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# Improving Restoration of Exotic Annual Grass-Invaded Rangelands Through Activated Carbon Seed Enhancement Technologies

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## Abstract

Cost-efficient strategies for revegetating annual grass-infested rangelands are limited. Restoration efforts typically comprise a combination of pre-emergent herbicide application and seeding to restore desired plant materials. However, practitioners struggle with applying herbicide at rates sufficient to achieve weed control without damaging nontarget species. The objective of this research was to determine if seed enhancement technologies using activated carbon would improve selectivity of the pre-emergent herbicide imazapic. Bluebunch wheatgrass (*Pseudoroegneria spicata*) seed was either untreated, coated with activated carbon, or incorporated into "herbicide protection pods" (HPPs) made of activated carbon through a newly developed seed extrusion technique. In a grow-room facility, bluebunch wheatgrass seeds were sown in pots that contained seed of the exotic-annual grass downy brome (*Bromus tectorum*). After planting, pots were sprayed with 70, 105, 140, or 210 g acid equivalent (ae) $\cdot$ ha$^{-1}$ of imazapic or left unsprayed. Where herbicide was not applied, downy brome biomass dominated the growing space. Imazapic effectively controlled downy brome and untreated bluebunch wheatgrass. Seed coating improved bluebunch wheatgrass tolerance to imazapic at 70 g ae $\cdot$ ha$^{-1}$. HPPs provided protection from imazapic at all application rates. When untreated seeds and HPPs are compared at the four levels of herbicide application (excluding the no herbicide level), HPPs on average were 4.8-, 3.8-, and 19.0-fold higher than untreated seeds in density, height, and biomass, respectively. These results indicate that HPPs and, to a lesser extent, activated carbon–coated seed have the potential to further enhance a single-entry revegetation program by providing land practitioners with the ability to apply imazapic at rates necessary for weed control while minimizing nontarget plant injury. Additional research is merited for further development and evaluation of these seed enhancement technologies, including field studies, before they can be recommended as restoration treatments.

**Key Words:** annual grasses, bluebunch wheatgrass (*Pseudoroegneria spicata*), downy brome/cheatgrass (*Bromus tectorum*), herbicide protection pod, revegetation, seed coating

## INTRODUCTION

Invasion of exotic annual grasses into native perennial plant communities poses a serious problem in many arid and semiarid regions throughout the world (Hobbs and Atkins 1988; D'Antonio and Vitousek 1992; Milton 2004; Davies 2011). Efforts to reseed desirable perennial species into annual-dominated rangelands have a high failure rate. If restoration of large areas is to be successful, new technologies will be needed (Rowland et al. 2006; Stohlgren and Schnase 2006; Davies et al. 2011).

In the sage-steppe ecosystem located in the western United States, downy brome (*Bromus tectorum* L.) and medusahead (*Taeniatherum caput-medusae* [L.] Nevski) are among the most prevalent exotic annual grasses displacing native perennial species (D'Antonio and Vitousek 1992; Davies et al. 2011). At the seedling stage, perennial sagebrush steppe species cannot effectively compete with exotic annual grasses (Clausnitzer et al. 1999). The ability of these annual weeds to outcompete seedlings of perennial species is generally associated with annuals having higher plant and seed bank densities (Young 1992), faster germination, greater germination potential (Clausnitzer et al. 1999), and higher growth rates (Arredondo et al. 1998; Monaco et al. 2003; Chambers et al. 2007). Subsequently, undesirable competitive species must be removed or greatly reduced prior to reseeding native species (Monson 2004). The most effective control of exotic annual grasses has been achieved with pre-emergent (soil active) herbicides (Monaco et al. 2005; Kyser et al. 2007; Davies 2010). Imazapic ([±]-2-[4,5-dihydro-4-methyl-4-{1-methylethyl}-5-oxo-1*H*imi-dazol-2-yl]-5-methyl-3-pyridinecarboxylic acid) is an example of a commonly used pre-emergent herbicide that can effectively control annual grasses when applied at appropriate rates (Kyser et al. 2007; Davies and Sheley 2011). However, imazapic's selectivity window is relatively narrow (Kyser et al. 2007). When imazapic is applied concurrently with reseeding, significant nontarget plant injury can occur if herbicide application rates are too high (Wilson et al. 2010; Sbatella et al. 2011; Hirsch et al. 2012).

