebastian et al. 2016*a*, 2016*b*, 2017*b*). Therefore, if paired with HPPs, indaziflam may increase the control of annual grasses, providing protected seeds more time to establish without competition from invasive annual grasses.

The purpose of this study was to determine the extent of protection offered by HPPs for a native shrub, Wyoming big sagebrush (*Artemisia tridentata* Nutt ssp. *wyomingensis*), and a native perennial grass, bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve), at low, medium, and high rates of indaziflam application in a lab setting. We hypothesized that following indaziflam application, seedling size (height, aboveground biomass, leaf number, leaf length, leaf width, and plant diameter) and density of both species would be greater when seeded in HPPs compared to being sown as bare seed.

Methods

Experimental Design

The study was conducted in a grow room at the Eastern Oregon Agricultural Research Center, in Burns, OR. Soil used in the experiments was collected in eastern Oregon from the Northern Great Basin Experimental Range (43°27′58.18″N, 119°41′49.11″W). The soil was a Gradon gravelly fine sandy loam and was sandy clay loam when textured (USDA NRCS 2018). Soil was sifted to exclude particles above a 6.35 mm. Medusahead seed was collected in Harney County, OR (43°43.845″N, 118°22.353″W, 1,138 m elevation) and was frozen for 2 days before planting to break dormancy and ensure maximum germination (Young et al. 1968). Wyoming big sagebrush and bluebunch wheatgrass seed were purchased from a commercial dealer.

Treatments were bare seed and seed incorporated into an AC pod (i.e. HPPs). HPPs were composed of 43% Ca bentonite, 33% AC, 6% worm castings, 14% compost, and 4% seed by dry weight. Dry materials were thoroughly mixed, then water was added so the material could be formed and passed through a pasta extruder (Model TR110, Rosito Bisani, Los Angeles, CA, U.S.A.). The AC mixture was extruded through an 8 mm diameter die, resulting in cylindrical strands which were then cut into pods approximately 15 mm long. Preemergent herbicide treatments were applied to sagebrush and bluebunch wheatgrass, at four indaziflam (Esplanade 200 SC, Bayer CropScience, Monheim am Rhein, Germany) rates, and replicated five times. The study was conducted in 53 cm × 42 cm × 11.5 cm boxes. Twenty boxes were filled and lightly packed to 2.5 cm below the top with soil. Each box was divided into five 10.6 cm × 42 cm × 11.5 cm containers with plastic dividers. Each box was randomly assigned one of the indaziflam application rates. The species and seed treatments (bare seed or HPP) were each randomly assigned to one of four containers in each box. One container in each box was planted with medusahead as a bioindicator of herbicide effectiveness. Seeds were planted at a rate of 50 pure live seeds per container for each species-treatment combination. Seed rate per container for HPP treatments was determined by estimating the number of viable seeds per pod. All pods were pressed gently into the soil and left uncovered. Medusahead and bluebunch wheatgrass bare seed were pressed into the soil and left uncovered while sagebrush bare seed was lightly covered with soil to prevent movement due to small size during watering. This resulted in each box containing one container each of bare seed bluebunch wheatgrass, HPPs bluebunch wheatgrass, bare seed sagebrush, HPPs sagebrush, and bare seed medusahead. Boxes were watered to field capacity the day before planting. Boxes were then weighed to determine weights at 75% field capacity for later watering. After seeding, indaziflam was applied at the following rates (1) 46.7 g ai·ha^{-1}(low), (2) 66.7 g ai·ha^{-1} (mid), (3) 93.4 g ai·ha^{-1} (high), and (4) zero (the control). Indaziflam was applied using a hand-operated backpack sprayer (Solo, Newport News, VA, U.S.A.). After indaziflam application, the boxes were placed 61 cm below PlatinumLED P1200 lights (PlatinumLED, Kailua, HI, U.S.A.) using a randomized design. The LED lights were set to a cycle of 12 hours of light (5:00–17:00) followed by 12 hours of darkness, per manufacturer specifications for germination and seedling growth. The grow room was set to 22°C temperature and 50% relative humidity. Boxes were watered daily to 75% field capacity by weight for 2 weeks, then every other day for the remainder of the experiment.

Measurements

The final density, height, leaf number, and leaf length for grasses were collected 7 weeks after planting. Final density was collected by digging up a container and separating and counting individual plants. Height, leaf number, and leaf length were measured on 10 randomly selected plants per container. If there were fewer than 10 plants in a container, all plants were measured. Height was measured from the base of the plant aboveground to the tallest green tip of the plant. Leaf length was measured to the end of the green portion of each leaf blade. After these measurements, each plant within a container was clipped as closely to the roots as possible and placed in a drying oven set at 50°C. Plants were pooled for each container and were dried for at least 72 hours then were weighed.

