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## Original Research

# Activated Carbon Seed Technology Protects Seedlings From Two Pre-emergent Herbicides Applied in Tandem <sup>☆,☆☆,★</sup>

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## ABSTRACT

In response to the challenge of simultaneously controlling invasive plant species and restoring desired species, seed technologies have been developed that use activated carbon to protect desired plants from pre-emergent herbicides that target invasive plants, such as herbicide protection pellets (HPPs). One ecosystem imperiled by this challenge is the sagebrush steppe of the Western United States. Land managers in the sagebrush steppe may use consecutive or concurrent applications of different pre-emergent herbicides in order to control invasive annual grasses while restoring desirable perennial vegetation that helps stabilize soil and reduce the frequency of wildfires. We conducted a pot study looking at the efficacy of HPPs for six perennial species with novel herbicide practices used by land managers: an application of both imazapic and indaziflam. The six test species included four bunchgrasses, one shrub, and one forb. The bunchgrass species responded well to the HPPs with similar seedling counts and biomass to bare seed when herbicide was not applied and higher seedling counts and biomass than bare seed when a double herbicide treatment was applied. Our results demonstrate that broader testing of HPPs with the application of both indaziflam and imazapic is needed, especially across wide climoedaphic field conditions.

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## Introduction

Effective control of exotic annual grasses and postcontrol restoration of perennial plant species in historically perennial plant-dominated ecosystems is critical in the Western United States (Sebastian et al. 2016). In the sagebrush steppe ecosystem,

exotic annual grasses are spreading at an incredibly fast rate, with an eightfold increase in extent since 1990 and spreading at a rate of  $>2300 \text{ km}^2 \cdot \text{yr}^{-1}$  (Smith et al. 2022). Although it was assumed that fire perpetuated the spread of exotic annual grasses, recent studies have demonstrated that exotic annual grasses are spreading regardless of fire, but fire is increasing because of exotic annual grass invasion (Smith et al. 2023). The rapid invasion and dominance of exotic annual grasses and increasing risk of fire threaten the persistence of hundreds of sagebrush steppe species of conservation concern and require simultaneous control of exotic annual grasses and re-establishment of perennial vegetation (Davies et al. 2011).

Over the past few decades, the herbicide imazapic (Plateau, BASF Corporation, Research Triangle Park, NC) has commonly been used to control exotic annual grasses in the sagebrush steppe (Kyser et al. 2007). However, many areas treated with imazapic are currently being reinvaded and require repeated management actions. The short duration of control provided by imazapic, 1 yr, may not be sufficient to effectively reduce exotic annual grasses and allow for a long enough establishment window for seeded species. Indaziflam (Rejuvra, Bayer Crop Science, Research Triangle Park, NC) was developed for long-term control of invasive

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species. Although testing is still in preliminary stages, indaziflam has demonstrated higher efficacy than imazapic in long-term control of exotic annual grasses in a sagebrush steppe ecosystem field study (Donaldson and Germino 2022). However, first-year annual grass control with indaziflam is often less effective than second-year control (Donaldson and Germino 2022; Davies et al. 2023). As such, applying imazapic in tandem with indaziflam can provide more effective control of exotic annual grasses (Donaldson and Germino 2022).

A major challenge following annual grass control in areas dominated by annual grasses with limited remnant perennial vegetation is establishing perennial vegetation (Svejcar et al. 2017). Seeding after annual grass control with pre-emergent herbicides can fail to establish seeded species because of nontarget herbicide damage (Davies et al. 2014). In a field test with imazapic and indaziflam applied in tandem, no seeded or hand-planted individuals survived when plantings were done at the time of herbicide application (Donaldson and Germino 2022). To counter this issue, seeding is often delayed until pre-emergent herbicide activity in the soil has decreased. Seeding is often delayed for 1 yr when imazapic is used (Davies 2010; Madsen et al. 2014), but it likely needs to be delayed multiple years with indaziflam (Davies et al. 2023) because of its longer-term soil activity (Sebastian et al. 2016). However, delaying seeding of perennial plants for several years is undesirable and potentially ineffective because exotic annual grasses may invade by the time it is possible to seed desired perennial species (Davies et al. 2014; Madsen et al. 2014; Davies et al. 2017, 2023).

