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Herbicide protection pod technology for native plant restoration: one size may not fit all

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Pre-emergent herbicides are frequently used to control exotic annual plants prior to seed-based restoration, but seeding must generally wait until herbicide toxicity has waned. The emerging seed-enhancement technology of herbicide protection pods (HPP) allows for simultaneous seeding and herbicide application by protecting desirable seeds inside pods or pellets containing activated carbon, allowing for single-entry and potentially cost-saving wildland restoration approaches. This technology has shown promise in multiple recent lab and field experiments. However, the effect of pod size on efficacy has not been formally investigated, and important small-seeded species have either not been tested or have shown less-promising results when used with this technology. Using emergence trials in two different laboratory environments with two small-seeded species important to restoration in the semi-arid western United States (Wyoming big sagebrush [Artemisia tridentata Nutt ssp. wyomingensis] and Sandberg bluegrass [Poa secunda J Presl]), we investigated if HPP size affected early performance and protection from herbicide (imazapic), as well as how different sizes of HPPs compared to bare seed. For both species, smaller HPP sizes selected to match optimal seeding depths showed up to two-fold higher emergence and aboveground biomass than larger pellets and still maintained protection from herbicide toxicity. Both species also showed 50–90% reductions in emergence and aboveground biomass due to incorporation into HPPs in general, resulting in only one species (bluegrass) showing the desired effect of HPPs: higher success than bare seed in the presence of herbicide. We suggest that additional experimentation to improve this promising technology is warranted.

Key words: activated carbon, Artemisia tridentata, extruded pellets, Poa secunda, seed enhancement technology, seed pellets

Implications for Practice

- Reducing herbicide protection pod (HPP) size may improve the performance of small-seeded species when used with this technology, while still maintaining protection from pre-emergent herbicide (imazapic).
- In the absence of herbicide, both species were negatively affected by the HPP technology in general, highlighting a priority for future HPP refinement.
- Additional experimentation with HPP formulation and dimensions to maximize success is highly warranted because it is likely to further enhance the already promising benefit of this technology.
- It is critical that additional experimentation include trials in field sites that mimic realistic restoration scenarios.

Introduction

Invasive annual grasses have devastating ecological effects in arid and semi-arid ecosystems (D'Antonio & Vitousek 1992). In Western North America, invasive annual grasses infest over 23 million hectares of formerly sagebrush and perennial grass-dominated ecosystems and cause tens of millions of dollars in

adverse effects annually (Duncan et al. 2004). Efforts to revegetate such ecosystems via seeding native species are widespread but often unsuccessful (Hardegree et al. 2016), due in part to the high competitive ability of invasive annual grasses (Nasri & Doescher 1995; Rafferty & Young 2002) and their effects on ecosystem processes like wildfire frequency and soil nutrient cycling (Brooks et al. 2004; Adair & Burke 2010). Treatments utilizing pre-emergent herbicides are increasingly recommended to abate invasive annual plants (Johnson & Davies 2015), but such treatments can have deleterious effects on desirable seeds (Davies et al. 2014), and require waiting periods—which may result in reinvasion—before reseeding can occur (Sheley et al. 2012).

Herbicide protection pod technology (HPP) was developed to protect desirable seeds from the effects of pre-emergent

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herbicides by extruding them in a pellet containing herbicide-adsorptive materials such as activated carbon (Madsen et al. 2014), thereby allowing seeded species to establish when herbicide effectiveness is highest and facilitating single-entry restoration strategies (Madsen et al. 2016; Davies et al. 2017). Multiple lab trials have demonstrated increased seedling density and size for HPPs compared to bare seeds of several species under a range of application rates of three pre-emergent herbicides (imazapic, Madsen et al. 2014; indaziflam, Clenet et al. 2019 and simazine, Brown et al. 2018). Field trials using imazapic have also demonstrated the effectiveness of HPP technology for introduced and native perennial grasses (Davies et al. 2017; Davies 2018; Clenet et al. 2020) and some promise for shrubs (Clenet et al. 2020).