It is common practice to postpone seeding efforts for up to a year following imazapic application, to allow herbicide activity to decline to a level that minimizes nontarget plant injury

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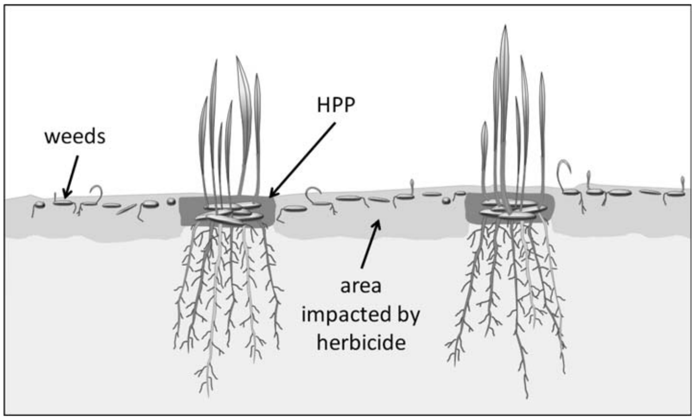


**Figure 1.** Illustration of a weed-infested area that was planted with seed that was incorporated within herbicide protection pods (HPPs). The site was treated with pre-emergent herbicide, which controlled weed species while activated carbon in the HPPs deactivates herbicide in the immediate vicinity of the sown seed and allows for plant growth.

(Davies 2010; Sbatella et al. 2011). However, when seeding is delayed, exotic annual grasses that were initially controlled by the herbicide may reinvade and outcompete seeded species (Sheley et al. 1996; Monaco et al. 2005; Sheley 2007). This reinvasion of annual grasses further reduces an already low success rate for establishing native perennial species. In addition, restoration that requires multiple steps is generally more expensive and energy demanding than single-entry approaches (Sheley et al. 2001).

Successful restoration of annual-dominated communities may be best achieved through methods that control invasive weeds while simultaneously establishing desired species during the time when competition from annuals is lowest. This requires that seeded species be planted at the same time invasive weeds are being controlled (Sheley 2007). Activated carbon has a high adsorption capacity for a wide range of organic compounds, including many herbicides (Coffey and Warren 1969). Activated carbon has been used in croplands to deactivate herbicides in the immediate vicinity of seeded species, which allows concurrent planting and weed control. Herbicide selectivity can be improved in row crops by applying a slurry of activated carbon in a band (2.54 cm or more) over the seed row to protect the crop from herbicide (Lee 1973). A limitation of activated carbon banding is that this technique does not provide complete control because weed seed within the band will also be protected from herbicide (Lee 1973).

It has been proposed that the selectivity of a range of herbicides can be further improved by coating crop seeds with activated carbon (Hagon 1977; Cook and O'Grady 1978; Scott 1989). Rotary and drum coaters are typically used to apply commercial seed coatings ranging in thickness from thin films up to around 1–2 mm (Gregg and Billups 2010). Unlike banding, an activated carbon seed coating provides protection only to the seed and potentially a thin layer around the seed. Protection afforded by such a thin layer of activated carbon may be inadequate for preventing herbicide uptake by the germinated seed as the radical extends into the surrounding unprotected soil.

To address these problems, we developed a new seed enhancement technology, herbicide protection pods (HPPs), that may offer both the protective ability of activated carbon banding and the improved selectivity of seed coating. HPPs are produced with extrusion equipment similar to that used in the food industry to pass a dough mixture containing seed, water-sensitive binders, activated carbon, and other additives through a rectangular die. The extruded dough material is then cut into short strips and dried. HPPs are sown flat with the top of the pod level or just below the soil surface (Fig. 1). This seeding method may allow for an efficient coverage of activated carbon over the seeded species to neutralize herbicide uptake, while maximizing the ability of the herbicide to control weed species. The objective of this research was to 1) determine how imazapic application rate influenced survival and growth of downy brome and a native perennial bunchgrass, bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh.) Löve), and 2) evaluate the efficacy of activated carbon–coated seeds and HPPs for improving imazapic selectivity.