Sagebrush final density, height, leaf number, longest leaf length, and canopy diameter were measured 10 weeks after planting. Diameter was estimated by averaging the width of the plant parallel to the long edge of the container and the second measuring the width of the plant perpendicular to the first width. Sagebrush aboveground biomass was determined using the same method as the grasses.

Statistical Analysis

Mixed model analysis of variance (ANOVA) was used to compare seeds incorporated into HPPs with bare seed at different levels of indaziflam application (SAS ver. 9.4; SAS Institute Inc., Cary, NC, U.S.A.). Treatment (i.e. HPPs or bare seed) and rate were fixed variables, while replicate and treatment by replicate were random variables in the models. Data were analyzed individually by species. Effects and differences in treatment means were considered significant if p values were ≤ 0.05 and means are reported with SEs (mean ± SE). Treatment means were separated using the least squares (LS) means procedure in SAS. All data reported were original data (nontransformed).

Results

Bluebunch wheatgrass density, height, leaf number per plant, mean leaf length, leaf width, and total container aboveground biomass were significantly affected by treatment, herbicide rate, and the interaction between herbicide rate and treatment ($p < 0.05$; Fig. 1A–F). In the absence of indaziflam, HPPs appear to have a slightly negative effect on height (Fig. 1B), leaf number (Fig. 1C), leaf length (Fig. 1D), and leaf width (Fig. 1E). However, when indaziflam was applied, bluebunch wheatgrass abundance and other measured characteristics were greater in the HPP treatment compared to the bare seed treatment ($p < 0.05$; Fig. 1A–F). Bare seed bluebunch wheatgrass failed to establish and survive for the duration of the study at mid and high rates of indaziflam application. Even with low indaziflam application, few bare seed bluebunch wheatgrass survived the duration of the study and growth was suppressed (Fig. 1A–F). Bluebunch wheatgrass density, height, leaf length, and container biomass generally decreased with increasing herbicide rate in the HPPs treatment (Fig. 1A, B, D, F).

Sagebrush height, diameter, and container biomass were affected by treatment, herbicide rate, and the interaction between treatment and herbicide rate ($p < 0.05$; Fig. 2B–D). Sagebrush density was influenced by herbicide rate and the interaction between herbicide rate and treatment ($p < 0.05$) but was not affected by treatment alone ($p = 0.10$; Fig. 2A). When indaziflam was not applied, sagebrush density and biomass were greater in the bare seed compared to HPPs treatment (Fig. 2A, D). When indaziflam was applied, HPPs had greater density, height, diameter, and biomass at all rates ($p < 0.05$; Fig. 2A–D). Sagebrush bare seed container biomass was more than four times greater than the biomass in the HPPs container without indaziflam application (Fig. 2D). Density of medusahead, the bioindicator of indaziflam effectiveness, varied by herbicide application rate ($p < 0.001$). Density was lower in low, medium, and high herbicide application rates compared to the control ($p < 0.001$; Fig. S1, Supporting Information). However, there was no difference between the low, medium, and high rates ($p > 0.05$; Fig. S1).

Discussion

HPPs have potential to be used with indaziflam to increase native perennial plant species establishment in annual grass-invaded rangelands. Increased establishment of native perennial species using HPPs and preemergent herbicide could help increase the success of restoration because perennial species could be established before invasive species begin to reinvade, providing a competitive barrier to reinvasion and reducing the likelihood of needing repeated herbicide treatments. The results of our study indicate that HPPs provide protection for two native species, a shrub and a perennial grass, from indaziflam at all application rates. Herbicide protection generally decreased as indaziflam application rate increased but was still effective at the highest rate of indaziflam application (93.4 g ai·ha^{-1}). This is the first study to evaluate use of HPPs with indaziflam applications and provides evidence that supports previous assumptions that HPPs will provide protection for seeded species from a variety of preemergent herbicides (Madsen et al. 2014; Davies et al. 2017). Additionally, this is the first study which provides evidence that HPPs can be used with shrubs, suggesting that HPPs may have wide applicability