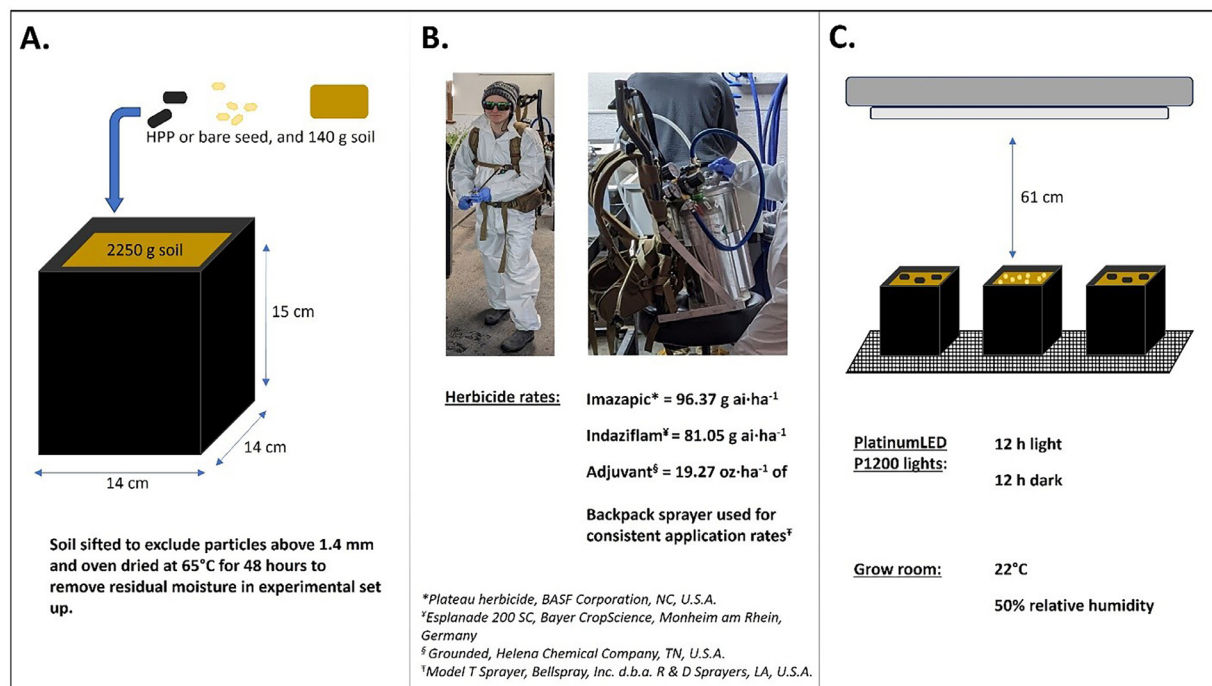
Activated carbon-based seed technologies that reduce herbicide toxicity to desired species and allow a single-entry, simultaneous seeding and herbicide application are being developed (e.g., Madsen et al. 2016; Clenet et al. 2020; Baughman et al. 2021). Although activated carbon-based technologies, such as herbicide protection pellets (HPPs), show promise for improving single-entry seeding and herbicide application in the field (Davies et al. 2017; Clenet et al. 2020; Baughman et al. 2021), no studies have evaluated whether HPPs have the ability to protect desired species when

imazapic and indaziflam are applied in tandem, which may have a larger impact on seeded species than either herbicide applied alone. We asked 1) whether HPPs alter early seedling growth of two native bunchgrasses, two nonnative bunchgrasses, one native forb, and one native shrub relative to bare seeds in the absence of herbicide and 2) whether HPPs improve early seedling growth of species compared with bare seed when two pre-emergent herbicides, imazapic and indaziflam, are applied.

