AN ABSTRACT OF THE THESIS OF

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Title: <u>Activated carbon incorporated into herbicide protection pods as a seed</u> amendment technology for revegetation.

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Kirk W. Davies

Reestablishing native perennial vegetation in annual grass-invaded rangelands is critical to restoring ecosystems, especially following wildfires. Controlling invasive annual grasses is essential to increasing revegetation success; however, pre-emergent herbicides used to control annual grasses prohibit immediate seeding due to nontarget herbicide damage. Thus, seeding is often delayed one year following herbicide application. This delay frequently allows for re-establishment of annual grasses, decreasing the success of revegetation efforts. Incorporating seeds into herbicide protection pods (HPPs) containing activated carbon (AC) permits concurrent high herbicide application and seeding because AC deactivates herbicides. However, only one pre-emergent herbicide and a limited number of species have been tested with HPPs thus far. In order to be an effective tool for land managers, HPPs must be effective with multiple pre-emergent herbicide and multiple restoration/revegetation species. We conducted two studies: 1) a lab study with two native species; (Wyoming big sagebrush (Artemisia tridentata Nutt ssp. wyomingensis) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve)), to assess the effectiveness of HPPs with a new pre-emergent herbicide, indaziflam, and 2) a field study with five bunchgrass species and two shrub species with high imazapic application rates to assess the effectiveness of HPPs with multiple species and functional groups in the field. HPPs protected seeded species at low, mid, and high rates of indaziflam. The abundance and size of plants was greater in HPPs compared to bare seed treatments. The results of the first study suggest that HPPs can be used to seed native grasses and shrubs simultaneously with indaziflam application. In the second study, HPPs significantly improved establishment of sagebrush (Artemesia tridentata Nutt. Spp. wyomingensis Beetle & Young), bluebunch wheatgrass (Pseudoroegneria spicata (Pursh) A. Love), and crested wheatgrass (Agropyron cristatum (L.) Gaertn.) over the two-year study. Three native perennial grass species were protected from herbicide damage by HPPs but had low establishment in both treatments. While establishment of native perennial bunchgrasses was low, this study demonstrates that HPPs can be used to protect seeded bunchgrasses and sagebrush from imazapic, prolonging establishment time in the absence of competition with annual grasses.

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Activated carbon incorporated into herbicide protection pods as a seed amendment technology for revegetation

by Danielle R. Clenet

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APPROVED:

Major Professor, representing Rangeland Ecology and Management

Head of the Department of Animal and Rangeland Sciences

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Danielle R. Clenet, Author

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Sagebrush Steppe Rangelands

The sagebrush ecosystem once dominated more than 60 million hectares in western North America (Davies et al., 2011; Rottler et al., 2015; Knick et al., 2003). Despite being the largest arid/semi-arid vegetation type, it has become increasingly fragmented and degraded and now only covers approximately 56% of its historic range (Knick et al., 2003; Schroeder et al., 2004). Approximately 45% of the area (39 million ha.) of the Great Basin, is sagebrush steppe and faces the same causes of fragmentation and degradation typical of the greater sagebrush ecosystem (Knapp, 1996).

Sagebrush ecosystems provide a wide variety of services for people and animals and are vital for agricultural production and ecosystem integrity (Box, 2006; Duncan et al., 2004; Hardegree et al., 2018; Mockrin et al., 2012). Approximately 70% of the Great Basin's sagebrush steppe is administered by the Bureau of Land Management (Gordon et al., 2014) and many ranchers depend on public grazing permits to provide livestock forage (Maher et al., 2013). In addition to livestock production, the sagebrush ecosystem provides many recreational opportunities for the public including camping, wildlife viewing, hiking, and hunting (Plieninger et al., 2012). The sagebrush steppe provides habitat for at least 170 vertebrate wildlife species (Rottler et al., 2015). Sagebrush ecosystems provide important winter forage for wild ungulates, including many big game species, because sagebrush taxa retain high nutrient content compared to grasses in the winter (MacCracken and Hansen, 1981; Wambolt, 1998). In Montana, all sagebrush species are important winter forage for mule deer (Wambolt, 2001). In Idaho, since access to agricultural fields has been declining since the 1980's, sagebrush steppe is the most important overwintering habitat type for mule deer does (Anderson et al., 2012). On top of being a forage source over winter, sagebrush shrubs also provide security and thermal cover for large grazers and browsers (Wambolt, 1998). In addition to providing many tangible services, sagebrush ecosystems also provide a wide range of less tangible services such as soil carbon sequestration, soil protection, biological diversity, decreased water runoff, and cultural and spiritual services for many people (Box, 2006; Brown et al., 2010; Havstad et al., 2007; Mockrin et al., 2012; Plieninger et al., 2012).

Perils of the Sagebrush Steppe of North America

Currently, the sagebrush ecosystem is one of the most imperiled ecosystems in the world (Noss et al., 1995). Settlement and development of the sagebrush steppe in North America has caused widespread degradation of the ecosystem due to historic misuse. Improper grazing (Knapp, 1996; Morris and Rowe, 2014; Young et al., 1972), purposeful spread of exotic species (Mack, 1981; Novak, 2004), accidental spread of exotic species (Mack, 1981; Morris and Rowe, 2014; Young et al., 1972; Young and Allen, 1997), conversion to agricultural uses (Morris and Rowe, 2014; Morris et al., 2011; Morris et al., 2014), conifer expansion (Miller and Rose, 1999; Miller and Tausch, 2001; Miller and Wigand, 1994; Rau et al., 2009), and changes to the historic fire regime (Bates et al., 2013; Bureau of Land Management, 1999; Young et al., 2015) have all impacted and shaped the present day landscape.

