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RESEARCH ARTICLE

Evaluating different rates of activated carbon in commercially produced seed coatings in laboratory and field trials

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Pre-emergent herbicides, commonly employed for managing invasive annual plants, often fail to meet restoration targets due to the absence of remnant perennial plants, which leaves sites vulnerable to re-invasion and hinders effective control of annual grasses. Combining an herbicide treatment with seeding is therefore desirable, but seeded plants can also be negatively impacted by pre-emergent herbicides. Herbicide protection (HP) seed technologies use activated carbon to adsorb herbicide near seeds and have shown promise for allowing simultaneous deployment of herbicide and seed, but recent research recommends numerous additional refinements be tested. We addressed some of these recommendations through one laboratory and a field trial replicated at multiple sites to explore whether commercially produced, single-seed HP coatings with two different rates of activated carbon can prevent herbicide-related damage to two perennial bunchgrasses native to the western United States. We also investigated how these coated prototypes compare in performance to the multi-seed extruded herbicide protection pellets (HPPs) tested in prior research. In the laboratory, neither coating treatment reduced total emergence, emergence rate, survival, or biomass in the absence of herbicide. In the presence of herbicide, both provided several-fold higher survival and aboveground biomass compared to untreated bare seed, but this represented incomplete protection from herbicide. In field trials where conditions were harsher than the laboratory, we found no evidence of HP from any treatment, and HPPs reduced seedling count for one species. We conclude that the tested HP coating prototype is an improvement over HPPs but requires additional refinements and testing.

Key words: HPP, imazapic, invasive annual grass, pre-emergent herbicide, seed enhancement technologies

Implications for Practice

- There is a great need to improve plant restoration success amid invasions of exotic annual plants, and herbicide protection (HP) seed technology could be a useful tool to make the most of preemergent herbicide applications by enabling the establishment of desired species during peak herbicide efficacy.
- An iterative and comprehensive process of refinement of HP technology prototype design, formulation, and delivery is needed to optimize their performance and ensure scalability.
- Recent refinements to HP seed technology tested here and elsewhere demonstrate progress being made despite the need for continued development and expanded assessment of performance in a broader spectrum of sites and site conditions.

Introduction

Many ecosystems across the globe are experiencing significant losses of biodiversity and function due to invasion by exotic annual plants (Duncan et al. 2004; Pejchar & Mooney 2009;

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Kerns et al. 2020). The use of pre-emergent herbicides is a globally popular method for temporarily controlling these annual plants (Weidlich et al. 2020), especially grasses (Kyser et al. 2013; Mangold et al. 2013; Clark et al. 2019). However, temporary control without the immediate release or restoration of desirable plant species will not lead to lasting improvements in ecosystem function (Davies et al. 2014; Davies & Johnson 2017). In sites where few desirable plants remain, successfully combining pre-emergent herbicide use with seeding can result in nontarget effects on seeded material (Sheley et al. 2012) or require multi-year approaches that apply seed one or more years after herbicide treatment, both of which risk re-invasion (Kyser et al. 2007).

Herbicide protection (HP) seed enhancement technologies have been developed to allow the simultaneous application of pre-emergent herbicide and seeding for improved dryland restoration outcomes. These seed technologies combine restoration seed with activated carbon, an ingredient which protects the seed from the deleterious effects of pre-emergent herbicide by adsorbing and locking up the herbicide in the immediate vicinity of the seed, allowing the seedling to establish free of competition (Madsen et al. 2014). HP technologies in the form of multi-seed, extruded herbicide protection pellets (HPPs) have shown promising results in both laboratory and field trials (Clenet et al. 2020; Baughman et al. 2023; Munro et al. 2023). However, researchers have expressed several challenges with extruded HPPs, including the incorporation of smaller-sized and oblong seeds (Baughman et al. 2020; Munro et al. 2023), issues with excessive hardness (Brown et al. 2023; Munro et al. 2023), and challenges to scaling up production due to their large size and production logistics (Holfus et al. 2021; Baughman et al. 2023; Duquette et al. 2024). Rotary seed coating, which creates a seed-shaped coating around individual seeds, is an alternative process for adding carbon to seeds (Terry et al. 2021; Brown et al. 2023). Developing a refined and effective HP seed coating would permit (unlike the extrusion pelleting process used to produce HPPs) production-level scaling and cost-efficiency using established processes and facilities, as well as enable the use of conventional seeding equipment. Initial laboratory and field trials using rudimentary carbon seed coatings give some positive evidence of efficacy (Terry et al. 2021; Munro et al. 2023; Duquette et al. 2024) and conclude that further refinements to HP seed coatings are possible.

The amount of activated carbon applied to each seed (hereafter, rate) as well as coating structural integrity (i.e. ability to break apart readily when wetted or when seeds swell and germinate) are two basic parameter of HP seed coating in need of evidence-based refinement (Duquette et al. 2024). The ideal carbon rate should be high enough to convey an acceptable level of HP and low enough to allow germinated seeds to emerge. The HP seed coating integrity should be low enough to reduce any inhibitory effect on emergence that may be incurred by high-integrity coatings, while ensuring that the coating is strong enough to handle transport and handling and to be compatible with typical seeding equipment. In this study, we conducted paired laboratory and field trials to evaluate the effects of two different rates of activated carbon incorporated in HP coatings in the presence and absence of pre-emergent herbicide on two species of perennial bunchgrasses native to the western United States. We also compared the performance of these coatings to the previously tested extruded HPPs as a benchmark of efficacy for the more recently developed, commercially produced coating prototypes. This work builds upon Duquette et al. (2024) by repeating one of their laboratory experiments and adding field trials using seed coating treatments that have lower structural integrity and should have a lowered physical barrier to seedling emergence.