## MATERIAL AND METHODS

### Soil and Plant Materials

Soil was obtained from a Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) steppe community type, located at the Northern Great Basin Experimental Range, 16 km southwest of Riley, Oregon (43°32′N, 118°9′W ). Soil on the site has a silt-loam texture and is classified as a fine-loamy, mixed, frigid Aridic Haploxeroll (Soil Survey Staff 2012). Soil was excavated from a maximum depth of 25 cm, with the top 2 cm of soil and litter discarded to remove existing seeds. Excavated soil was used to fill square 14-cm–wide by 14-cm–deep growing pots that were placed in a grow-room at the Eastern Oregon Agriculture Research Center, located in Burns, OR.

Species used in the study included the native bunchgrass 'Anatone' bluebunch wheatgrass and the non-native annual weed downy brome. Bluebunch wheatgrass was chosen because it is often a major component of native plant communities in the sage-steppe ecosystem of western North America and is commonly used in rangeland seeding efforts (Ogle et al. 2010). Like many grasses used for restoration, bluebunch wheatgrass is injured by imazapic applied at rates required for downy brome control (Shinn and Thill 2004). Germination potential of bluebunch wheatgrass and downy brome was 92% and 98%, respectively (as tested on blue blotter paper in 130-mm diameter Petri dishes, with 25 seeds $\cdot$ dish$^{-1}$ replicated four times per species).

### Study Design

Bluebunch wheatgrass seeds were untreated, coated with activated carbon, or incorporated into HPPs containing activated carbon. Pots with the sown seeds were sprayed with 0, 70, 105, 140, or 210 g acid equivalent (ae) $\cdot$ ha$^{-1}$ of the pre-emergent herbicide imazapic (Panoramic 2SL, Alligare, Opelika, AL) (3 seed treatments $\times$ 5 herbicide application rates $=$ 15 experimental treatments). The study was arranged in a

**Table 1.** Batch formulations applied to bluebunch wheatgrass and amount of product applied per seed to produce activated carbon–coated seed and herbicide protection pods. A single untreated bluebunch wheatgrass seed weighed approximately 3.05 mg.

| | Seed coating | | Herbicide protection Pod | |
|---|---|---|---|---|
| Ingredients | Batch (g) | Seed (mg) | batch (g) | seed (mg) |
| Nuchar | 228.0 | 6.10 | 551.2 | 44.10 |
| diatomaceous earth | | | 460.9 | 36.87 |
| Selvol-205 | 19.8 | 0.53 | 14.4 | 1.15 |
| Water | 208.2 | | 1 420.7 | |
| Seed | 114.0 | | 12.5 | |
| Total | 570.0 | 6.60 | 2 459.6 | 82.10 |

randomized complete block design with eight replicates per treatment.

**Activated Carbon Seed Enhancements.** Seeds receiving the seed coating treatment were coated with powdered activated carbon (Nuchar AG, MWV, Richmond, VA) at 200% weight of product to weight of seed ($w_p \cdot w_{-s}$) using a RP14DB rotary coater (BraceWorks Automation and Electric, Lloydminster, SK, Canada; Table 1). Using standard seed-coating methods, activated carbon was attached to the seeds with the partially hydrolyzed polyvinyl alcohol binder Selvol-205® (Sekisui Specialty Chemicals, Dallas TX; Table 1) at 17% $w_p \cdot w_{-s}$. Selvol-205 was prepared with an 8% solid content, according to Sekisui Specialty Chemicals solution preparation guidelines (Sekisui Specialty Chemicals 2009).

The formulation used for producing HPPs contained by weight of the total dry material 53% activated carbon, 44% diatomaceous earth, 1.4% Selvol-205, and 1.2% seed (Table 1). Following standard procedures used for forming dough and pasta, the dry materials (i.e., activated carbon, diatomaceous earth, and seed) were first thoroughly mixed, after which liquid Selvol-205 prepared with a 1% solid content was incorporated with the dry material to form a dough. Dough material was passed through a handheld extruder (Model no. 468, Lem Products, West Chester, OH) that had a rectangular 8 mm × 16-mm–wide die. Extruded material was cut into 16-mm lengths, producing pods that were 8-mm thick, 16-mm wide, and 16-mm long. Average number of seeds within a pod was equal to $5.4 \pm 0.4$ (mean ± SE, n = 15), which is equal to 5.0 pure live seeds (PLSs).