Early settlers of Great Basin used grazing techniques that had been effective on the Great Plains but that were not suitable on the sagebrush steppe (Knapp, 1996). These techniques often resulted in improper livestock grazing, which was the greatest cause of early rangeland deterioration (Williams et al., 1968). Sheep and cattle were introduced in large numbers to the western Great Basin in the 1850s and 1860s, respectively, to supply food for the increasing mining populations (Knapp, 1996). By 1874, there were more than 180,000 cattle grazing in Nevada alone (Knapp, 1996) and most of the sagebrush ecosystem in North America was subjected to unregulated grazing by sheep and cattle by the late 19th and early 20th century (Morris and Rowe, 2014). Grazing by migratory sheep bands increased herbivory pressures and land degradation in the Great Basin because sheep could access areas cattle could not (Knapp, 1996; Young et al., 1972). Additionally, starting in the 1870s, horses also contributed to overgrazing of the Great Basin rangelands (Knapp, 1996). This unregulated grazing of sheep, cattle, and horses caused 57.5% of western rangelands to be considered severely depleted, and left only 16% in reasonably good condition by 1936 (Morris and Rowe, 2014).

Congress responded to the degradation of grazing lands in 1934 by enacting the Taylor Grazing Act, which was designed to 'stop injury to the public grazing lands by preventing overgrazing and soil deterioration' (Knapp, 1996). Unfortunately, the policies did not often ameliorate rangeland conditions as much as intended and improper grazing management continued (Knapp, 1996). Legacy effects of improper grazing management can be seen today as degraded herbaceous understories and increased dominance of shrubs (Davies et al., 2016; Miller and Eddleman, 2000). Unfortunately, cessation of grazing in areas with significant improper grazing legacies rarely elicits recovery of the degraded understories and more direct restoration must be applied (Davies et al., 2016)

Cultivation was also a significant land use of the Great Basin area during the late 19th and early 20th century, primarily driven by the Homestead Act of 1862 (Morris and Rowe, 2014). Nearly a half-million hectares in the arid sagebrush steppe of the Intermountain West were cultivated during the early 20th century (Morris et al., 2011). Cereal grains and hay were the main crops cultivated by dry-farming (farming without irrigation) in the Great Basin (Morris and Rowe, 2014). Plowing techniques used in the sagebrush steppe were those modified from use in the Great Plains and, like the grazing techniques from the Great Plains, were not suited for use in the

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sagebrush steppe (Mack, 1981). Cultivation had severe impacts on the sagebrush ecosystem because it introduced new forms of disturbance, like plowing, and was instrumental in the introduction of non-native species as seed contaminants (Morris and Rowe, 2014). Cultivation, similar to improper grazing, also has a legacy effect on plant communities (Morris et al., 2011; Morris et al., 2014). Even 90 years after farming has ceased, native species' density and cover can still be impacted and reduced (Morris et al., 2011). Cultivation legacy effects have also been shown to decrease revegetation success in old crop fields in the Great Basin (Morris et al., 2014).

Since 1860, pinyon (*Pinus* spp. L.) and juniper (*Juniperus* spp. L.) trees have expanded by at least 60% from their pre-European historic range into higher elevation zones in the Great Basin (Rau et al., 2009). In 1994, western juniper (*Juniperus occidentalis* Hook.) occupied one million ha in the Great Basin (Miller and Wigand, 1994) and by 2001, pinyon-juniper woodlands had spread to occupy approximately 18 million ha (Miller and Tausch, 2001). Conifer expansion in the sagebrush ecosystem has been driven primarily by climate changes, increased atmospheric CO₂, improper livestock grazing, and fire reduction (Miller and Rose, 1999; Miller and Tausch, 2001; Miller and Wigand, 1994; Rau et al., 2009). A period of mild climate and greater precipitation from 1850 to 1916 coincided with the initial peak in conifer expansion (Miller and Tausch, 2001; Miller and Wigand, 1994) and the continued historically mild climate has allowed conifers to expand further (Romme et al., 2009). Increased atmospheric CO₂ worldwide has also been cited as a reason for woody encroachment (Miller and Rose, 1999). Increased atmospheric CO₂ can result in the CO₂ fertilization effect and encourage physiological changes which can potentially enhance growth rates and make trees more resistant to drought (Romme et al., 2009).

Overstocking and improper grazing may also have increased conifer expansion by limiting herbaceous species which are competitive with tree seedlings (Miller and Rose, 1995; Miller and

Tausch, 2001), by increasing shrub cover, thereby increasing optimal tree establishment sites because shrubs often serve as nurse plants (Burkhardt and Tisdale, 1976; Miller and Tausch, 2001), and by decreasing fine fire fuels (Miller and Rose, 1995; Miller and Tausch, 2001). Fire reduction is an important driver of conifer expansion because western juniper and other conifers in the Great Basin are easily killed by fire when trees are relatively young (less than 50 years old) (Burkhardt and Tisdale, 1976). Historically, fire intervals restricted stands of trees to areas that had low fire risk and therefore limited expansion (Burkhardt and Tisdale, 1976; Miller and Rose, 1999).