Specifically, using laboratory and field trials, we asked:

(1) Are there differences in final emergence, emergence rate, survival, aboveground biomass, and/or seedling size between seed treatments (bare seed, high-rate activated carbon single-seed coating, low-rate activated carbon single-seed coating, and multi-seed HPP) in the presence of herbicide?
(2) Using the same evaluation metrics, do any of the tested seed treatments inhibit seedling performance in the absence of herbicide?

In the presence of herbicide, we expected seed coatings and HPPs to elicit improved responses (more and larger seedlings) compared to bare seed, with higher rates of carbon conferring larger improvements. In the absence of herbicide, we expected the best-performing seed coatings to have little or no effect on responses compared to bare seed, and for HPPs to have greater inhibitory effects than seed coatings.

Methods

Overview

The goal of our methodological approach was to use a similar experimental design and the same two study species in both the laboratory (one trial) and realistic field conditions (one trial replicated at five sites) to evaluate the ability of several HP treatments to provide measurable protection from pre-emergent herbicide to native bunchgrasses, as well as identify any effects of the HP seed treatments on normal seed-seedling development of those bunchgrasses in the absence of herbicide. Our experiments focused only on the early developmental stages (emergence and multi-leaf seedling), using a 52-day laboratory trial and 8-month field trials.

Study Species and Seed Coating

We used two species of perennial bunchgrasses native to the *Artemisia* spp. (sagebrush) steppe region of the western United States: bluebunch wheatgrass (*Pseudoroegneria spicata*) and bottlebrush squirreltail (*Elymus elymoides*). Both species have similar sized seeds (3.5–4.9 mg) and show no physiological or physical dormancy aside from a brief after-ripening period. We used a single lot of one commercially available seed source for each species ("Anatone" bluebunch wheatgrass, "Turkey Lake" bottlebrush squirreltail), and assessed viability via tetrazolium stain tests for each species and treatment immediately prior to experiments so that the seeding rate could be adjusted to ensure matching numbers of viable seeds per sample. Viability for bottlebrush squirreltail was 92% for bare seed and HPP, and 95% for both coating treatments, and for bluebunch wheatgrass was 98% for bare seed and both coatings, and 90% for HPP.

Three prototypes of HP seed technology were used in our trials: single-seed coating with a low rate of activated carbon (hereafter, low-rate coating), single-seed coating with a high rate (150% of the low rate) of activated carbon (hereafter, high-rate coating), and multi-seed extruded HPPs containing activated carbon and other materials (only used in the field trial). Germains Seed Technology (Gilroy, CA, U.S.A.) produced coating prototypes using a proprietary formula. The specific rates of activated carbon in each coating prototype are currently protected as intellectual property, but the amount per seed was the same across species for each coating prototype. We produced HPPs in-house following the "small" (16 mm long and 8 mm wide) pellet specifications described by Baughman et al. (2023). The single type of activated carbon used in all prototypes is manufactured for the amendment of herbicide-exposed agricultural soils, has a particle size of 149–44 μm, is highly alkaline (pH 10.6–11.3), and is sourced from coal. Both coating prototypes have a physical integrity (hardness) that is high enough to hold together through storage and seeding, but low enough to allow for seedling emergence and/or disintegration by precipitation. The coatings tested here were made with a lower structural integrity formula than those with the same rates of activated carbon tested by Duquette et al. (2024), which were found to reduce the emergence of bluebunch wheatgrass in the absence of herbicide in a laboratory trial.

Laboratory Trial

The single laboratory trial occurred at the USDA ARS Seed Laboratory (Burns, OR, U.S.A.) in a single climate-controlled grow room (maintained at approximately 23°C, approximately 35–50% RH). We used a randomized complete block design with 10 replicates and three factorial effects: species (bottlebrush squirreltail and bluebunch wheatgrass), seed treatment (bare seed, low-rate coating, and high-rate coating) and pre-emergent herbicide treatment (present and absent). This design resulted in 120 experimental units consisting of square plastic pots (14 cm^2 and 2.5 L) filled with 2100 mL of a 1:1 mixture of course mortar sand and sandy loam field soil sieved to 1 mm. Pots were spread across two identical tables exposed to a 12 hours per day photoperiod (Platinum LED P1200 lights, Platinum LED LLC, Kailua, HI, U.S.A.), and saturated with tap water 24 hours prior to seeding to attain field capacity at the time of sowing.