## Planting, Herbicide Application, and Growing Conditions

Each pot was seeded with 20 PLSs (1 000 PLSs $\cdot$ m$^{-2}$) of downy brome, and 10 PLSs (500 PLSs $\cdot$ m$^{-2}$) of bluebunch wheatgrass. Two pods were added to each pot designated to receive HPPs. Herbicide was applied immediately after planting, with 2 ml of water $\cdot$ pot$^{-1}$, using a handheld fine mist sprayer (Model no. 26028, Mid-States Distributing Co., St. Paul, MN). After spraying, pots were incubated in an environmental grow-room set at a constant temperature of 21°C, 12-hr day length, and 632 W $\cdot$ m$^{-2}$ of fluorescent lighting. The study was conducted for 47 d. During the first 7 d of the study pots were watered daily to field capacity (–0.01 MPa), and then every 2 to 3 d for the remainder of the study. Response variables recorded at the conclusion of the study included 1) plant density, 2) shoot height, and 3) oven dried (65°C for 72 h) aboveground biomass.

**Table 2.** Degrees of freedom (df), *F*, and *P* ($Pr > F$) values from analysis of variance (ANOVA) for the effect of seed technology, and imazapic rate, on plant density, average height and aboveground biomass production. *P* values in bold are statistically significant ($P < 0.05$).

| | | Density | | Plant height | | Biomass | |
|---|---|---|---|---|---|---|---|
| Effect | df | *F* | *P* | *F* | *P* | *F* | *P* |
| Downy brome | | | | | | | |
| Seed technology (ST) | 2 | 0.4 | 0.668 | 1.0 | 0.385 | 0.8 | 0.465 |
| Imazapic rate (IR) | 4 | 293.5 | < **0.001** | 220.8 | < **0.001** | 467.9 | < **0.001** |
| ST × IR | 8 | 1.5 | 0.158 | 1.0 | 0.477 | 0.5 | 0.875 |
| Bluebunch wheatgrass | | | | | | | |
| Seed technology (ST) | 2 | 23.0 | << **0.001** | 34.2 | < **0.001** | 35.7 | < **0.001** |
| Imazapic rate (IR) | 4 | 7.0 | << **0.001** | 6.5 | < **0.001** | 1.9 | 0.110 |
| ST × IR | 8 | 3.4 | **0.002** | 3.8 | **0.001** | 5.8 | < **0.001** |

## Data Analysis

Bluebunch wheatgrass and downy brome response data were analyzed separately in SAS (Version 9.3; SAS Institute, Cary, NC) using a two-way randomized complete block analysis of variance (ANOVA; Proc Mixed). Effects tested were seed treatment, imazapic application rate, and their interactions. Block was considered a random factor. For bluebunch wheatgrass, seed treatment × imazapic application rate interactions were significant; therefore, the LSMEANS procedure was used to compare seed treatment means within imazapic application rate levels (15 comparisons). The resultant *P* values were adjusted using a Bonferroni post hoc test. Significance was determined at $P \leq 0.05$.

# RESULTS

Imazapic effectively controlled downy brome and impaired untreated bluebunch wheatgrass at all application rates (Table 2; Fig. 2). Averaged across the study, the herbicide reduced downy brome density, height, and biomass by 84.7%, 87.5%, and 99.4%, respectively. Where herbicide was not applied, downy brome biomass dominated the growing space, producing approximately 3-fold more plants and 13-fold more aboveground biomass than bluebunch wheatgrass. Neither of the activated carbon seed enhancement technologies applied to bluebunch wheatgrass reduced imazapic control of downy brome (Table 2; Figs. 2A–2C).

Bluebunch wheatgrass seed coated with activated carbon showed some resistance to imazapic at 70 g ae $\cdot$ ha$^{-1}$ (Figs. 2D–2F). At this rate, aboveground biomass produced from coated seed was 10.0-fold higher than nontreated seed (Fig. 2F). While higher on average, biomass from activated carbon–coated seed was not significantly different from nontreated seed when imazapic was applied above 70 g ae $\cdot$ ha$^{-1}$. Bluebunch wheatgrass density and height were statistically similar for activated carbon–coated and nontreated seeds at all imazapic application rates.