Conifer encroachment into sagebrush steppe ecosystems can have many negative impacts. For example, it eliminates sagebrush and can significantly decrease herbaceous cover (Bates et al., 2005; Davies et al., 2011; Miller et al., 2000). When western juniper reaches 50% of its maximum potential, sagebrush declines to only a quarter of its maximum potential (Miller et al., 2000). Since increased juniper or pinyon cover decreases inter-canopy vegetative cover, conifer encroachment can also increase the risk of soil erosion (Pierson et al., 2007; Pierson et al., 2013; Roundy et al., 2016), decrease habitat for species like the greater sage grouse (Baruch-Mordo et al., 2013; Farzan et al., 2015; Severson et al., 2017), reduce forage available for livestock (Bates et al., 2005; Farzan et al., 2015), and change the fire regime (Bates et al., 2013; Young et al., 2015). As pinyon-juniper woodlands develop to a state where trees are dominant and shrubs and herbaceous layers are reduced, higher tree canopy fuels influence fire behavior and increase fire severity (Bates et al., 2013; Roundy et al., 2014; Young et al., 2015). Higher fire severity may also increase the chance for invasion by exotic annual grasses, which are extremely problematic in the sagebrush steppe, by further decreasing perennial grasses and sagebrush shrub cover (Bates et al., 2005; Bates et al., 2013; Condon et al., 2011).

Invasive Winter Annual Grasses: Medusahead and Cheatgrass

In the United States, arid and semi-arid regions are the most negatively impacted by invasive annual grasses (D'Antonio and Vitousek, 1992). The two most detrimental invasive species in the western portion of the sagebrush ecosystem are cheatgrass (Bromus tectorum L.) and medusahead (Taeniatherum caput-medusa (L.) Nevski) and the consequences and expansion of these two species have been especially severe in low-elevation sagebrush plant communities and in the Wyoming big sagebrush alliance (Boyd and Davies, 2012). Cheatgrass and medusahead are invasive annual grasses that were introduced accidentally from Eurasia to North America (Mack, 1981). Both species are winter annual grasses which commonly germinate in the fall, though they can also germinate in the spring (Mack, 1981; Sebastian et al., 2017c). They have similar life history strategies with a few, yet important, differences (Young, 1992). Medusahead matures two to four weeks after cheatgrass and other annual grasses, its seed caryopsis is covered with small barbs of silica, a considerable deterrent to any species that would use them as a food source, and its seeds require a temperature-related, after-ripening period to germinate (Young, 1992). Since cheatgrass and medusahead are adapted to similar rangeland climates as those common in the Intermountain West, they have been able to establish readily in some sites regardless of disturbance or degradation (Young and Allen, 1997).

Cheatgrass was likely introduced as a seed contaminate in grain seed used for cultivation and/or packing material in the 1790's but only began to become noticeably prominent by 1889 (Novak, 2004). After introduction, cheatgrass spread by discarded bedstraw, along railway sidings, through continued contamination of grain seed, and by livestock (Mack, 1981). It was also spread purposely for a time because overgrazing had reduced the native grasses so much that ranchers were looking for a new grass that could serve as forage (Mack, 1981). Cheatgrass was sold as a lauded "100-day" grass fix for degraded pasture (Mack, 1981). Medusahead was likely introduced as a seed contaminate in the late 1800s and has spread in similar ways as cheatgrass, invading much of the sagebrush steppe (Novak, 2004).

Medusahead infests 972,683 hectares and cheatgrass infests 22,680,785 hectares in the 17 western-most states (Duncan et al., 2004). In the Great Basin area, cheatgrass infests and dominates over 20,000 km², approximately 7% of the land area (Boyte et al., 2016). While the economic impact of both grasses are poorly understood, it is estimated that they cost millions to billions of dollars annually (Duncan et al., 2004) by decreasing forage quality and availability, through costs associated with habitat restoration, and from costs associated with increased wildfire (Bradley, 2009).

Problems Caused by Invasive Winter Annual Grasses

Exotic grass invasion occurs around the globe and often has substantial impacts from the population to the ecosystem level (D'Antonio and Vitousek, 1992). Invasive grasses are detrimental in the continental U.S., Africa, New Zealand, Hawaii, and Australia (D'Antonio and Vitousek, 1992; DiTomaso, 2000; Lamoureaux and Bourdôt, 2014; Musil et al., 2009; Parker-Allie et al., 2009). Invasion can cause loss of biological diversity, alter nutrient flows, and degrade ecosystem function (D'Antonio and Vitousek, 1992; Milton, 2004). Perhaps most importantly, invasive grasses can significantly alter the disturbance regime of an ecosystem, decreasing the likelihood of native plant establishment and enhancing invasive grasses dominance (Brooks et al., 2004; D'Antonio and Vitousek, 1992; Milton, 2004). Alterations to ecosystem processes can completely transform an ecosystem by removing native species and decreasing palatable forage for wildlife and livestock (Brooks et al., 2004; Davies and Svejcar, 2008; Milton, 2004; Reynolds and Trost, 1980; Wright and Klemmedson, 1965; Young and Allen, 1997). Restoration of invaded

areas is imperative in order to reestablish a functioning ecosystem that supports native flora and fauna and enables multiple land uses (Davies et al., 2014*a*).