Pots received 25 seeds followed by an even dusting of sieved soil to achieve a planting depth of 2–3 mm. The 60 pots assigned to the herbicide treatment were immediately treated with a mix of herbicide and tap water equal to 730 mL/ha (10 oz./acre) formula of Plateau (BASF Corporation; Research Triangle Park, NC, U.S.A.) via a hand-held spray bottle. The active ingredient in Plateau is ammonium salt of imazapic, composing 23.6% of the formula. This rate of Plateau, also used in the field trial, is on the higher end of the range used by land managers in the study region (438–876 mL/ha; 6–12 oz./acre). We chose this rate because we presume that differences in HP among prototypes under higher rates of herbicide are more useful for continued refinement of the technology than differences under lower rates. The remaining pots were wetted without herbicide using the same method. All pots were placed in complete randomized blocks across two tables, then covered with a clear plastic tarp for 3 days (before lights were engaged) and misted with approximately 5 mL of water daily. We implemented this 3-day period to keep soil moisture high and temporarily offset the need for watering, ensuring herbicide remained concentrated in the seed zone during imbibition and germination. Tarps were then removed, lights engaged, and pots watered with 25–50 mL of tap water daily, as needed, to maintain consistent moisture levels. We monitored daily for emergence (visible cotyledon above soil surface) for the entire 52-day study. We recorded the number of living seedlings (any green tissue) at the end of the study, and measured aboveground biomass by harvesting all living tissue at the root collar, drying at 75°C for greater than 48 hours, and weighing with a microbalance to the nearest tenth of a milligram.

Field Trials

We seeded and applied herbicide to field trials across five sites during 1–18 September, 2021. This timing is a compromise between typical herbicide application timing, which is often a few weeks before this window, and seeding, which is often a few weeks after. The sites, experimental design, and seeding methods were identical to the 2020 planting year described in Baughman et al. (2023), and are only summarized here. The five sites, located in different U.S. states (Idaho [ID], Nevada [NV], Oregon [OR], Utah [UT], and Wyoming [WY]), were all in invaded plant communities in which exotic annual grasses made up at least 80% of the total vegetative cover. Our design consisted of 0.5 × 1.0 m experimental units arranged in a randomized split-plot (split represented by the presence or absence of herbicide application) with seven whole plots per site. We created three 1 m long, 2–3 cm deep furrows simulating drill-seeder rows by hand in each subplot. We hand-sowed samples of each seed treatment that each contained approximately 276 viable seeds per subplot into the furrows and tamped seeds by hand without burial. We used this seeding rate, which is approximately twice the standard rate for wildland seedings (Jensen et al. 2001), to ensure most subplots had at least some emergence in the event of poor establishment conditions. Herbicide was applied via a backpack sprayer within 48 hours of seeding at the same rate as the laboratory trial. We omitted bluebunch wheatgrass at the UT site due to a shortage of coated seed for both prototypes.

Our field trials were marked by notably dry early and mid-spring conditions, with every site experiencing moderate to extreme drought for every month relevant to germination, emergence, and first-season establishment (November–May; Fig. S1). Mean monthly precipitation during the critical window of seedling establishment (February–May, 2022) was 85, 61, 68, 64, and 112% of the 30-year normal for the ID, OR, NV, UT, and WY sites, respectively (PRISM Climate Group 2023). We monitored seedling count and size for seeded species by inspecting the entire subplot area. The count of invasive annual grasses and forbs was assessed by inspecting the entire subplot area unless densities were so high that these counts would regularly be in the hundreds for a given site, in which case the sampling area for that plant group for the entire site and sampling event were downscaled to a portion of the subplot area that would regularly produce fewer onerous counts. These downscaled counts were later extrapolated back to the full subplot area before analysis. We monitored during 2–16 May, 2022, which is considered late in the growing season for first-year seedlings of these species under drought conditions.

Response Variables

In the laboratory, we calculated emergence rate (days to 50% emergence) following Farooq et al. (2005), final emergence as the percent of viable seed sown that emerged at any point during the 52-day trial, survivorship as the percent of emerged plants that were alive at the end of the trial, and aboveground biomass as mean per-seedling biomass by dividing total pot biomass by the number of living seedlings in the pot. We calculated the late spring field seedling count as the percent of viable seed sown that was alive (possessed green or purple tissue). We measured average plant height and leaf count in the field by measuring up to three randomly selected seedlings per subplot. We included dead leaves of living plants in measurements in both the laboratory and field.

Analyses

We assessed emergence (%), survivorship (%), and mean aboveground biomass (mg) in SAS (v 9.4) using the UNIVARIATE procedure to examine data distributions, assess normality, and identify outliers. We analyzed responses separately for each species in a general linear model using the PROC GLM procedure with seed treatment (low-rate coating, high-rate coating, and bare seed), herbicide (present and absent), and their interaction as fixed effects and block as a random effect. We applied a square root-transformation and removed two outliers to improve normality of model residuals for aboveground biomass. We evaluated significant main or interactive effects with post hoc Tukey's honest significant difference tests.

Field trial data consisted of late spring seeded seedling count (%), seeded seedling height (mm), and leaf count (number), and invasive annual grass and forb count (number). We performed analyses separately for each species in a standard least squares mixed model performed in JMP (SAS Institute, Carey, NC, U.S.A.) with seed treatment (low-rate coating, high-rate coating, HPP, and bare seed), herbicide (present and absent), and field site (five sites) as main effects, with all interactions, and whole plot (seven; nested within each site) as a random effect. We applied a natural log transformation to improve normality of model residuals for seedling leaf count. We evaluated significant main or interactive effects with post hoc Tukey's honest significant difference tests. Results were considered significant at $p \leq 0.05$ in all laboratory and field tests.