The majority of studies using HPP technology indicate success with relatively large-seeded perennial grass species using pillow-shaped pellets measuring 8 mm × 16 mm × 16 mm or cylindrical pellets measuring 8 mm × 16 mm. For these dimensions of HPPs, each seed is surrounded or buried by up to 8 mm of the pellet matrix. This depth is generally less than or equal to the recommended seeding depths for the larger-seeded species being tested (Ogle 2006; Ogle et al. 2009). However, smallerseeded species, which often require shallower seed burial, have shown less success in experimental trials when used in these HPP sizes, perhaps because they struggle to emerge from the relatively large pellets (Davies 2018; Clenet et al. 2019). Therefore, given the importance of many small-seeded species for restoration in various regions, refinement or modification of HPP technology is justified for small-seeded species (Clenet et al. 2019).

We conducted a laboratory experiment in dissimilar laboratory growth environments using two common, small-seeded species native to the western United States, Sandberg bluegrass (*Poa secunda* J Presl.) and Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) to determine (1) if HPP size affects emergence (total and rate) and aboveground biomass, (2) if HPP size affects its protection from a pre-emergent herbicide, and (3) how various sizes of HPPs compare to bare seed with regard to emergence (total and rate), aboveground biomass, and protection from herbicide.

Methods

We used a fully factorial, five replicate experimental design to examine the effects of a seed treatment (small pellet vs. large pellet vs. pillow vs. bare seed control) on the growth of two native species (Wyoming big sagebrush and Sandberg bluegrass) under two herbicide treatments (herbicide vs. no herbicide). We performed identical trials in two separate, dissimilar laboratory environments (grow room vs. growth chamber) to detect environment-related differences in responses.

Seed Selection and Seed Lot Assessment

Wyoming big sagebrush (hereafter, sagebrush), a long-lived perennial shrub preferring 0–3.2 mm seed burial (Tilley et al. 2008a), and Sandberg bluegrass (hereafter, bluegrass), a

shallow-rooted perennial bunchgrass preferring no more than 6.4 mm of seed burial (Tilley et al. 2008b), were chosen due to their small seed size, preference for shallow seeding depths, and importance in regional restoration. The number of whole seeds per bulk gram for each seed lot was determined by averaging 6 sub-gram counts of approximately 100 seeds each. Viability was determined via germination tests in Petri dishes in a growth chamber under conditions described below.

HPP Size Selection

For both test species, we used two HPP sizes (Fig. 1) similar to those used in previous research: a pillow-shaped pellet (8 mm × 16 mm × 16 mm, hereafter "pillow"; Madsen et al. 2014; Clenet et al. 2020), and a cylindrical large pellet (9.5 mm \times 16 mm, hereafter "large pellet" Davies et al. 2017; Davies 2018; Brown et al. 2018; Clenet et al. 2019, 2020) (Fig. 1). In addition, a smaller, species-specific pellet size (hereafter, "small pellet") was developed based on each species' recommended seeding depth to avoid over-burial within the pellet. For bluegrass, small pellets measured 6.4 mm diameter × ~14 mm length to achieve the average preferred seeding depth of 0-6.4 mm. For sagebrush, optimal seeding depth is 0-3.2 mm, but prior research indicated a seed coating with a similar thickness of activated carbon showed inadequate herbicide protection (Madsen et al. 2014). Therefore, for sagebrush, we developed a small pellet size of 4.8 mm diameter $\times \sim 11$ mm length for sagebrush to minimize average seed burial while maintaining herbicide protection. Targeting "average" rather than precise seed burial depths in HPPs is a result of the mixing and production method described below, which generates random seed placement within the HPPs.

HPP Production

Production of HPPs followed Madsen et al. (2014). Teflon dies used to extrude HPPs were drilled and beveled (on inside edge) to produce the specific HPP diameters being tested. Different HPP batches differed only in the amount and type of seed added, and the die used, and contained bentonite clay, activated carbon, compost and worm casting fines, and fungicide (Table S1). Water was added at a rate of 0.647 mL/g of non-seed dry material. Calculations to determine the amount of seed added to each batch utilized seed viability and seeds per bulk gram estimates for each lot, with a target of eight viable seeds per HPP, regardless of size or species.