Invasive annual grasses are able to displace native perennial species through efficient seeddispersal mechanisms, high seed production, and rapid germination and emergence early in the spring (Belnap and Phillips, 2001; Chambers et al., 2007; Evans and Young, 1970; Jones et al., 2010; Sperry et al., 2006; Young, 1992). Plant species diversity, perennial bunchgrass density, total native herbaceous densities, perennial bunchgrass cover, perennial forb cover, sagebrush cover, total native vegetative cover, biological soil crust cover, perennial bunchgrass biomass, and total native herbaceous biomass are all negatively correlated with increasing medusahead density (Davies, 2011). Sandberg bluegrass and large perennial bunchgrass cover were 24 and 15 times lower in communities where medusahead had invaded (Davies and Svejcar, 2008). Similarly, total herbaceous cover was 8.7 times lower, species richness 2 times lower, and total native biomass production 7.8 times lower in invaded communities (Davies and Svejcar, 2008). Loss of diversity caused by annual grass invasion limits restoration success due to a limited seed source for species that have diminished with the presence of the exotic species (Davies and Svejcar, 2008).

Invasion of cheatgrass into perennial bunchgrass communities also significantly altered soil biota in Utah (Belnap and Phillips, 2001). Cheatgrass dominance increased gross rates of nitrogen mineralization and net nitrification leading to faster rates of nitrogen cycling compared to bunchgrass communities (Belnap and Phillips, 2001). Soil beneath cheatgrass also had higher concentrations of soil organic carbon and nitrogen (Belnap and Phillips, 2001). Soil biota changes can influence the availability of nutrients in the soil and energy flow of the ecosystem (Belnap and Phillips, 2001). These changes may create a positive soil-plant feedback that promotes the competitive advantage of cheatgrass (Sperry et al., 2006; Stark and Norton, 2015).

Litter accumulation, which is a common consequence of both medusahead and cheatgrass invasion, produces more favorable conditions for germination and establishment of invasive annuals by keeping soil temperature more constant and above critical temperatures for germination, by stabilizing soil moisture, and by decreasing soil moisture depletion rates (Evans and Young, 1970). Litter accumulation is especially pronounced for medusahead due to slow decomposition caused by high silica content of the plant (Bovey et al., 1961) and further inhibits the growth of other species by forming a physical barrier between seeds and soil (Young, 1992). Annual grass litter also decreases and changes the composition of biological soil crusts which are important for maintaining soil fertility, reducing erosion, and affect nutrient distribution in sagebrush ecosystems (Serpe et al., 2013). Winter annual grass invasion alters ecosystem functionality in many ways, however, the most significant alteration occurs through a decrease in the fire return interval (Davies and Nafus, 2013).

Before the introduction of invasive species, fire return intervals were likely between 50-100 years or more in native dominated low-elevation sagebrush steppe ecosystems (Baker, 2006; Bukowski and Baker, 2013; Mensing et al., 2006; Whisenant, 1990). Fires were historically infrequent in these areas because the cover and distribution of perennial bunchgrasses produces a discontinuous fuel bed that required extreme fire weather and fuel conditions to burn (Knick and Rotenberry, 1997; Whisenant, 1990). In general, the introduction and dominance of invasive grasses in many areas of the sagebrush steppe have decreased the fire return interval to ten years or less (Bureau of Land Management, 1999). In the Snake River Plains in Idaho, many areas now burn every three to five years (Whisenant, 1990).

Exotic annual grass invasion results in a larger quantity of continuous fine fuels that greatly increases the likelihood of ignition and spread of a fire. In addition, exotic annual grasses often recover more rapidly than native species, and thereby create a positive feedback loop for

expansion and invasion (Brooks et al., 2004; D'Antonio and Vitousek, 1992; Knick and Rotenberry, 1997; Whisenant, 1990). Compared to European population, North American populations of cheatgrass seed had a higher tolerance to heat shock during fire and had greater height and biomass following fire, thus producing plants with enhanced flammability potential (Fenesi et al., 2016). These traits suggest that North American cheatgrass have enhanced fire response traits that make them even more invasive and likely to take over a community in a firefeedback cycle (Fenesi et al., 2016). Cheatgrass invaded rangeland vs. uninvaded communities can have fine fuel biomass that is 2-3 times greater, fine fuel cover that is over 2 times higher, and average continuous fine fuel cover length that is 9-17 times greater, while uninvaded sites can have higher fuel moisture (Davies and Nafus, 2013). There is a positive correlation between cheatgrass abundance and fire risk and frequency (Whisenant, 1990; Link et al., 2006). When cheatgrass is the dominant vegetation cover, double the amount of land burns during a fire compared to land dominated by native vegetation (Balch et al., 2013). Additionally, once a cheatgrass stand is burned, it is likely to burn again due to an increase in post-burn cheatgrass cover (Reed-Dustin et al., 2016). Post-burn cheatgrass cover has been found to increase by 135% in previously sagebrush dominated sites (Reed-Dustin et al., 2016).