Bluebunch wheatgrass seeds incorporated into HPPs were protected from imazapic at all application rates, including the

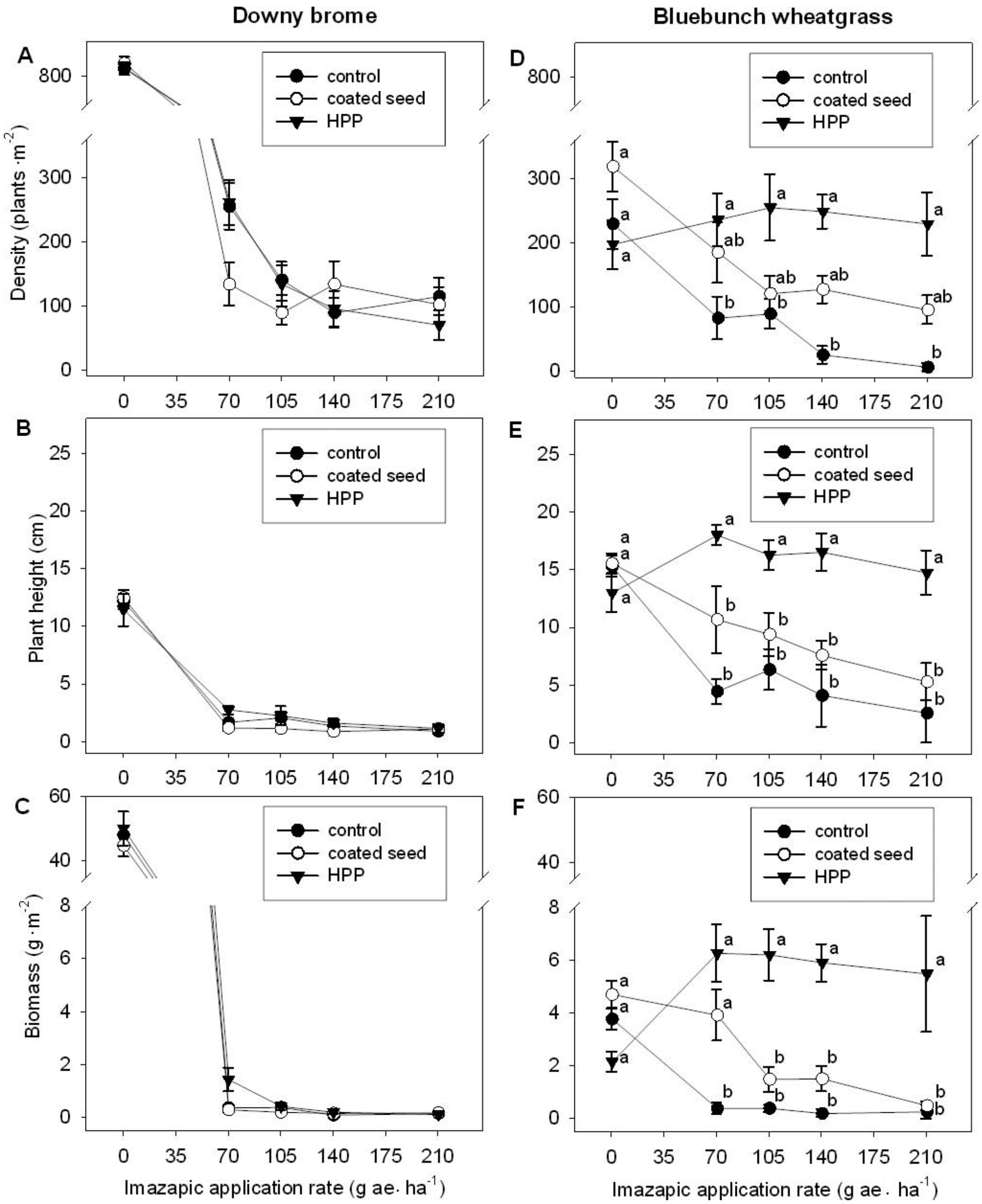


**Figure 2.** Downy brome (**A–C**) and bluebunch wheatgrass (**D–E**) density, plant height, and aboveground biomass production in response to imazapic application rates and seed treatments. Seed treatments were only applied to bluebunch wheatgrass and included 1) uncoated seed, 2) activated carbon–coated seed, and 3) herbicide protection pods (HPPs). Mean seed treatment values with different lowercase letters differ by $P \leq 0.05$, within an imazapic application rate level.