## Methods

The experiment was conducted in a grow room at the Eastern Oregon Agricultural Research Center in Burns, Oregon. The soil used in the experiment was collected in eastern Oregon from the Northern Great Basin Experimental Range (43.489770, –119.710736). The soil is a Gochea sandy loam (US Department of Agriculture Natural Resources Conservation Service (NRCS) 2019). Seeds for the experiment were sourced from commercial seed providers. Four perennial species commonly used in sagebrush steppe restoration were used: two native bunchgrasses, bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve), and two nonnative bunchgrasses, crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.) and Siberian wheatgrass (*Agropyron fragile* [Roth] P. Candargy). Given the low success of native bunchgrasses in many areas, especially at lower elevations, nonnative bunchgrasses are often used by land managers in restoration efforts (Svejcar et al. 2017). We also used a common perennial forb, yarrow (*Achillea millefolium* L.), and shrub, blackbrush (*Coleogyne ramosissima* Torr.).

Seed treatments were bare seed and seed incorporated into HPPs. The HPPs were made following the formula and methodology of Clenet et al. (2019). Each seed treatment then either had a pre-emergent herbicide mix applied (herbicide mix) or not applied (control), resulting in four treatments, and each treatment was replicated five times. The study was conducted in square pots (Fig. 1A), which were filled with soil and completely saturated



**Figure 1.** Experimental setup of the study including planting strategy (A), herbicide application (B), and grow room setup and conditions (C). Pellets were cylindrical in shape and made with a pasta extruder (Model TR110, Rosito Bisani, Los Angeles, CA) following the recipe and methodology of Clenet et al. (2019). HPP indicates herbicide protection pellet.

**Table 1**Six perennial species commonly used in restoration efforts in the sagebrush steppe were tested in this study.<sup>1</sup>

Species	Functional type	Origin	PLS/g	PLS/HPP	Total HPPs/pot	Total PLS: HPP	Total PLS: bare seed
<i>Achillea millefolium</i> L.	Forb	Native	8598	18	2	40	40
<i>Agropyron cristatum</i> (L.) Gaertn.	Grass	Nonnative	248	14	5	70	70
<i>Agropyron fragile</i> (Roth) P. Candargy	Grass	Nonnative	472	22	2	44	44
<i>Coleogyne ramosissima</i> Torr.	Shrub	Native	57	3	5	15	15
<i>Elymus elymoides</i> (Raf.) Swezey	Grass	Native	125	5	5	25	25
<i>Pseudoroegneria spicata</i> (Pursh) Á. Löve	Grass	Native	190	10	5	50	50

<sup>1</sup> The number of pure live seed (PLS) per herbicide protection pellet (HPP) differed by species because the formula used for the HPPs was based on weight (Clenet et al. 2019). Thus, small-seeded species had higher seed counts within a pellet than large-seeded species. A minimum of two pellets and a maximum of five pellets per pot were used when planting to account for variability within pellets and to ensure enough uncovered soil was present around pellets for herbicide movement, respectively.

with water 24 h before planting. Seed weight is used to determine quantity of seed added to ensure flowability of material through equipment in the production process, so HPPs contained different total amounts of pure live seed (PLS) per species, which led to differences in total PLS rates per pot (Table 1). The HPPs were all 8 × 15 mm in size and cylindrical. Bare seed was seeded on top of the soil surface and was covered with 140 g of soil in order to prevent seed movement during watering. The HPPs were gently pressed into the soil to improve seed-soil contact.