Results

Laboratory Trial

The percent of viable seed sown that emerged varied by species and seed treatment in the presence of herbicide (Fig. 1). For bluebunch wheatgrass, emergence from both coatings was 42–47% greater than bare seed (seed treatment × herbicide interaction; $F_{[2,45]} = 14$, $p < 0.001$) and was equivalent to emergence among bare seeds not treated with herbicide. For bottlebrush squirreltail, there were no effects of seed treatment or herbicide treatment. In the absence of herbicide, there were no effects of seed treatment on seedling emergence for either species.

Emergence rate (days to 50% emergence) varied by species, seed treatment, and herbicide treatment (Fig. 1). For bluebunch wheatgrass, emergence rate did not differ among seed treatments in the absence of herbicide (4.3–4.6 days), but emergence rates of both coatings (6.2–6.7 days) were almost twice as fast as bare seed (11.9 days) in the presence of herbicide (seed treatment × herbicide interaction; $F_{[2,43]} = 6.63$, $p = 0.003$). There were no differences in emergence rate in the presence compared to the absence of herbicide for either coating. There was no effect of any experimental factor on the emergence rate of bottlebrush squirreltail.

Survival of emerged seedlings and aboveground biomass varied by seed treatment and herbicide treatment in similar patterns for both species (Fig. 1). In the presence of herbicide, for both species, survival and biomass were higher for low- and high-rate coatings than for bare seed. Specifically, bottlebrush squirreltail survival was 200–340% greater and biomass was 250–350% greater for coatings than for untreated bare seed, and bluebunch wheatgrass survival was 70–80% greater and biomass was 150–200% greater for coatings than untreated bare seed (seed treatment × herbicide interactions for both responses; bottlebrush squirreltail: $F_{[2,44–45]} = 9.2–12.3$, $p < 0.001$; bluebunch wheatgrass: $F_{[2,44–45]} = 6.4–17.5$, $p < 0.004$). Survival and biomass were lower in the presence than the absence of herbicide for both species for all seed treatments (particularly bare seed, as described above), with a greater difference for bottlebrush squirreltail (67–93% lower survival and 84–97% lower biomass) than bluebunch wheatgrass (21–57% lower survival and 80–93% lower biomass). In the absence of herbicide, there was no effect of seed treatment for either species on survival or aboveground biomass. There were no differences between low- and high-rate coatings for any response for either species.

Field Trials

There were differences in seedling count among the seed treatments that varied by species as well as site (Figs. 2 & S2). For bottlebrush squirreltail, seedling count of both low- and high-rate coating did not differ from bare seed at any of the five sites, and HPP had equal or lower seedling count than other treatments depending upon the site (site × seed treatment interaction, $F_{[12,210]} = 3$, $p = 0.001$). Specifically, HPP had lower bottlebrush squirreltail seedling count than all other treatments in NV, lower than bare seed in OR and UT, lower than low-rate coating in OR, and did not differ from any treatment in