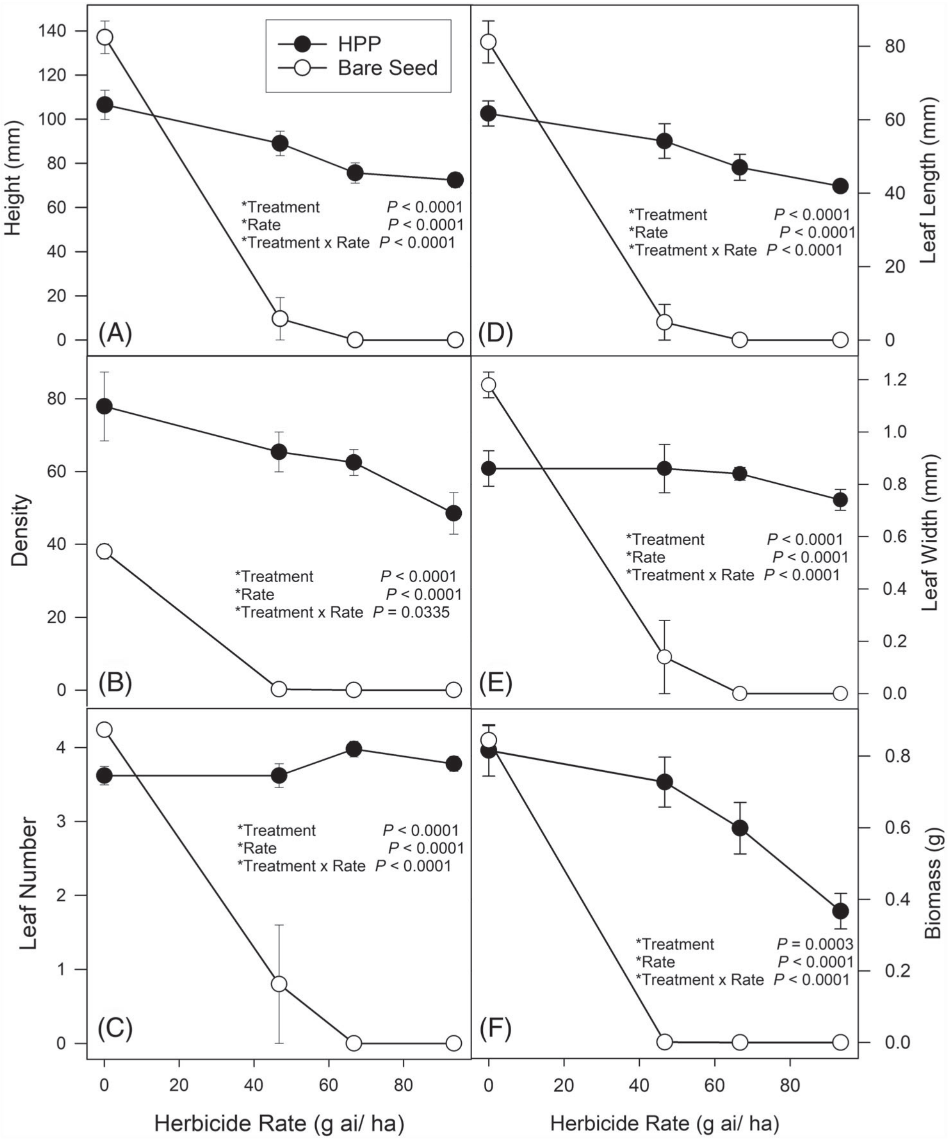

Figure 1. Bluebunch wheatgrass aboveground height (A), density (B), leaf number (C), leaf length (D), leaf width (E), and total container aboveground biomass (F) (means ± SE) for bare seed (open circle) and HPP (solid circle) treatments across increasing indaziflam application rates.

for restoration of multiple plant functional groups in exotic plant-invaded communities.

Integrating HPPs with indaziflam application contributed to larger plants and greater abundance of bluebunch wheatgrass and Wyoming big sagebrush in a grow room study. This method should be researched in the field because presence of invasive species is often one of the major limiting factors to restoration success (Masters et al. 1996). Invasive annual grasses limit establishment of native perennial grass seedlings through physical litter barriers (Evans & Young 1970; Young 1992) and competitive use of soil water and nutrients (Booth et al. 2003; Humphrey & Schupp 2004; Burnett & Mealor 2015). Decreased competition during early seedling growth may substantially improve native perennial vegetation establishment (Burnett & Mealor 2015). Thus, if use of HPPs increases native bunchgrass establishment, once established, native perennial vegetation can effectively compete with invasive annual grasses and help prevent annual re-dominance (Davies & Johnson 2017).