After seeding, the two pre-emergent herbicides were applied with adjuvant, which increases herbicide effectiveness per manufacturer recommendations (Fig. 1B). After herbicide application, the pots were placed on grow benches below PlatinumLED P1200 lights (PlatinumLED, Kailua, HI; Fig. 1C) using a complete randomized block design. Pots were watered with ~50 mL of water every other day for the first week of the study to simulate wet spring conditions (Davies and Svejcar, personal observation) and account for higher rates of drydown under the grow lights versus in field conditions. Then, pots were watered to ~50% of field capacity by weight every other day for the final 5 wk. Seedling emergence was checked every few days for the first 3 wk. All grass species appeared to emerge within the second week, but count data were not recorded. Final counts of live seedlings were collected, and then all live individuals were harvested for aboveground biomass 6 wk after planting. Live individuals included plants with at least 75% green biomass because grow lights can occasionally singe the top portion of grown plants, and we did not want to exclude those individuals. Aboveground biomass samples were dried in a drying oven for 72 h at 65°C and then weighed. One of the herbicide treatment pots for *C. ramosissima* was planted as an *A. millefolium*, which resulted in four replicates of *C. ramosissima* and six replicates of *A. millefolium* for the HPP-herbicide treatment combination.

### Statistical analysis

We analyzed data to evaluate whether HPPs impact seedling emergence (no herbicide applied) and are effective in protecting seedlings from herbicide (double herbicide treatment applied) using both count and biomass data. We implemented all analyses in R version 4.2.3 (R Core Team 2023). Species and herbicide treatment were all analyzed separately, with seed treatment (HPP and bare seed) used as the fixed effect. The four bunchgrass species had strong responses to all treatments, but the forb and shrub species had overall low emergence. Thus, for bunchgrass analyses, we used generalized linear models for biomass and count data (stats, glm, v.3.6.2) with a Gaussian and Poisson error distribution, respectively. A log link transformation was used when the dependent variable was count data (Zuur et al. 2007). Log-scale least square means and 95% confidence intervals were back-transformed to the original count scale for clarity in interpretation (emmeans, v.1.8.7; du Prel et al. 2009; Lenth 2023). Given the uneven number

of replicates and low emergence of *A. millefolium* and *C. ramosissima*, a simple Kruskal-Wallis test for the count and biomass data (stats, kruskal.test, v 3.6.2) was used. Model results (Fig. 2) and raw data (Fig. S1; available online at [insert URL here]) are visualized using ggplot2 (v.3.4.2; Wickham 2016).

## Results

### Test of seed treatment: no herbicide applied

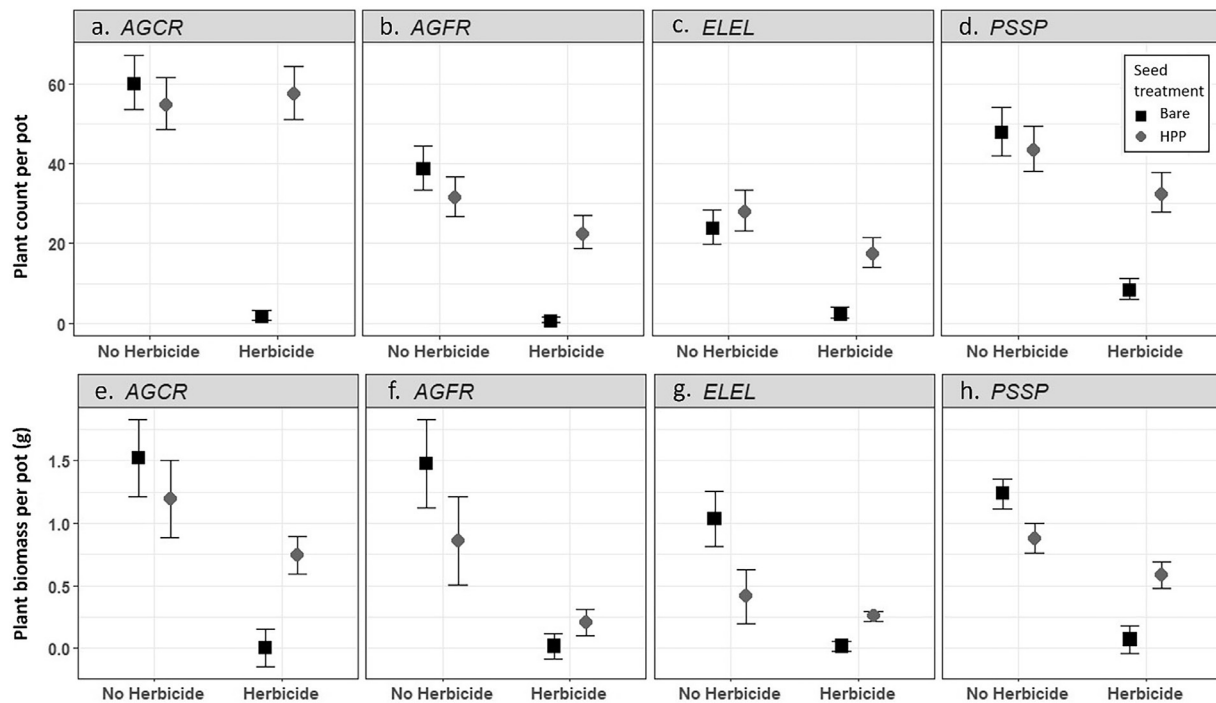
There was no statistical difference in seedling counts between bare seed and HPPs for the four bunchgrasses when grown without any herbicide application (Figs. 2a–d). Similarly, there was no statistically significant difference between seed treatments for *C. ramosissima* ( $P = 0.749$ , Fig. S1a). *A. millefolium* did demonstrate a statistically significant difference between seed treatments for seedling counts ( $P = 0.046$ , Fig. S1a), with HPP treatment having 2.7 times more emergent seedlings compared with the bare seed treatment.