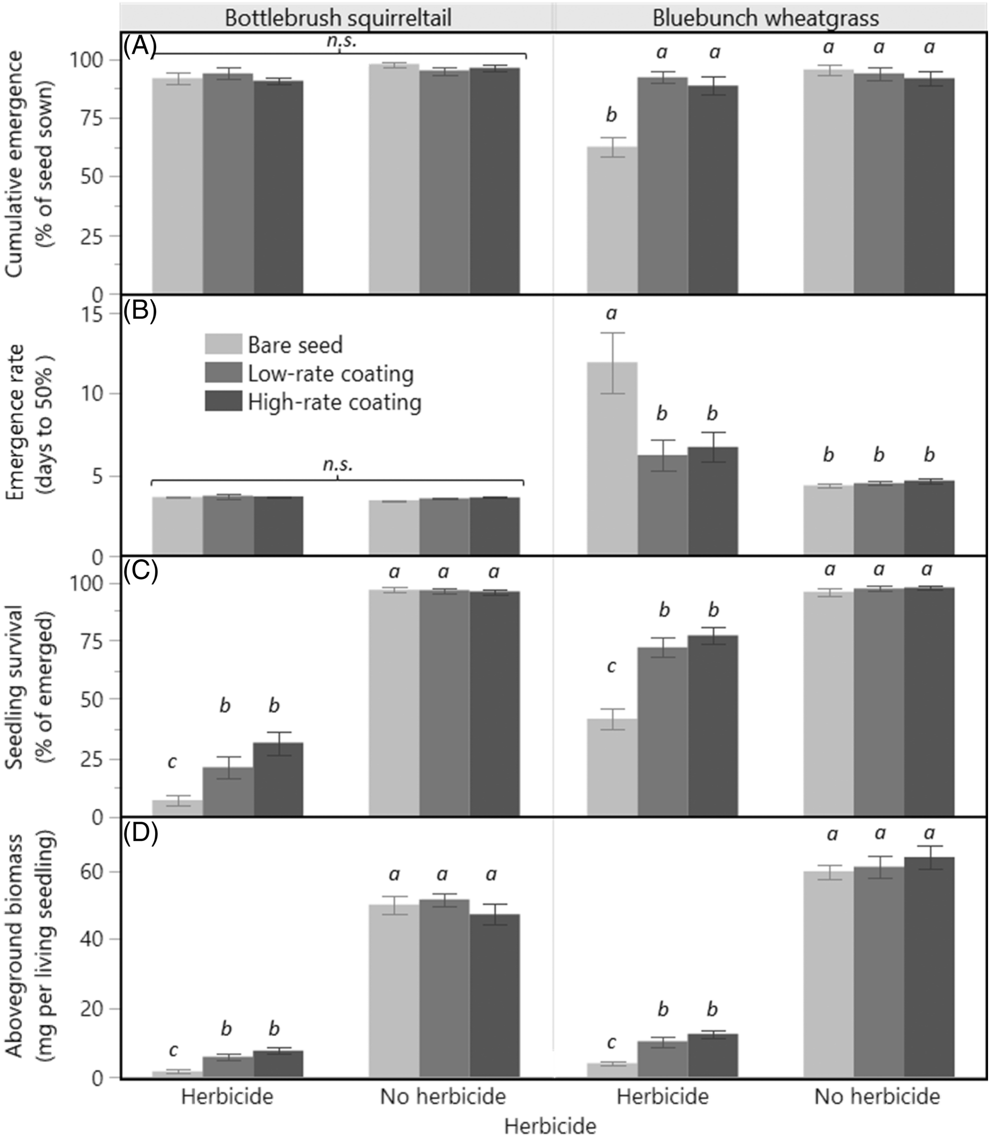

Figure 1. Effects of seed treatment on cumulative emergence (panel A), emergence rate (B), seedling survival (C), and aboveground biomass (D) of bottlebrush squirreltail and bluebunch wheatgrass seedlings in the 52-day laboratory trial. For each species and response variable, bars that do not share letters are significantly different as determined by post hoc Tukey's Honestly significant difference (HSD) tests at the $p = 0.05$ level, and bars marked "n.s." had no significant main or interactive model effects. Error bars are ± SE.

ID and WY. This pattern of differences among seed treatments was observed in both the presence and absence of herbicide (all interactions involving seed treatment and herbicide: $F_{[3–12,30–120]} < 2.2$, $p > 0.085$). Seedling count for bottlebrush squirreltail was 20–81% lower in the presence than the absence of herbicide, regardless of seed treatment, with the largest decrease in WY (81% lower) and ID (63% lower) and the least in UT (22% lower) and OR (20% lower) (site × herbicide interaction; $F_{[4,210]} = 7.2$, $p < 0.001$; not shown). For bluebunch wheatgrass, differences among seed treatments varied by herbicide presence (Fig. 2). In the presence of herbicide, there were no differences in seedling count among seed treatments (herbicide × seed treatment interaction, $F_{[3,167]} = 2.9$, $p = 0.036$), and all seed treatments had 22–74% lower seedling count compared to the absence of herbicide, with the greatest reduction in WY (74% lower) and NV (60% lower), and the least in OR (22% lower) (site × herbicide interaction; $F_{[2,167]} = 19.2$, $p < 0.001$; not shown). In the absence of herbicide, the seedling count of the high-rate coating was 38–50% greater than bare seed and HPP, while the low-rate coating had an intermediate seedling count and did not differ from any other treatment (herbicide × seed treatment interaction, above).

Seedling size (height and leaf count) varied primarily by species, site, and herbicide treatment. There were only two minor instances of differences in size that were related to seed treatment, for seedling height of bottlebrush squirreltail and leaf count of bluebunch wheatgrass. For bottlebrush squirreltail, seedling height did not differ among seed treatments in either