Our results show that HPPs are effective when used with indaziflam. Although not currently registered for use on grazing lands (Bayer 2018), our results indicate that indaziflam may be a promising restoration tool for annual grass-invaded communities. Indaziflam has longer soil activity compared to other common preemergent herbicides (Sebastian et al. 2016*a*, 2016*b*, 2017*a*, 2017*b*) that affords seeded restoration species a longer establishment window before experiencing competition from reinvading annual grass. However, land managers cannot seed until indaziflam soil activity significantly diminishes in order to avoid desired-species damage. The delay in seeding

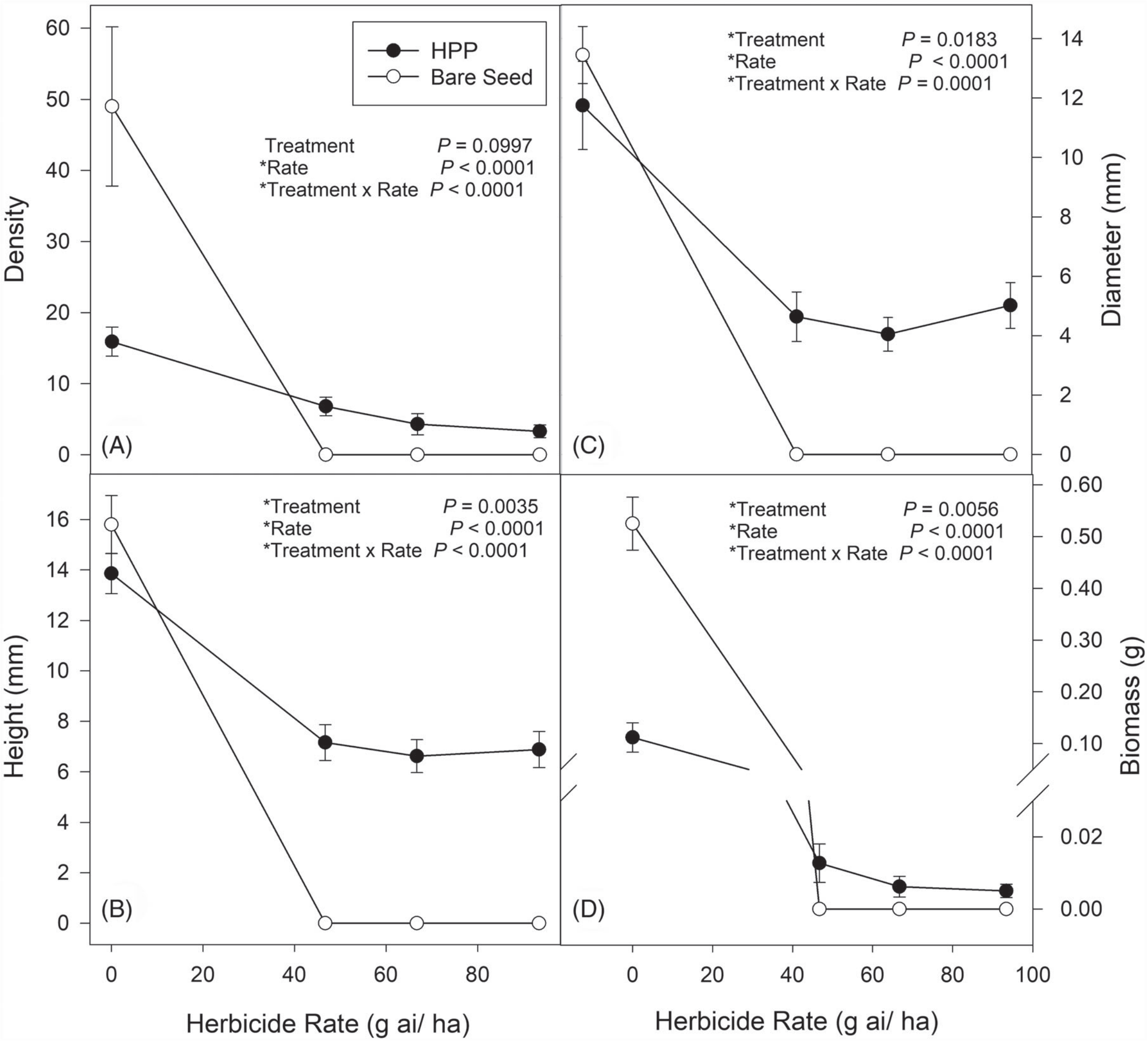

Figure 2. Wyoming big sagebrush density (A), aboveground height (B), diameter (C), and total container aboveground biomass (D) (means ± SE) for bare seed (open circle) and HPP (solid circle) treatments across increasing indaziflam application rates.

after indaziflam would therefore be longer than the delay after application of other, common preemergent herbicides. Integrating HPPs with indaziflam also increases the time that seeded species have to grow when competition from annual grasses is limited. This may lead to greater establishment and growth of seeded species, increasing the likelihood that they would limit reinvasion by exotic annuals.