Greater differences between seed treatments without herbicide application were found for biomass (Figs. 2e–h). The two native bunchgrasses, *E. elymoides* and *P. spicata*, had higher biomass in the bare seed compared with HPP treatments (2.5 and 1.4 times greater,  $P < 0.001$  and  $P = 0.001$ , respectively). *A. fragile* also had a difference between treatments ( $P = 0.023$ ), with bare seed having 1.7 times greater biomass than HPP treatments, but confidence intervals overlapped (Fig. 2b). *A. cristatum*, *A. millefolium*, and *C. ramosissima* did not differ statistically for biomass ( $P = 0.122$ ,  $P = 0.601$ , and  $P = 0.754$ , respectively).

### Test of dual herbicide: herbicide applied

For all bunchgrass species, HPP treatments had higher final counts of seedlings than bare seed treatments when herbicides were applied (Figs. 2a–d; all four species:  $P < 0.001$ ). The two non-native bunchgrasses had particularly large differences in counts between seed treatments, with *A. cristatum* and *A. fragile* having 36 and 56 times more seedlings, respectively, in HPP treatments than bare seed treatments. The two native bunchgrasses, *E. elymoides* and *P. spicata*, also had greater counts of seedlings in HPP treatments relative to bare seed (8 and 4 times greater, respectively). No individuals of *A. millefolium* or *C. ramosissima* survived in the bare seed treatment, but HPP treatments averaged 10.8 and 2.8 individuals per pot, respectively (*A. millefolium*  $P = 0.007$ , *C. ramosissima*  $P = 0.004$ ; Figs. S1a and S1b).

Similar to plant counts, biomass measurements of bunchgrasses were greater for HPP treatments relative to bare seed treatments for pots with herbicide application (Figs. 2e–h). The greatest difference between seed treatments was found in *A. cristatum* (117 times greater biomass for HPP relative to bare seed treatments;  $P < 0.001$ ; Fig. 2e). HPP treatments also had greater biomass than bare seed for *A. fragile* (12 times greater;  $P = 0.018$ ), *E. elymoides* (14 times greater;  $P < 0.001$ ), and *P. spicata* (8 times greater;  $P < 0.001$ ). Given