Increased frequency of fire can lead to declines and even local extinctions of native species by disrupting their recovery (Whisenant, 1990). For instance, sagebrush can re-establish post-fire but if a second fire occurs before plants are mature (i.e. produce seed to replenish the sagebrush seedbank), the local population is persistently diminished (Whisenant, 1990). Following multiple historic fires, sagebrush cover was almost entirely absent at low-elevation sites as areas became dominated by early-successional weedy species like cheatgrass (Davies et al., 2012). Additionally, fires started by cheatgrass often lead to loss of shrub patches and more fragmented sagebrush stands that further contribute to the spread and dominance of cheatgrass (Knick and Rotenberry, 1997). In a study that performed experimental burns on four species of perennial bunchgrass, significant damage to basal area for *Hesperostipa comata* (Trin. & Rupr.) Barkworth, and *Achnatherum thurberianum* (Piper) Barkworth was measured (Wright and Klemmedson, 1965). While basal damage was not present for all species in the study, it is important to note that these measurements were taken after a single burn (Wright and Klemmedson, 1965). Subsequent burning shortly after the first would likely increase the damage since the plants would still be in the process of recovery from the prior fire (Wright and Klemmedson, 1965). Regardless, these results suggest that even after a single burn, some species may experience significant mortality in their populations (Wright and Klemmedson, 1965). Cheatgrass changes the seasonality of wildfires and increases fire risk, fire intensity, and completeness of burns and, in turn, creates a cycle that supports the establishment of an annual grass monoculture (Davies and Nafus, 2013).

Once an invasive annual grass has established as a monoculture, the grazing quality of the land decreases (Young and Clements, 2007). Exotic winter annuals, like cheatgrass, tend to green up as soon as moisture is available early in the spring but the subsequent green feed period is shorter than those provided by most perennial grass species. Once mature, cheatgrass is a protein deficient forage similar to other grasses, however, unlike in more diverse plant communities, livestock cannot graze other species to increase diet quality in annual grass monocultures (Young and Clements, 2007). Additionally, cheatgrass and medusahead seeds can be injurious to the eyes and mouths of livestock and are generally avoided once they are mature (Turner et al., 1963; Young and Clements, 2007). The largest issues with relying on winter annual grass monocultures as forage for livestock are that annual grass production within the Great Basin is extremely variable due to high variability in annual precipitation and because forage for the year can be decimated by a single annual grass-promoted wildfire (Knapp, 1996; Young and Clements, 2007; Young et al., 1987).

Although medusahead and cheatgrass have similar life cycles, medusahead tends to mature later than cheatgrass, making it more competitive on certain sites (Hironaka, 1961; Young et al., 1999). While cheatgrass can be a useful spring forage for livestock production, medusahead is considerably less palatable (Turner et al., 1963) and decreases forage capacity by at least 50-90% (Davies and Svejcar, 2008; Hironaka, 1961). Although livestock will graze medusahead during the vegetative stage, perennial grasses and cheatgrass are preferred (DiTomaso et al., 2008; Young, 1992). Even with nutritional supplementation, medusahead intake by sheep in early and late reproductive stages is extremely limited (Hamilton et al., 2015). Medusahead has comparable protein content to cheatgrass and other grass species (Bovey et al., 1962). However, it has much higher ash content than other species, 70% of which is silica (Bovey et al., 1961). High silica content is likely the reason medusahead is highly unpalatable to livestock (Bovey et al., 1961) since silica dilutes nutrients, affects mastication, and inhibits digestibility in ways similar to lignin (Hamilton et al., 2015).

At least 170 vertebrate wildlife species are in some way associated with or are dependent on the sagebrush ecosystem (Rottler et al., 2015). Loss of sagebrush shrubs translates into a loss of habitat for many species (Rottler et al., 2015). Annual grass invasion promotes fire return intervals that preclude establishment and maintenance of non-sprouting shrubs like sagebrush (Knick and Rotenberry, 1997), leading to a change in plant community that negatively affects wildlife species (Rottler et al., 2015). As sagebrush is lost, avian species diversity and nest density decreases, small mammal density declines, large mammal density and diversity decreases, and lizard density diminishes (Reynolds and Trost, 1980). The native fauna of sagebrush ecosystem evolved to be highly dependent on sagebrush shrubs and some, like the sage thrasher, sage sparrow, and Brewer's sparrow, are sagebrush obligates and decline as the system is degraded by annual grasses (Reynolds and Trost, 1980). The greater sage grouse (*Centrocercus urophasianus*) is another sagebrush obligate because of their dependence on sagebrush as their sole food source in winter (Wallestad and Eng, 1975). The greater sage-grouse is a species of special interest because it is a continued potential candidate for federal listing under the endangered species act in the U.S. (Schroeder et al., 2004; USFWS, 2015) and has already been listed an endangered species in Canada (Aldridge and Brigham, 2003). The range and population of greater sage-grouse coincides with the sagebrush ecosystem and declines with loss and fragmentation of the ecosystem as sagebrush landscape is converted to annual grassland (Schroeder et al., 2004).