Laboratory Emergence Trial

Following Clenet et al. (2019), reinforced plastic $56 \text{ cm} \times 34 \text{ cm} \times 7 \text{ cm}$ trays were filled with 13 L of dry, sifted (1.0 mm) soil, and subdivided into eight separate sample zones (hereafter, plots). The soil was collected from a nearby field site at which both study species occur naturally. Twenty trays were watered until the soil was saturated and let rest 24 hours, then, a sample of each treatment (three different HPP sizes plus bare seed \times 2 species) was randomly assigned to each plot and planted. Each



Figure 1. Various sizes of herbicide protection pods (HPPs) and the seeds they contain, including $8 \text{ mm} \times 16 \text{ mm}$ "pillows" with bluebunch wheatgrass seed (far left), $9.5 \text{ mm} \times \sim 16 \text{ mm}$ "large pellets" with bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) seed (middle left), $6.7 \text{ mm} \times \sim 13 \text{ mm}$ "small pellet" with Sandberg bluegrass seed (middle right), and $4.8 \text{ mm} \times \sim 10 \text{ mm}$ "small pellet" with Wyoming big sagebrush seed and chaff (far right). All HPP sizes shown here, but only bluegrass and sagebrush seeds, were used in this study.

HPP sample consisted of 10 HPPs, with weight recorded, placed end-to-end in two equally spaced rows with about one-half an HPP length between each. Each HPP was gently pushed into the soil just enough to ensure it would not roll when trays were moved. For the bare seed treatment, samples of approximately 80 viable seeds were weighed using mean viable seeds per gram estimates and hand broadcast over the same size area occupied by the two rows of HPPs, then evenly covered with 1–2 mm of sifted soil so that seeds would not float away when watered.

After planting, 10 trays were randomly selected and treated with a 105 g ai·ha⁻¹ application of imazapic herbicide using a backpack sprayer. The other 10 were not treated. Five trays from each herbicide treatment were placed in one of two growth environments, both with 12 hour photoperiods: (1) open benches in a grow room at a constant approximately 21-24°C, 24 in. below Platinum LED P1200 light panels (hereafter, grow room), and (2) in Hoffman SG2-22 growth chambers mounted with 500 K, 2360 lm LED lights (hereafter, growth chamber). Two different growth environments were used to improve our ability to detect changes associated with our treatments rather than those arising from a single environment. Trays in both environments were watered with a diffuse spray nozzle on day 2 and then whenever both the pellet and soil surface were dry to the touch, which was every 1-3 days in the grow room; trays in the growth chamber remained saturated and were not watered after day 2. Therefore, the grow room plots experienced repeated drying and wetting cycles while those in the growth chamber did not.

Data Collection and Analysis

Total number of emerged seedlings per sample was recorded five times weekly for 40 days. Emergence was defined as a visible cotyledon. After 40 days, all aboveground live biomass of seeded species was harvested at soil level, dried at 50°C for 4 days, and weighed for each plot.

The final rate of whole seeds per gram of HPP was determined via destructive sampling of five samples of 15 pellets per batch, and multiplying by the germination percent gave a final estimate of viable seeds per gram HPP. Gross counts of emergent seedlings per sample were normalized by this updated estimate prior to analysis. The resulting metric was the percent of viable seeds per sample that emerged (hereafter, emergence of viable seed). Aboveground biomass of the entire sample was divided by the number of seedlings in the sample to obtain per-seedling biomass, and by the estimate of viable seed planted per sample to obtain biomass per viable seed planted. Emergence rate was calculated as time (in days) to reach 50% of maximum emergence (hereafter, time to 50% emergence) following Farooq et al. (2005).

Response variables were final (40-day) emergence of viable seed, maximum 25-40-day emergence of viable seed (see the following paragraph), final aboveground biomass per viable seed planted, final aboveground per-seedling biomass, and time to 50% emergence. Data were analyzed via mixed model ANOVA in JMP v.13, with species (SPEC; bluegrass and sagebrush), seed treatment (TRT; small pellet, large pellet, pillow, and bare seed control), herbicide application (HERB; herbicide treatment and no treatment), and growth environment (ENVR; grow room and growth chamber) as main factors, and planting tray (1-20) as a random factor. Significant differences among factor levels were examined using Student's t tests and linear contrasts on least significant means. A square root transformation was used on all responses (except time to 50% emergence) to improve normality of full-model residuals. Effects and interactions were considered significant if p values were less than 0.05.