**Figure 2.** Least square means of seedling counts (a–d) and seedling total biomass per pot (e–h) with no herbicide and herbicide applied. Least square means are presented with 95% confidence intervals. Seeding rates per pot were based on seed size, which differed, and as such, comparisons are within species, not between species. Seed treatments include bare seed (Bare) and seed incorporated into activated carbon-based seed technologies (HPP). AGCR indicates *Agropyron cristatum*; AGFR, *Agropyron fragile*; ELEL, *Elymus elymoides*; HPP, herbicide protection pellet; and PSSP, *Pseudoroegneria spicata*.

that no individuals survived for either *A. millefolium* or *C. ramosissima*, the bare seed treatment was zero, and for the HPP treatment, it was 0.03 g per pot for both species ( $P = 0.007$  and  $P = 0.004$ , respectively; Figs. S1c and S1d).

## Discussion

Overall, HPPs did not limit the number of individual plants that emerged and survived when no herbicides were applied. However, there was a reduction in total biomass for the two native bunchgrass species, *E. elymoides* and *P. spicata*. These findings are in line with previous research that found that certain growth characteristics of tested native bunchgrasses were inhibited by HPPs when tested in laboratory conditions and no herbicide was applied (Madsen et al. 2014; Clenet et al. 2019). The only species to have a strong positive effect from the HPP treatment when no herbicide was applied was the forb *A. millefolium*, which had increased counts of individuals, but the biomass between HPPs and bare seed was similar.

When both herbicides were applied, there was a strong positive difference in plant density between seed treatments for all species. Previous laboratory studies found similar results for both native and nonnative bunchgrass species and shrubs (Madsen et al. 2014; Clenet et al. 2019; Baughman et al. 2021). However, we did not find as strong of a difference when accounting for plant biomass and in particular for *A. fragile* and *E. elymoides*. Two factors may have contributed to the lesser difference in biomass. First, there may have been a delay effect on emergence from the HPPs that we did not capture. Second, seedlings may have been impacted by the herbicides even though they were still alive after the short duration of our study. Field studies of HPP technologies demonstrate variability in seedling survival for native and nonnative bunchgrasses using either imazapic or indaziflam (Clenet et al. 2020; Terry et al. 2021; Baughman et al. 2023; Davies et al. 2023). The long-term effects of imazapic and indaziflam applied in tandem on seedling growth

and survival from HPPs versus bare seed require further testing in the field.

Testing of HPP technologies is still in development, with most research to date focusing on native and nonnative perennial grass species (Madsen et al. 2014; Davies et al. 2017; Brown et al. 2019; Clenet et al. 2019; Terry et al. 2021; Baughman et al. 2023) and one shrub species, big sagebrush (*Artemisia tridentata* Nutt.; e.g., Davies et al. 2018; Clenet et al. 2020; Baughman et al. 2021, 2023). Our study demonstrates that HPPs may be effective when used with forb and shrub species other than *A. tridentata*. Both the forb and shrub species we tested (*A. millefolium* and *C. ramosissima*, respectively) demonstrated greater survival and biomass when incorporated in HPPs when herbicides were applied. *A. millefolium* also had greater emergence and survival in HPPs relative to bare seed, even when herbicides were not applied. However, both species had overall low emergence, which may be the result of seed storage conditions or specific germination requirements (Allison 2002; Meyer and Pendleton 2005). Our study demonstrates that HPPs may alleviate the challenge of restoring certain species while controlling invasive annual grasses under some conditions. Larger scale field studies testing the effects of various HPP technologies in protecting against simultaneous applications of indaziflam and imazapic for a diversity of species and through both space and time are warranted.

## Declaration of competing interest

Authors declare that they have no competing interests.

## CRediT authorship contribution statement

**Lauren N. Svejcar:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Danielle R. Clenet:** Conceptualization,

Writing – original draft, Writing – review & editing, Methodology. **Christie H. Guetling:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Kirk W. Davies:** Conceptualization, Writing – original draft, Writing – review & editing.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.rama.2024.05.006](https://doi.org/10.1016/j.rama.2024.05.006).

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