Our results suggest that HPPs protection may decrease with increasing indaziflam application rate. This was evident as the size and density of bluebunch wheatgrass decreased with increasing indaziflam application rate. This was likely because AC has a maximum adsorption capacity for any given substance (Lladó et al. 2015). Additionally, as herbicide application rates increased, more herbicide may have leached underneath the HPPs where it could contact plants' roots as they grew into the soil beneath the HPP and may inhibit growth. A decrease in HPP's protection with increasing herbicide application rate was less obvious for Wyoming big sagebrush. This may have been because the smaller seeds had more AC per seed to act as an herbicide adsorbent, sagebrush roots did not grow past the protective barrier of the HPP as bluebunch wheatgrass roots may have (personal observation), or because indaziflam mainly targets annual grasses and broadleaf weeds, not shrubs (EPA 2010). It is also possible that relatively small effects of different herbicide application rates were not detectable due to sagebrush's reduced emergence and density in the HPPs compared to bluebunch wheatgrass. Despite decreases in protection afforded by HPPs with increasing herbicide application, HPPs still provided protection for seeded species at the highest application rate.

Growth characteristics and abundance for both species were generally greater for bare seed than for seed incorporated into HPPs when indaziflam was not applied. This indicates that HPPs may hinder the emergence and growth of plants. This trend was more pronounced in sagebrush compared to bluebunch wheatgrass. It is possible that sagebrush was more inhibited by

HPPs because sagebrush seed is very small, only has the ability to emerge from a depth of approximately 5 mm, and can be easily restricted by soil crusts (Jacobson & Welch 1987; Madsen et al. 2012, 2016*b*). The clay and powdered AC used in the HPPs may have compacted when compressed through the die and thus presented a physical barrier to seedling emergence similar to a soil crust. Additionally, since HPPs have a diameter of 8 mm, some sagebrush seed may have been too deep to emerge. Further research is needed to refine the HPP formulation to reduce its inhibition of small-seeded species emergence. This may include reducing the clay component of the formula or by adding a fibrous component to help limit compaction. The HPPs used in this study also had a similar, though smaller, effect on the emergence of bluebunch wheatgrass, a much larger seeded species. Despite the limits to seedling density and growth, the benefits of HPPs could outweigh their costs when used in combination with preemergent herbicides because they increase potential seedling establishment.

HPPs expand our options to restore exotic annual-invaded wildlands. Long-term control of invasive weeds is often limited with herbicides alone and results in rapid reinfestations before native plants are restored (Sebastian et al. 2017*a*). HPPs, when combined with a preemergent herbicide, may enhance the control of invasive weeds by increasing the establishment of desired species and limiting reinfestation during seedling growth. Invasive weeds are problematic worldwide and therefore HPPs may have broad applicability to increase success of restoration efforts. HPPs in combination with preemergent herbicide may be especially useful in areas where exotic annual species have become problematic such as in Australia and the Qinghai-Tibetan Plateau (Dong et al. 2005; Prober & Thiele 2005). HPPs could also be used in instances where invasive perennial grasses are first controlled with a contact herbicide and then a preemergent herbicide is used to control reestablishment from seed, such as *Aristida junciformis* in Africa (Wiseman et al. 2002). They may also be useful in areas of the world where land management objectives include limiting herbicide use because they may prevent the need for repeated herbicide application by increasing the establishment of competitive desirable species.

Future research in the field to validate the results of this study are necessary because grow room experiments generally limit the amount of stress that seedlings experience. In contrast, rangelands have high annual climactic variability and heterogeneous landscapes. Additionally, field experiments evaluating long-term survival of seedlings established within HPPs are crucial. Soil organic matter content, soil volumetric water content, soil texture, indaziflam application rate, and rooting depth of plants all affect the amount of injury caused by indaziflam to postgerminative establishment of grass species (Gomez de Barreda et al. 2013; Jones et al. 2013; Schneider et al. 2015; Jeffries & Gannon 2016). The long-term effects of HPPs have not been studied and it is unknown if they will limit indaziflam injury beyond early seedling growth.

Despite the limits of a grow room study, there is a growing body of evidence that HPPs are an effective strategy to prevent preemergent herbicide damage to seeded perennial grasses (Madsen et al. 2014; Davies et al. 2017). Though more testing and further refinement of the HPP formula are warranted, our current research suggests that HPPs will likely limit preemergent herbicide effects on other plant functional groups and may be an important new strategy to be used in restoration of annual-invaded ecosystems.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Bare seed medusahead density (mean ± SE) across increasing indaziflam application rates.

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