Two models were used. A reduced model with the bare seed treatment excluded was used to address questions 1 and 2, which are concerned only with differences among the pellet treatments. A full model including the bare seed treatment was used to address question 3, which concerns pellet treatment efficacy in

comparison to not using the technology (bare seed). Model results for final emergence of viable seed were compared to those of maximum 25–40-day emergence of viable seed to determine if small levels of seedling attrition that occurred before day 40 were unduly affecting results. No changes in main or interactive effects were detected between these two measures of emergence, so we present only final emergence. Similarly, the results for aboveground biomass per viable seed planted were compared to those for per-seedling aboveground biomass, and a lack of notable differences led us to present only aboveground biomass per viable seed planted (hereafter, biomass).

The lack of repeated watering in the growth chamber (see the previous section) likely reduced our ability to assess herbicide effectiveness or protection, because imazapic requires water infiltration to spread into the soil profile (Tu et al. 2001). To determine if this issue was leading to misinterpretation of herbicide-related findings, results from models including both environments were compared to those in which growth chamber data were excluded. No changes in significance to the remaining main or interactive effects were observed, so data from both environments are presented to avoid drawing conclusions from a single environment/trial.

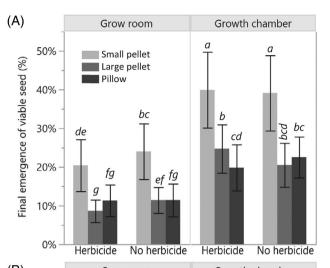
Results

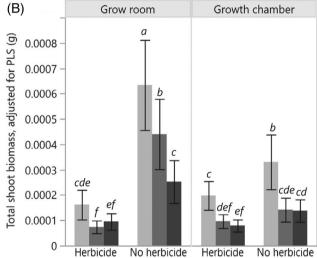
Effect of HPP Size on Seedling Emergence, Biomass, and Emergence Rate

For both species and in both growth environments, the small pellet supported 1.6–2.2-fold higher final emergence of viable seeds than the pillow or large pellet (Fig. 2A, Table S1), regardless of herbicide treatment. This effect was 27% larger for sagebrush than bluegrass (Table S1, SPEC*TRT interaction, reduced model, $F_{2,80} = 8.39$, p = 0.0005). In addition, a significant TRT*SPRAY*ENVR interaction was present ($F_{2,80} = 4.76$, p = 0.0111), but linear contrasts on least significant means indicated final emergence was significantly higher for small HPPs than other sizes in all cases (p < 0.001 for each contrast, not shown).

Mean biomass was significantly higher for the small pellet than for the large pellet (Fig. 2B, Table S1), and in all but one case (when treated with herbicide in the grow room), higher than the pillow as well (TRT*SPRAY*ENVR interaction, reduced model, $F_{2,80} = 4.98$, p = 0.0092). Biomass for the large pellet was generally not different from the pillow except for when untreated with herbicide in the grow room (TRT*SPRAY*ENVR interaction, reduced model). Mean biomass across all HPP sizes was 12-fold lower for sagebrush than bluegrass (SPEC main effect, $F_{1,80} = 636$, p < 0.0001).