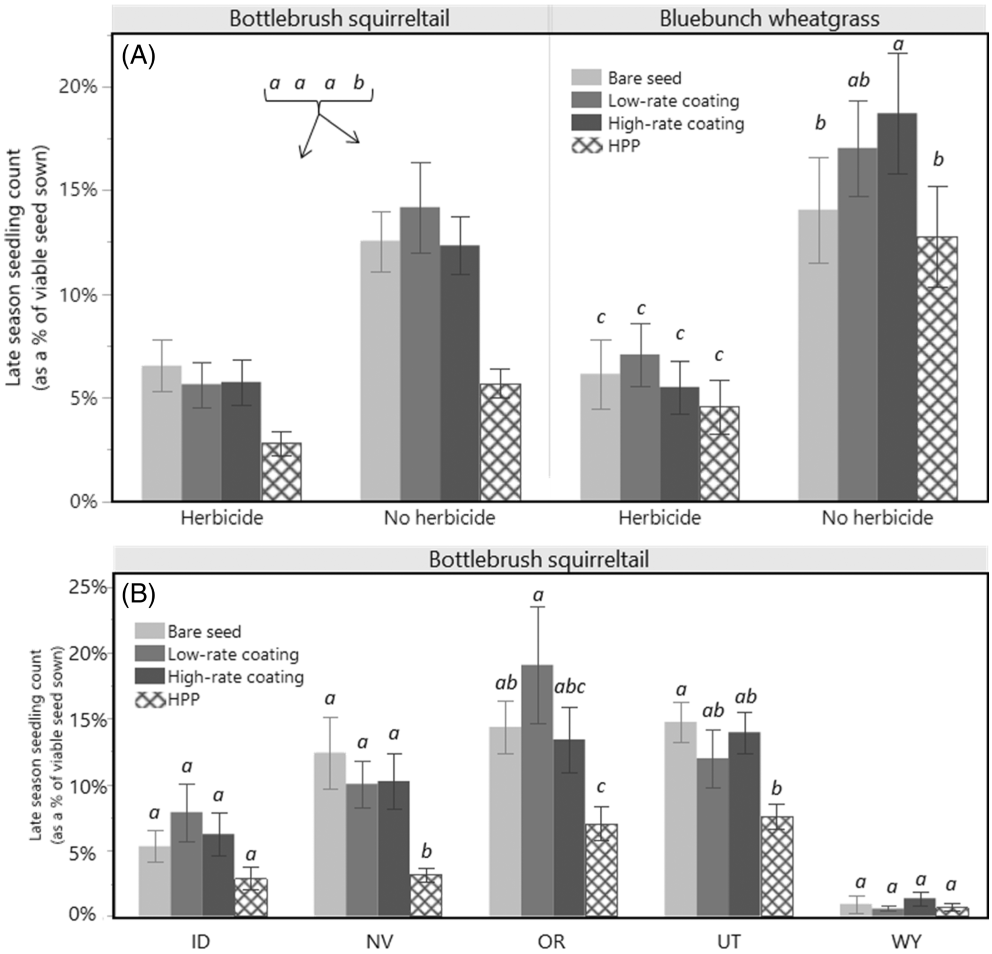

Figure 2. Effects of seed treatment (different shades of bars) on late season seedling count (as a percent of viable seed sown) in the field trials by species for bottlebrush squirreltail and bluebunch wheatgrass and herbicide treatment (panel A) as well as by field site (B; ID, Idaho; NV, Nevada; OR, Oregon; UT, Utah; WY, Wyoming) for bottlebrush squirreltail. For each species in panel (A) and for each site in panel (B), bars not sharing any letters are significantly different as determined by post hoc Tukey's Honestly significant difference (HSD) tests at the $p = 0.05$ level. In panel (A) for bottlebrush squirreltail, there was no herbicide × seed treatment interaction, so Tukey lettering is only given for the main effect of seed treatment. Error bars are ±1 SE. In panel (B), the original lettering from the Tukey HSD tests on the site × seed treatment interaction (available in Fig. S5) has been simplified for interpretability, and therefore letters should only be compared among treatments within a site. There was no treatment by site interaction for bluebunch wheatgrass, so it is omitted from panel (B).

the presence or absence of herbicide, but herbicide was associated with a decrease in height for bare seed (Fig. S3), with no such effect of herbicide for any carbon treatment (treatment × herbicide interaction $F_{[3,190]} = 4.0$, $p = 0.009$). There was no effect of seed treatment on bottlebrush squirreltail leaf count (not shown), and herbicide reduced leaf count by 22% in WY, increased it by 75% in NV and 36% in UT, and had no effect in ID and OR (state × herbicide interaction, $F_{[4,193]} = 16.7$, $p < 0.001$; Fig S3). For bluebunch wheatgrass, there was no effect of seed treatment on seedling height, and the presence of herbicide reduced seedling height at only one site, WY, by 67% (state × herbicide interaction, $F_{[3,145]} = 8.06$, $p < 0.001$; Fig S4). Bluebunch wheatgrass leaf count varied by seed treatment only in WY, and only in the presence of herbicide, with HPP having 28–30% leafier seedlings than all other treatments including bare seed (site × treatment × herbicide interaction, $F_{[9,145]} = 2.09$, $p = 0.034$; not shown). Bluebunch wheatgrass leaf count was higher in the presence than the absence of herbicide in OR (46% higher) and NV (35% higher), but 12% lower in the presence of herbicide in WY and unaffected by herbicide in ID (state × herbicide interaction, $F_{[3,146]} = 13.8$, $p < 0.001$).

Herbicide application significantly reduced the density of invasive annual grasses at all sites and invasive annual forbs at all but the WY site (Fig. S5). Reductions in the density of living invasive annual grass by the late spring was 91–98% for all sites except for UT, where it was 59% (herbicide × site interaction, $F_{[4,760]} = 64.5$, $p < 0.001$). Reductions in invasive annual forbs were 90–99% in all sites but WY, where there was no reduction associated with herbicide application and forb densities were lower than at any other site (herbicide × site interaction, $F_{[4,760]} = 83.8$, $p < 0.001$).

Discussion

HP seed technologies are being developed for use in the western United States (Madsen et al. 2014; Clenet et al. 2020; Baughman et al. 2023) and western Australia (Brown et al. 2018; Brown et al. 2023; Munro et al. 2023) to facilitate simultaneous application of pre-emergent herbicides and reseeding of desirable species. We compared several HP technologies in laboratory and field trials for two perennial bunchgrass species, including multi-seed pellets (HPP) that have been tested by Madsen et al. (2014), Davies et al. (2017), and Brown et al. (2018) as well as several single-seed coatings that are more refined than those tested by Holfus et al. (2021) and Baughman et al. (2023) and have a lower physical integrity than those tested by Duquette et al. (2024). A successful demonstration of HP seed technology efficacy should include clear evidence of deleterious herbicide effects on untreated bare seed (control), a dramatic reduction in the densities of targeted invasive species associated with herbicide in the field, improved outcomes of treated compared to untreated bare seed in the presence of herbicide, and no deleterious effects on germination, emergence, or early growth of the treated seed.