Sagebrush Steppe Invasibility

Invasibility of the sagebrush-steppe depends on current and historic land use and on a multitude of environmental characteristics (Beckstead and Augspurger, 2004; Brooks and Chambers, 2011; Chambers et al., 2007; Davies and Johnson, 2017; McGlone et al., 2011; Whisenant, 1990). Land use factors include current disturbance (Beckstead and Augspurger, 2004; Brooks and Chambers, 2011; Whisenant, 1990) and the legacy effects of historic disturbance (Chambers et al., 2007; Knapp, 1996). Environmental characteristics influencing invasion risk include topography (Chambers et al., 2007), climate (Chambers et al., 2007), and pre-disturbance plant community (Davies and Johnson, 2017; McGlone et al., 2011). Combined, environmental and land use variables have an impact on a plant community's resilience to disturbance which has a direct relation to that community's resistance to invasion (Brooks and Chambers, 2011). Resilience is an ecosystem's ability to withstand disturbance before changes in ecosystem structure and processes occur (Brooks and Chambers, 2011). Resilience typically increases if there is a tight coupling of resource availability and plant uptake (Blank et al., 2007; Brooks and Chambers, 2011).

When there is not a tight coupling of resource availability and resource use, site resistance to invasion decreases as nutrients and soil water are made available for invasive species' use (Beckstead and Audpurger, 2004; Brooks and Chambers, 2011; Chambers et al., 2007; McGlone et al., 2011). If a site experiences a sudden increase in resource availability and either more resources are available than the established species can use or use quickly enough, invasion is more likely, regardless of other site characteristics (Davis et al., 2001). Fast-growing exotic annual grasses, like cheatgrass and medusahead, can take advantage of nutrient pulses more quickly than perennial grasses (Blank et al., 2007). This may be the reason why ecosystems that seem like they should be resistant to invasion can become invaded (Davis et al., 2001). Native species normally dominate and remain resistant to invasion by exotics when high levels of nutrients are consistently available in a system (Chambers et al., 2007). However, even brief spikes in nutrient availability due to inconsistent precipitation or disturbance, increases the likelihood of invasion (Chambers et al., 2007). Plant resources, such as soil nutrients and moisture, are spatially and temporally variable in the sagebrush-steppe so plant communities' susceptibility to invasion fluctuates over time and space (Blank et al., 2007).

Despite these fluctuations, patterns emerge where sites with certain characteristics are generally more or less invasible. In general, sites with greater resources, especially water, and higher productivity are more resistant to invasion (Brooks and Chambers, 2011). In contrast, sites with fewer resources tend to have decreased competitive resistance to exotic species due to harsher conditions (Chambers et al., 2014; Davies et al., 2012). Climate can be the major determining factor for resource availability and has been strongly linked to determining which areas are susceptible to severe invasion by annual grasses (Bradford and Lauenroth, 2006). One study found that as aridity and soil temperature increase, invasive grass cover increases as well, indicating that climate has a strong relationship with annual grass invasion (Bansal and Sheley, 2016). North facing slopes tend to be cooler and wetter favoring higher productivity and faster recovery of native plants, therefore allowing native plants to compete more effectively against invasive species

(Chambers et al., 2014). Following a fire, bluebunch wheatgrass recovered to pre-burn cover within 1-2 years in plots with a north facing aspect, while recovery occurred between 2-5 years post-burn on plots with a south facing aspect (Reed-Dustin et al., 2016). Plant communities on higher elevation sites are also generally more resilient to repeated disturbances and more resistant to invasion (Blank et al. 2007; Davies et al., 2012). In contrast, low-elevation sites experiencing warmer and drier environmental conditions are more likely to be severely degraded post-disturbance (Blank et al. 2007; Davies et al., 2012).

In general, disturbance from fire, biomass removal, vegetation mortality, and physical alteration of the landscape, decrease resistance to invasion by decreasing the number of mature native plants and making nutrients available to invasive species (Beckstead and Augspurger, 2004; Blank et al., 2007). The severity of the effects of disturbance are often dependent on the predisturbance site characteristics, characteristics of the disturbance, and post-disturbance climate (Blank et al., 2007). Sagebrush steppe ecosystems exhibited higher resilience 17 years post fire when they were in good ecological condition pre-fire, post-fire disturbances were limited, and unburned islands within the fire provided a native seed source (Ellsworth et al., 2016).

Certain plant functional groups possess traits which limit invasion of exotic annual grasses more than others (Sheley and James, 2010). In the sagebrush-steppe, mature, established perennial grasses decrease the invasibility of a site more than other functional groups (Davies, 2008; Sheley and James, 2010). Even with repeated disturbance and increased levels of cheatgrass seed availability, sites where native species were already established were highly resistant to invasion by cheatgrass (McGlone et al., 2011). Non-native bunchgrass species, such as *Agropyron desertorum* (Fisch. ex Link) Schult., are also effective at limiting the invasion of exotic annual grasses (Davies et al., 2010).