Time to 50% emergence differed among HPP sizes only in a few instances and only for sagebrush (Fig. 2C, Table S1). Specifically, sagebrush pillows took around 10 days longer to reach 50% emergence than both large and small pellets in the herbicide treatment, and around 3 days longer than large pellets (but not small pellets) when untreated with herbicide, regardless of the environment (Fig. 2C, SPEC*TRT* SPRAY interaction, reduced model, $F_{2.78} = 7.23$, p = 0.0013). The effect of the





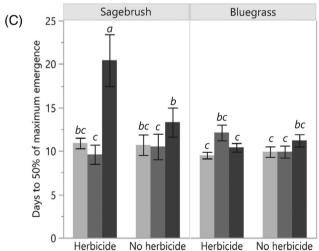


Figure 2. Final emergence as a percent of viable seed planted per plot (A), total dry shoot biomass (grams) per viable seed planted per plot (B), and days to 50% of maximum emergence per plot (C) for three sizes of herbicide protection pods (small pellet, large pellet, and pillow; see Methods), with bare seed control treatment excluded. Within each panel (A, B, C), bars sharing the same lowercase letters are not significantly different based on Student's t test ($\alpha = 0.05$). Error bars represent \pm SE of untransformed data.

random factor (planting tray) was not significant on any response in the reduced model (p > 0.05 for all), explaining less than 7.5% of variation in responses.

Effect of HPP Size on Herbicide Protection

There was no main effect of herbicide treatment on final emergence of viable seeds for HPP treatments, but a significant TRT*SPRAY*ENVR interaction (Fig. 2A; Table S1, reduced model, $F_{2,80} = 4.76$, p = 0.0111) revealed that herbicide application significantly reduced final emergence for large and small pellets in the grow room, but not for pillows in the grow room or any size HPP in the growth chamber. This effect was regardless of species. Biomass was significantly reduced by herbicide treatment for all HPP sizes regardless of species or environment, with the exception of large pellets in the growth chamber, where no effect was observed (Fig. 2B, TRT*SPRAY*ENVR interaction, reduced model). In all cases, the effect of herbicide on HPPs was less in the growth chamber than in the grow room.

Comparison of HPPs to Bare Seed Seedling Emergence, Biomass, and Emergence Rate

When the bare seed treatment was included as a control for seed treatment, final emergence of viable seeds varied by seed treatment, species, herbicide treatment, and environment (Fig. 3A, Table S1, full model). The bluegrass bare seed treatment produced significantly higher emergence than any HPP size when untreated with herbicide, and significantly lower emergence than any size HPP when treated. For sagebrush, bare seeds had higher emergence of viable seed than any size of HPP regardless of herbicide treatment or growth environment, although this difference was less when treated than when untreated with herbi-(SPEC*TRT*SPRAY cide interaction, model, $F_{3.112}$ = 8.56, p < 0.0001), stemming largely from the growth chamber, where the effect of herbicide on bare seed plots of both species (a 37-51% reduction) was significantly less than in the grow room (a 96-99% reduction; TRT*SPRAY*ENVR interaction, full model, $F_{3,112} = 25.9$, p < 0.0001).

When untreated with herbicide, biomass was significantly higher in bare seed plots than for any HPP size, regardless of species or growth environment (TRT*SPRAY interaction, Fig. 2B, Table S1, full model, $F_{3,112} = 125$, p < 0.0001). When treated with herbicide, for bluegrass, the small pellet produced significantly higher biomass than bare seed and other HPP sizes regardless of environment. For sagebrush, HPPs never produced greater biomass than the bare seed control, regardless of environment, or herbicide treatment.

Differences between HPPs and bare seeds for time to 50% emergence varied considerably by species, and to a lesser degree by herbicide treatment. For sagebrush, the bare seed treatment always had less time to 50% emergence than the pillow, and, when treated with herbicide, faster emergence than the small pellet (SPEC*TRT*SPRAY interaction, Fig. 3C, Table S1, full model, $F_{3,110} = 7.14$, p = 0.0002). For bluegrass, the bare seed treatment had less time to 50% emergence than all HPP sizes, except the small pellet when treated with herbicide. Plots of both