The first step in determining whether these prototypes provided protection from the deleterious effects of the pre-emergent herbicide imazapic was to confirm that the herbicide was a notable barrier to the success of seedlings from untreated bare seed. We observed consistently reduced laboratory survival, laboratory biomass, and field seedling density of bare, untreated seed in the presence of herbicide. Herbicide application also led to greater than 90% decreases in the density of targeted invasive annual grasses and forbs in four of five field sites. Interestingly, herbicide reduced the cumulative laboratory emergence and emergence rate of bluebunch wheatgrass but not bottlebrush squirreltail, suggesting that these two species could have different tolerance to the herbicide. Others have reported interspecific variation in imazapic effects on native grasses and forbs of this region (Sheley et al. 2007; Elseroad & Rudd 2011) but these differences are inconsistent and variable.

At first glance, our laboratory trial appears to demonstrate that both the low- and high-rate activated carbon seed coatings provided a high degree of protection from herbicide due to the several-fold greater seedling survival and biomass of coated treatments than of untreated bare seed for both species in the presence of herbicide. However, evidence of HP in the laboratory trial was only partial, because both coating treatments for both species showed reductions in survival (65–70% and 20–23% for bottlebrush squirreltail and bluebunch wheatgrass, respectively) and biomass (70–76% for both bottlebrush squirreltail and bluebunch wheatgrass) in the presence compared to the absence of herbicide. Furthermore, in the field trials, we saw no direct evidence of any of the seed treatments leading to improved seeding outcomes (number or size of seedlings) compared to bare seed in the presence of herbicide. The HPP treatment was associated with seedling counts that were often lower than bare seed and both low-rate and high-rate coatings. Similar side-effects of HPPs have been observed in other recent studies in our region that included both HPPs and coatings (Duquette et al. 2024), though positive effects of HPPs were observed on multiple species in other field tests in our region (Clenet et al. 2020) as well as on some Banksia Woodland species (Brown et al. 2023; Munro et al. 2023). Variability in effectiveness has been common throughout the history of HPP evaluation, and we concur with Clenet et al. (2020) in suspecting that this is likely related to how post-seeding weather does or does not aid in breaking down the durable pellets to facilitate the emergence of germinating seeds.

Previous research using less refined single-seed coatings also observed some HP in laboratory tests (Holfus et al. 2021; Duquette et al. 2024) but effects measured in field tests in the presence of herbicide have ranged from mixed (Terry et al. 2021; Brown et al. 2023; Munro et al. 2023) to no clear benefit (Baughman et al. 2023). The discrepancy between our laboratory and field results is likely due to the ability of seedlings to survive and grow in the near-optimal laboratory conditions despite some deleterious, herbicide-induced effects, whereas the challenging conditions of the field trials led to larger consequences of herbicide. Additionally, faster degradation of imazapic has been found in soil that is warmer and wetter (Su et al. 2019), and our laboratory soils likely met these criteria in comparison to our field soils for most of the study period. Either explanation could have led seedlings in field conditions to experience a stronger relative effect of the herbicide. The pre-emergent herbicide imazapic used in these trials inhibits amino acid production, resulting in diminished or halted protein synthesis and cell growth (Little et al. 1991). Young seedlings require rapid root growth, and seedlings with underdeveloped roots can survive, albeit with reduced growth, if near-surface soil moisture levels are sufficient, as they were in the laboratory trial that was watered almost daily. However, even minor differences in seedling root development in semiarid field settings for the species we tested can be highly consequential (Rowe & Leger 2011; Atwater et al. 2015; Foxx & Kramer 2021), and our field trials across three sites occurred in a notable drought year. Our results reflected this logic: laboratory survival of coated seeds was indeed higher than untreated bare seeds in the presence of herbicide (demonstrating partial protection), but the biomass of those plants was nearly as low as the biomass of the few surviving plants from unprotected seed. If those low-growth plants affected by herbicide failed to survive in the drier-than-laboratory conditions of typical field sites, we would expect to see lower overall seedling survival and no difference in seedling counts among treatments in the presence of herbicide, as well as few if any differences in measurements of seedling size (a proxy for biomass). These are precisely the patterns we observed in the field trials. Therefore, increased protection in laboratory settings is needed before detectable protection in most restoration-relevant semiarid field settings should be expected, at least for the rate of herbicide tested here. Additionally, lower herbicide application rates might lead to higher protection efficacy for the tested prototypes, and the lowest rates of this herbicide (imazapic) commonly used by regional land managers can be as much as 40% lower than the rates used in this study. Thus, trials including multiple rates of herbicide are warranted.

In addition to delivering HP, it is also imperative that seed treatments do not impede the development of seeds into seedlings. In both the laboratory and field trials, we found no instances of single-seed coatings impeding any seeding outcomes in the presence or absence of herbicide when compared to bare seed. This is an improvement over similar low- and high-rate activated carbon coatings produced using a high structural integrity formulation (see experiment 2, Duquette et al. 2024), which reduced emergence in the absence of herbicide relative to bare seed. Additionally, bluebunch wheatgrass seedling density in our field trials was slightly higher for the high-rate coating than bare seed in the absence of herbicide, suggesting the treatment may have inadvertently improved germination or emergence conditions for this species. Conversely, HPPs reduced the field density of one species, bottlebrush squirreltail, in the absence of herbicide. Together, these results suggest that our single-seed coatings, which have been purposefully designed to have lower physical integrity are well-formulated to allow the natural progression of germination and emergence. Future coating prototype refinements must continue to avoid increasing the physical integrity of coatings or modifying other characteristics that could result in impeding germination and early seedling development. Additionally, other barriers to early seedling survival could be addressed using one or more other seed enhancement technologies (Berto et al. 2021; Brown et al. 2021; Donovan et al. 2024).