Historic disturbance, such as overgrazing and cultivation, alter the resistance of the

ecosystem by changing native plant community composition, decreasing the abundance and competitive ability of native bunchgrasses, introducing invasive species seeds, and reducing the seedbank of native species (Brooks and Chambers, 2011). Some invaded plant communities that have been persistently changed by repeated, long-term, historic disturbances cannot return to a pre-invasion vegetative community without human intervention (Masters and Sheley, 2001). Since some sites have more inherent resistance to invasive species, these sites should be the focus of restoration efforts because, once restored, they are more likely to resist reinvasion (Brooks and Chambers, 2011).

Future predictions of climate change indicate that altered temperature and precipitation regimes may open new areas to invasion which are currently at low risk (Bradley, 2009). Predicting the invasion risk contains significant amount of uncertainty because of difficulty in predicting how climate change will occur and complexity of interactions between plants, climate, and atmospheric gases (Bradley, 2009; Polley et al., 2013). For example, one study's models predicted that decreasing summer precipitation increases land area at risk to invasion by cheatgrass by 45%, while increased precipitation reduced land susceptible to invasion by 70% (Bradley, 2009). However, a different study, found that both cheatgrass and native grasses responded negatively to warming and drying, indicating that climate change which follows those two patterns may decrease the number of sites invadable by cheatgrass (Larson et al., 2017). For the most part, studies in the Pacific Northwest indicate that annual grasses will expand into areas that were once considered safe from invasion due to expected climate changes (Bradley, 2009; Creutzburg et al., 2015; Polley et al., 2013; Sandel and Dangremond, 2011). Thus, effective restoration techniques and informed management will be crucial to practically manage the sagebrush steppe for long-term goals (Creutzburg et al., 2015).

Current Restoration Shortcomings

Habitats which have been overtaken by medusahead and/or cheatgrass are difficult to reliably revegetate with perennial bunchgrass species because of competition with invasive exotics at the seedling stage (Clausnitzer et al., 1999; Davies et al., 2015; Humphry and Schupp, 2004; Young et al., 1999), limited and unpredictable precipitation (Call and Roundy, 1991; Chambers et al., 2007; Young et al., 1999), factors of disturbance (Call and Roundy, 1991; Chambers et al., 2007), episodic perennial seedling recruitment (Call and Roundy, 1991; Young et al., 1999), and high landscape heterogeneity (Boyd and Davies, 2012; Call and Roundy, 1991; Madsen et al., 2016a). Similar to site invasibility, ability to revegetate a site once it has been invaded by invasive annual grasses can be helped or hindered by site characteristics and success is often lowest in low elevation areas or on south facing slopes due to harsher local climate and lower resilience to disturbances (Chambers et al., 2014; Davies et al., 2012; Reed-Dustin et al., 2016). Revegetation success can be increased by seeding sites that were not an invasive annual grass monoculture preburn and there is a "rapidly closing window of opportunity" for successful revegetation post-fire because invasives re-establish quickly (Eiswerth et al., 2009). For this reason, successful revegetation early is crucial to restoration of the rangeland landscape to a desirable condition before it becomes nearly impossible to achieve without considerable expense (Eiswerth et al., 2009).

Use of native perennial bunchgrass species results in seeding success estimated to be as low as 10% (Madsen et al., 2016*a*). A success rate of 10% may also be an overestimate since few negative results are reported in literature (Hardegree et al., 2011; Sheley, 2007). Nonnative species, such as crested wheatgrass (*Agropyron cristatum* [L.] Gaertm. and *Agropyron desertorum* [Fisch.] Schult.), have been used for revegetation of annual-invaded communities because they are competitive with invasive annual grasses (Davies et al., 2010; Davies et al., 2015; Thacker et al., 2009), they establish more often and in poorer conditions than the native perennial grass species (Asay et al., 2001; Knutson et al., 2014; Robins et al., 2013), they provide good forage (Asay et al., 2001), and they are less costly than seeding native perennial grass species (Boyd and Davies, 2010; Epanchin-Niell et al., 2009). Revegetation with native seed can cost as much as 15% more per acre than revegetation with non-native crested wheatgrass (Epanchin-Niell et al., 2009). Unfortunately, these species also tend to dominate a community and create a monoculture which limits the reintroduction of native perennial species (Davies et al., 2013; Rinella et al., 2016). In large-scale restoration projects, once crested wheatgrass populations are established they often increase gradually over the years and outcompete other seeded species, including native bunchgrasses (Rinella et al., 2016; Nafus et al., 2015; Hamerlynck and Davies, 2019). As management objectives have changed to provide greater ecosystem services and habitat to various species, the use of native perennial grass species in seed mixes intended for restoration have increased (Leger and Baughman, 2015).

Revegetation of rangelands tends to be most successful and have more lasting success when following control of invasive annuals because seeded species experience decreased competition (Huddleston and Young, 2005; Masters et al., 1996; Sheley and Krueger-Mangold, 2003; Young et al., 1999). Additionally, revegetation of rangelands after control of invasive annuals is needed to limit re-dominance of invasives after the initial control measures degrade (Davies et al., 2015; Masters et al., 1996). Combining burning, pre-emergent herbicide application, and seeding can create a plant community that will resist future invasion (Masters et al., 1996; Sheley et al., 2007). However, pre-emergent herbicides, while an important and effective part of management, can also cause damage to desirable seedlings (Hirsch et al., 2012; Munson et al., 2015; Wilson et al., 2010). This leads to a multiple-entry approach wherein herbicide is applied and a year or more passes before desired species are seeded. For example, Huddleston and Young (2005) applied herbicide in November 2007 and did not apply seeding treatments until April 2008. Waiting a year to plant limits damage to non-target species but may also increase the likelihood that the invasives will begin to re-establish (Madsen et al., 2014). Although a multiple-entry approach can be successful at establishing perennial grasses and rehabilitating invaded areas (Davies, 2010), it is also very costly (Davies et al., 2014*a*; Sheley, 2007).