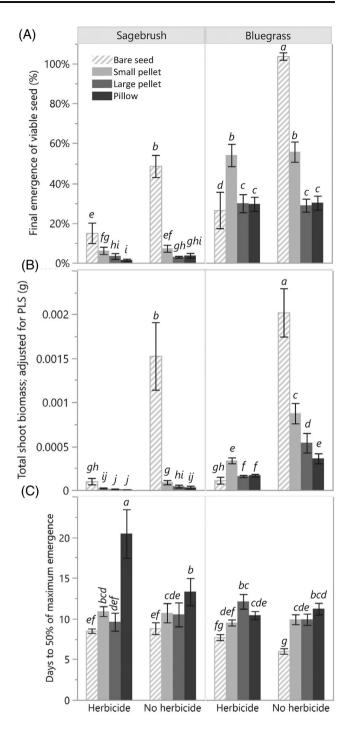


Figure 3. Final emergence as a percent of viable seed planted per plot (A), total dry shoot biomass (grams) per viable seed planted per plot (B), and days to 50% of maximum emergence per plot (C) for three sizes of herbicide protection pods (small pellet, large pellet, and pillow; see Methods) and bare seed control. Within each panel (A, B, C), bars sharing the same lowercase letters are not significantly different based on Student's ttest (α = 0.050). Error bars represent \pm SE of untransformed data.

species had approximately 36% less time to 50% emergence in the grow room than the growth chamber (Table S1, ENVR main effect, $F_{1.16} = 35.3$, p < 0.0001).

Other complex interactions existed when bare seed treatment was included in the full model (Table S1), but none of them significantly altered the general patterns described above relating to comparisons of bare seed with HPPs. The effect of the random factor (planting tray) was not significant on any response in the full model (p > 0.05 for all), explaining less than 3.5% of variation in responses.

Discussion

Herbicide protection technology is a promising new approach to allow for the successful, simultaneous application of seeds and pre-emergent herbicides in wildland restoration settings (Madsen et al. 2014). The technology is promising (Davies et al. 2017), but because it is still in development, there is considerable room for refinement and improvement, especially for small-seeded species, which have often had poor performance to date in existing studies (Brown et al. 2018; Davies 2018; Clenet et al. 2019).

Our laboratory study found that smaller HPPs of both species consistently produced 1.6–2.2-fold higher final emergence and 1.4–2.5-fold higher aboveground biomass per viable seed planted, and provided similar levels of protection from a standard rate of pre-emergent herbicide (105 g ai·ha⁻¹ imazapic) when compared to larger HPP sizes. In addition, our finding of no consistent effect of HPP size on time to 50% emergence suggests that higher total emergence and biomass of small pellets over other sizes during these relatively short (40 day) trials was not simply related to effects on emergence rate. Together these results indicate that the success of the small-seeded species used here, which prefer shallower seed depths than afforded by larger HPP sizes, may be improved with smaller pellets.

While there was a clear advantage of smaller over larger HPPs for the species tested, we found that the intended benefit of HPP technology—enhanced performance of pelleted seed over bare seeds in the presence of herbicide—was present for bluegrass but not for sagebrush in this laboratory trial. In the presence of herbicide, bluegrass HPPs showed 1.1-2-fold higher emergence and 1.5–3-fold higher biomass than bare seeds (with the highest values always belonging to the small pellet), but sagebrush HPPs showed 2.5-7.5-fold lower emergence and 4.5-16-fold lower biomass than bare seed. In contrast, Clenet et al. (2019, 2020) found that HPPs provided clear protection from herbicide for sagebrush in both the lab and field. However, they too found lower laboratory performance of sagebrush than a grass species in 8 mm × 16 mm pellets across a range of indaziflam rates and they speculated that sagebrush, with a seed around 10 times smaller than the tested grass, may have physically struggled to emerge from the pellet (Clenet et al. 2019). In, the field, Clenet et al. (2020) found more promising results for sagebrush, with 8 mm × 16 mm HPPs producing 7-fold higher sagebrush seedling densities 2 years after sowing than a bare seed treatment, under a 210 g ai·ha⁻¹ imazapic application. These discrepancies in sagebrush performance in HPPs may be explained by differences in herbicides and/or variations in pellet structure degradation caused by differences in moisture and temperature regimes. Nonetheless, our study is the first to manipulate pellet size and

suggests this factor can influence HPP performance, although additional field tests are required to confirm and better understand this influence. Alternative methods for generating herbicide protection should also continue to be explored, such as coating individual seeds with activated carbon (Madsen et al. 2014; T. Terry, Brigham Young University, Provo, UT, personal communication), which can also modify the effective seed burial depth but, unlike the pelleting described here, can ensure precise seed placement within the added material.