highest rate recommended by the herbicide manufacturer (210 g ae · $ha^{-1}$; see Panoramic 2SL specimen label). Plant density, height, and biomass production from HPPs were slightly higher in the imazapic-treated pots than in pots without imazapic (Figs. 2D–2F). Averaged across the four levels of herbicide application (excluding the no herbicide level), bluebunch wheatgrass density, height, and biomass produced from HPPs were 1.7-, 8.5-, and 10.8-fold higher than downy brome, respectively, and 4.8-, 3.8-, and 19.0-fold higher, respectively, than that produced from the untreated bluebunch wheatgrass seeds (Fig. 2).

Seedling density produced from HPPs was not statistically higher than activated carbon–coated seed (Fig. 2D). Seedling height produced from HPPs was between 1.7- and 2.7-fold higher than activated carbon–coated seed under the four levels of herbicide application. Aboveground biomass was between 3.9 and 11.1-fold higher for HPPs than for activated carbon–coated seed when imazapic application rates were above 70 g ae · $ha^{-1}$ (Figs. 2D–2F).

## DISCUSSION

These results indicate that HPPs and, to a lesser extent, activated carbon seed coatings, may make it possible for land managers to use a single-entry system to plant desired species while simultaneously applying imazapic for weed control. In this study, imazapic was effective for controlling downy brome; however, significant nontarget plant injury occurred to seedlings growing from nontreated bluebunch wheatgrass seed. Under the conditions of our study, we consider biomass production and plant height to be more important than plant density as an indicator of protection from imazapic injury. While activated carbon–coated seed improved emergence, growth was suppressed for the majority the seedlings subjected to imazapic application rates above 70 g ae $\cdot$ ha$^{-1}$. We anticipate that stress typical of field conditions would have prevented establishment of the stunted seedlings. Activated carbon coatings may be effective in limiting nontarget plant injury only at low imazapic application rates (below 70 g ae $\cdot$ ha$^{-1}$). In contrast, HPPs demonstrated superior plant protection even at high imazapic application rates (up to 210 g ae $\cdot$ ha$^{-1}$).

Our results, similar to Madsen et al. (2012), indicate that agglomerated seeds (i.e., seed grouped together in clusters) can outperform seeds that were spaced apart. Madsen et al. (2012) demonstrated that by agglomerating seeds together within the same pellet seedling emergence is improved because multiple emerging seedlings in the same location generate greater emergence thrust than a single seedling. Our research demonstrates another benefit of agglomeration plantings: by agglomerating the seeds together, greater amounts of seed enhancement materials can be grouped around the seeds. In this study, improved performance of HPPs over activated carbon–coated seed is most likely due to the HPPs' having a larger amount of activated carbon to provide protection from herbicide. We estimate that a single HPP contains 36.1-fold (214.4 mg $\cdot$ seed$^{-1}$) more activated carbon than a single coated seed (Table 1). As with carbon banding techniques (Lee 1973), there is an umbrella effect provided by the HPPs where the microsite underneath the pod is protected from the herbicide (Fig. 1). With respect to the plane parallel to the soil surface, each HPP had approximately 256-mm$^2$ surface area (16 mm width $\times$ 16-mm–long pellet). The coated bluebunch wheatgrass seed used in this study had roughly a 24 mm$^2$ area parallel to the soil surface (estimates based on 2.6-mm–wide $\times$ 9-mm–long coated seed). Subsequently, an HPP has around 10.3-fold more area parallel to the soil surface.

We primarily attribute improved performance of HPPs and activated carbon–coated seeds to the ability of activated carbon to neutralize imazapic within the microsite of the seed. However, activated carbon may provide additional benefits beyond protecting seeds and seedlings from soil active herbicide. Plants can compete against each other through the release of allelopathic chemicals into the soil (Mahall and Callaway 1992). For example, it has been suggested that some invasive weeds like Russian and spotted knapweed can use allelopathic chemicals to promote their success by limiting growth of native plant species (Callaway and Aschehoug 2000; Bais et al. 2003; Hierro and Callaway 2003). Activated carbon soil amendments have been proposed as a restoration tool to limit allelopathy (Cipollini 2002; Kulmatiski and Beard 2005; Cipollini et al. 2008). It may be possible that activated carbon applied to seed could improve restoration success in environments limited by allelopathy. Activated carbon may also improve plant growth through improving nutrient availability. Lau et al. (2008) demonstrated that activated carbon increased plant growth when mixed in potting media and attributed this increase to greater nitrogen availability.