We observed no differences between the high- and low-rate coatings in seedling number or growth in the laboratory or field, and both coatings had higher laboratory survival and biomass than bare seeds. While this may suggest no additional advantage of the higher rate of carbon relative to the low rate, the clear absence of benefits from either the low- or high-rate coating in the field in the presence of herbicide does not support this interpretation. The high-rate coating contains the highest reasonable rate of activated carbon given the rotary coating methods used by our commercial producer. Higher rates are possible but would result in notable increases in the coating structural integrity and hardness (R. Rios 2023, USDA Agricultural Research Service, Burns, OR, personal communication). This is likely to result in reduced seedling emergence due to physical obstruction and is a significant hurdle in low- and variable-moisture environments such as semiarid sites. This tradeoff of additional carbon potentially reducing emergence, combined with no observed difference in HP between the low- and high-rate coatings tested, leads us to believe that further increases in the rate of the specific activated carbon used are not likely to improve the performance of the coatings.

An important caveat of our five-site field experiment is that the single year of the study occurred during a notable drought, and year effects can have strong and complex influence on community assembly in post-restoration scenarios (Werner et al. 2020). Under more mild or wetter than average conditions, it is likely that we could have observed different performance of the seeded species, different effects of the seed treatments on that performance, different effects of herbicide on seed treatments, and thus a different combined influence of all these effects on seeding outcomes. Prior field studies on these and other similar seed treatments have also often occurred during drought years (Davies 2018; Clenet et al. 2020; Baughman et al. 2023). The complex combined influence of different factors makes it difficult to speculate on whether the HP seed treatments tested here might perform better or worse under more mild conditions. However, as described above in comparing laboratory to field results, drought conditions in the field could serve to accentuate small differences in seedling health and vigor among treatments that may be less notable under more optimal conditions.

Evidence of partial protection being achieved in the laboratory for single-seed coatings, along with no evidence of the coatings impeding normal seedling development, leads us to support continued refinement of single-seed coating prototypes until substantially higher protection in the laboratory is achieved or all practical refinements have been exhausted. As mentioned earlier, additional trials exploring whether lower rates of herbicide will increase protection while still adequately controlling invasive plants are another means of refining the HP approach. We also recommend that multi-year, multi-site field trials continue to accompany laboratory trials to better gather evidence of field efficacy to account for the highly variable and often low seeding success that is typical for semiarid drylands (Hardegree et al. 2016; Shackelford et al. 2021; Svejcar et al. 2023). Previous field and laboratory trials have produced mixed results regarding whether HPPs perform similarly to single-seed coatings (Baughman et al. 2023), give limited positive co-benefits to survival unrelated to HP (Munro et al. 2023), or demonstrate aspects of lower performance such as reduced emergence (Duquette et al. 2024). Combining these mixed results with the challenges of producing, transporting, and seeding HPPs at large scales (Baughman et al. 2023) and the complications of successfully incorporating seeds of some species (Baughman et al. 2020; Munro et al. 2023), we continue to recommend further development of single-seed coatings over HPPs for eventual use with landscape-scale restoration efforts seeking to restore on the order of thousands or tens of thousands of hectares. However, we are not discounting the potential of HPPs, as they continue to be refined (e.g. Brown et al. 2023), to be valuable technologies at smaller scales or in more resource-intensive efforts.

One attribute of HP technologies that has yet to be tested in depth is the type of activated carbon used. Only a narrow selection (four types) of activated carbon have been used in nearly all prior HP seed pellet and coating research for native plants, and there have been no published comparisons among multiple sources of activated carbon of their capacity to adsorb imazapic or other modern pre-emergent herbicides when used in seed coatings or pellets. Yet, evidence from other research sectors suggests that the adsorption capacity of different carbons varies with carbon type (Díaz-Terán et al. 2001). Identifying which sources of activated carbon maintain the highest adsorption and retention, even after being combined with other necessary seed coating ingredients, is an important but unaddressed research goal that should be the focus of future studies.

Ourselves and others continue to recommend further research into HP treatments (Baughman et al. 2023; Brown et al. 2023; Munro et al. 2023), despite often mixed results or only modest success. Numerous opportunities exist for continued iterative refinement of these treatments and the general approach of HP. Additionally, achieving even partial, but consistent HP can confer significant restoration benefits given the scale of restoration and rates of failure in invaded dryland systems.

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Supporting Information

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

Figure S1. Output from U.S. Drought Monitor for the western United States, from November 2021 through May 2022.
Figure S2. Panel (B) from Figure 1 with original Tukey HSD lettering, which allows comparison across all sites and treatments.
Figure S3. Mean subplot seedling height for bottlebrush squirreltail, across all sites.
Figure S4. Effect of herbicide treatment (different shades) on seedling height (top) and seedling leaf count (bottom) for both species.
Figure S5. Effects of herbicide treatment (different shades) on the late spring density of living invasive annuals grasses (panel A) and forbs (B) by field site.

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