Similarly, while smaller HPPs showed clear advantages over other sizes, the HPP technology in general had a negative effect on seedling emergence and growth in the absence of herbicide in our trials. In the absence of herbicide treatment, all HPP sizes reduced emergence and biomass of both bluegrass and sagebrush by an average of 63–90% and 71–96%, respectively, when compared to bare seeds. Similar to our results, Clenet et al. (2019) reported that seeded sagebrush abundance and biomass were negatively impacted when seeds were incorporated into HPPs compared to bare seed in the absence of herbicide treatment. Other trials using alternative ingredients (diatomaceous earth, polyvinyl alcohol binders, and/or superabsorbent polymers) found fewer or no negative effects of this nature on large-seeded grasses (Madsen et al. 2014; Brown et al. 2018). While HPP technology is not intended for use in the absence of herbicide, additional experimentation to optimize recipe formulation is warranted and should include field trials. It is possible that a portion of this "cost" of HPPs for the small-seeded species tested in this study may be due to not enough germination force to successfully root or emerge from the HPP, with a smaller HPP resulting in less cost, but future research is needed to confirm.

We conducted trials in two laboratory environments to reduce the chance of drawing conclusions from a single environment or trial, and therefore our discussion has focused on results that are generally robust across both environments. However, some differences between the two environments, such as the lack of water infiltration and repeated wet-dry cycles in the growth chamber, likely limited the efficacy of the herbicide and influenced the pellet hardness and structural decomposition. These potential effects suggest that the growth chamber environment is less well suited than the grow room for the questions posed here. In any case, while these environments are useful for testing concepts, it is critical to conduct multi-site and multi-year field tests to estimate the true efficacy of different sizes of HPPs specifically and pellet technology in general. Finally, we did not manipulate the application rate of herbicide, imazapic, in this experiment (105 g ai·ha⁻¹), and HPP effectiveness may vary by application rate, which can range from 35–210 g ai·ha⁻¹ on range and pasturelands.

In summary, we found that reducing the size of seed pellets significantly improved the laboratory performance of HPP technology for two small-seeded species, but that significant costs to performance associated with the technology remained. For Sandberg bluegrass, we found that the benefit of HPP technology in the presence of herbicide outweighed the costs for only the smaller pellet, showing that pellet size can be a barrier to HPP efficacy for some species. For Wyoming big sagebrush,

however, the performance costs were larger than the benefit in the presence of herbicide for all pellet sizes in this laboratory-only study, demonstrating that additional factors may need to be addressed when using HPPs with this species. We conclude that HPP size is an important factor to consider when applying this technology, and there is room for additional refinements in HPP technology to improve efficacy, and these refinements may need to be species-specific. It is critical to conduct additional tests examining the effects of pellet size, the HPP technology in general, and other means to provide herbicide protection in field environments that represent realistic restoration sites and include competition from invasive annual weeds.

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LITERATURE CITED

- Adair CE, Burke IC (2010) Plant phenology and life span influence soil pool dynamics: *Bromus tectorum* invasion of perennial C3-C4 grass communities. Plant and Soil 335:255–269
- Brooks ML, D'Antonio CM, Richardson DM, Grace JB, Keeley JE, Diomaso JM, Hobbs RJ, Pellant M, Pyke D (2004) Effects of invasive alien plants on fire regimes. BioScience, 54:677
- Brown VS, Ritchie AL, Stevens JC, Harris R, Madsen MD, Erickson TE (2018)
 Protecting direct seeded grasses from herbicide application: can new
 extruded pellet formulations be used in restoring natural plant communities? Restoration Ecology 27:488–494
- Clenet DR, Davies KW, Johnson DD, Kerby JD (2019) Native seeds incorporated into activated carbon pods applied concurrently with indaziflam: a new strategy for restoring annual-invaded communities? Restoration Ecology 27:738–744
- Clenet DR, Davies KW, Johnson DD, Kerby JD (2020) Herbicide protection pods (HPPs) facilitate sagebrush and bunchgrass establishment under imazapic control of exotic annual grasses. Rangeland Ecology and Management, 73:687–693. https://doi.org/10.1016/j.rama.2020.07.002
- D'Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass/fire cycle and global change. Annual Review of Ecology and Systematics 23:63–87
- Davies KW (2018) Incorporating seeds in activated carbon pellets limits herbicide effects to seeded bunchgrasses when controlling exotic annuals. Rangeland Ecology and Management 71:323–326
- Davies KW, Madsen MD, Hulet A (2017) Using activated carbon to limit herbicide effects to seeded bunchgrass when revegetating annual