The combined use of imazapic and HPPs or activated carbon–coated seeds has a strong potential to decrease resource competition from invasive weeds. For example, downy brome is considered a strong competitor against native perennial grass seedlings (Melgoza et al. 1990; Humphrey and Schupp 2004; Blank 2010). The use of activated carbon may allow seeding to occur simultaneously with downy brome grass control, as compared to the traditional practice of waiting 1 yr for herbicide toxicity to decrease. This restoration approach should allow seeded species 1 more yr of growth with relatively minimal competition from exotic annual grasses. Because established perennial bunchgrasses are competitive with downy brome and other exotic annual grasses (Clausnitzer et al. 1999; Davies 2008), it is probable that long-term control of cheatgrass and other exotic annual weeds can be achieved by coupling pre-emergent herbicide with activated carbon seed enhancements.

The use of HPPs and activated carbon–coated seeds may also decrease the cost of restoration in annual grass–invaded rangelands. Traditional restoration efforts that require two entry points (one to apply the pre-emergent herbicide and the second to plant after phytotoxicity levels have subsided) continue to be less feasible as energy costs increase (Sheley et al. 2012). Activated carbon–treated seed could provide a single-entry restoration approach by allowing seeding and pre-emergent herbicide to be applied at the same time. Sheley et al. (2012) demonstrated a single-entry approach in a medusahead invaded community by applying a low imazapic application (i.e., 60 g ae $\cdot$ ha$^{-1}$) and seeding at the same time. This low herbicide rate many not be adequate at all sites. Past studies have shown there is a high degree of variability in the amount of imazapic that is required to control annual grasses due to differences in soil characteristics, climate, application timing, litter cover, and other factors (Monaco et al. 2005; Kyser et al. 2007; Sheley 2007; Morris et al. 2009). HPPs and, to a much lesser extent activated carbon–coated seeds, may provide a more consistent exotic annual grass control by allowing higher pre-emergent herbicide application rates to be used without compromising establishment of seeded species.

Methods for incorporating activated carbon seed coatings and HPPs into rangeland restoration efforts merit further study. In general, professional seed-coating companies possess technical knowledge and infrastructure for producing activated carbon–coated seeds (Hagon 1977; Cook and O'Grady 1978; Scott 1989; Gregg and Billups 2010). Because buildup of material around the seed is minimal, standard rangeland drilling or broadcast methods could be used to plant activated carbon–coated seeds. Extrusion equipment for producing our HPP technology is not currently available, but we anticipate systems used in the dough and pasta industries could be modified for producing HPPs and other seed extrusion technologies. Because HPP technology is new, specific field seeding techniques will require further testing.

## MANAGEMENT IMPLICATIONS

Our results indicate that HPPs and to a lesser extent activated carbon seed coatings may further enhance a single-entry revegetation program by providing land practitioners with the ability to apply imazapic at rates necessary for weed control without causing nontarget plant injury to seeded species. This restoration approach may enhance the establishment of seeded species by providing a longer window before seedlings experience significant competition from exotic annual grasses. Concepts tested in this study for revegetating annual grass-infested rangelands may apply to a variety of agricultural systems where soil active herbicide is applied at the time of seeding. The approach outlined in this study should also open new lines of research to improve seeding establishment. Field research will be needed to take HPPs past the proof of concept stage before it can be recommended as a restoration technology. There is a host of potential studies that should be done to further improve the efficacy of HPP technology. For example, future developmental studies could be conducted to determine the 1) optimal size and number of seeds per HPP, 2) amount and type of activated carbon required to overcome phytotoxicity of specific herbicides, 3) extension to other species and soil types (specifically with respect to soil organic matter and texture), 4) potential of adding other seed enhancements to address other barriers to revegetation (e.g., fertilizers, inoculates, biopolymers, growth regulators, fungicides, insecticides, and rodent deterrents), and 5) appropriate planting methods and equipment for seeding HPPs.

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