- grass-invaded rangelands. Rangeland Ecology and Management 70: 604-608
- Davies KW, Madsen MD, Nafus AM, Boyd CS, Johnson DD (2014) Can imazapic and seeding be applied simultaneously to rehabilitate medusaheadinvaded rangeland? Single vs. multiple entry. Rangeland Ecology & Management 67:650–656
- Duncan CA, Jachetta JJ, Brown ML, Carrithers VF, Clark JK, Ditomaso JM, Lym RG, Mcdaniel KC, Renz MJ, Rice PM (2004) Assessing the economic, environmental, and societal losses from invasive plants on rangeland and wildlands. Source: Weed Technology 18:1411–1416
- Farooq M, Basra SMA, Ahmad N, Hafeez K (2005) Thermal hardening: a new seed vigor enhancement tool in rice. Journal of Integrative Plant Biology 47:187–193
- Hardegree SP, Jones TA, Roundy BA, Shaw NL, Monaco TA (2016) Assessment of range planting as a conservation practice. Rangeland Ecology and Management 69:337–347
- Johnson DD, Davies KW (2015) Reestablishing perennial-dominated plant communities in medusahead-invaded sagebrush rangeland. Great Basin Factsheet Series, https://www.sciencebase.gov/catalog/item/ 5a15e762e4b09fc93dd170f5 (accessed 12 June 2019)
- Madsen MD, Hulet A, Phillips K, Staley JL, Davies KW, Svejcar TJ (2016) Extruded seed pellets: a novel approach for enhancing sagebrush seedling emergence. Native Plants Journal 17:230–243
- Madsen MD, Davies KW, Mummey DL, Svejcar TJ (2014) Improving restoration of exotic annual grass-invaded rangelands through activated carbon seed enhancement technologies. Rangeland Ecology and Management 67:61–67
- Nasri M, Doescher PS (1995) Effect of competition by cheatgrass on shoot growth of Idaho fescue. Journal of Range Management 48:402–405
- Ogle DG (2006) Plant guide—crested wheatgrass. USDA Plants Database, http://plants.usda.gov (accessed 12 June 2019)
- Ogle DG, John LS, Jones TA (2009) Plant guide—Bluebunch wheatgrass (*Pseudoroegneria spicata*). USDA Plants Database, http://plants.usda.gov/accessed 12 June 2019)
- Rafferty DL, Young JA (2002) Cheatgrass competition and establishment of desert needlegrass seedlings. Journal of Range Management 55:70–72
- Sheley RL, Bingham BS, Davies KW (2012) Rehabilitating medusahead (*Tae-niatherum caput-medusae*) infested rangeland using a single-entry approach. Weed Science 60:612–617
- Tilley D, Ogle D, Majerus M, Hybner R, Holzworth L, Stannard M (2008a) Plant guide—Sandberg bluegrass. USDA Plants Database, http://plants.usda.gov (accessed 12 June 2019)
- Tilley D, Ogle DG, St. John L, Benson B (2008b) Plant guide—Wyoming big sagebrush. USDA Plants Database, http://plants.usda.gov (accessed 12 June 2019)
- Tu M, Hurd C, Randall JM (2001) Weed control methods handbook: tools & techniques for use in natural areas. The Naure Conservancy. https://www.invasive.org/gist/products/handbook/methods-handbook.pdf (accessed 5 Mar 2020)

Supporting Information

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

Table S1. Full factorial ANOVA results for reduced model, with bare seed treatment excluded (top) and the full model (bottom).

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