In response to increased costs and the potential re-establishment of exotic species with multiple-entry approaches, a single-entry treatment has been attempted to revegetate invaded rangelands (Sheley, 2007; Sheley et al., 2007; Sheley et al., 2012). The single-entry approach simultaneously applies herbicide and seeding in the hope that desired species will have time to establish before competition increases with re-dominance of invasives (Davies et al., 2014*a*). The single-entry approach used in the fall was successful when reseeding a rangeland invaded by Russian knapweed (*Rhaponticum repens* L.) and green rabbitbrush (*Chrysothamnus viscidiflorus* (Hook.) Nutt.) (Sheley, 2007).

However, responses of different seeded species to herbicide application are variable and some species can experience significant damage when herbicide is applied simultaneously with seeding (Sheley, 2007; Sheley et al., 2007). Research demonstrates that a rate of at least 70 g ha⁻¹ of pre-emergent imazapic is required for adequate control of the invasive annual grass medusahead into the second year and that higher rates result in better, longer-lasting control (Kyser et al., 2007; Sheley et al., 2007). Unfortunately, higher rates of imazapic decrease vigor in all perennial grass species, though some have greater tolerance than others (Kyser et al., 2007). For this reason a single-entry approach will likely necessitate relatively low pre-emergent herbicide rates to reduce the negative impact of herbicides on desirable plant species' seed. A relatively low rate of pre-emergent imazapic (52 g ha⁻¹) may be used so that non-target species

are not severely damaged in a single-entry application but has been shown to result in a relatively low decrease in biomass of medusahead of 52% (compared to the control) which only lasted one year (Sheley et al., 2012). A higher rate of herbicide application would likely have increased medusahead control allowing for better revegetation of desired in species (Monaco et al., 2005) but is impractical with a single-entry approach unless a seed technology can be applied to limit herbicide damage to seeded species.

One other issue with the multiple-entry approach is that since a delay in seeding allows invasive annual species to re-dominate (Madsen et al., 2014), increasing the likelihood that revegetation efforts fail. This results in multiple herbicide applications at the same site. Aside from being costly, repeated herbicide use can also lead to herbicide resistance in exotic species via selection pressures as all but herbicide resistant individuals are controlled or eradicated (Klein et al., 2008; Norsworthy et al., 2012). Currently, no resistance has been reported for medusahead, however another species in the Triticeae tribe (hare barley (*Hordeum murinum*) has evolved resistance to two herbicidal modes of action and it is possible for medusahead to evolve a resistance as well (Kyser et al., 2014). Unfortunately, cheatgrass has already exhibited resistance to PSII inhibitors, ACCase inhibitors, and ALS inhibitors in grass crop fields (Heap, 2017; Sebastian et al., 2017a). In order to combat the evolution of herbicide resistance, rangeland managers must diversify their use of weed management techniques, increase desired plant competitiveness, and use revegetation tools that increase the likelihood of success after one application (Norsworthy et al., 2012).

Indaziflam: A new herbicide tool

The use of a new herbicidal mode of action is one way to diversify management (Klein et al., 2008). Indaziflam is a new pre-emergent herbicide that acts as a cellulose biosynthesis inhibitor in

both monocots and dicots but has a different molecular target than preexisting cellulose biosynthesis inhibitors (Brabham et al., 2014). Indaziflam is more lipophilic than imazapic, aminocyclopyrachlor, picloram, and aminopyralid, likely resulting in a longer soil residue and longer control than other herbicides (Sebastian et al., 2017*b*). Tank mixing herbicides with indaziflam provided increased invasive control up to four years after treatment for dalmatian toadflax and cheatgrass in one study (Sebastian et al., 2017*b*). Additionally, indaziflam alone increased cheatgrass control two and three years after treatment compared with imazapic, glyphosate, and rimsulfuron (Sebastian et al., 2016*a*). Other direct comparisons between imazapic and indaziflam suggest indaziflam application results in superior invasive winter annual grass control two years after treatment (Sebastian et al., 2017*a*).

Additionally, indaziflam can be applied at lower rates and still decrease biomass by 50% for six invasive winter annual grasses including medusahead and cheatgrass (Sebastian et al., 2016*b*). Indaziflam alone compared to aminopyralid and aminocyclopyrachlor reduced plant biomass to 50% at lower rates and was 3-145 times more active for nine different species (Sebastian et al., 2017*b*). These data suggest that when indaziflam gets approved for grazing lands, it may be extremely helpful in restorative revegetation efforts by increasing control of invasive annual winter grasses for up to 4 years. While indaziflam has better control efficacy than other evaluated herbicides, it still poses a risk to non-target species (Jeffries et al., 2014). Further research is needed to determine non-target species damage risks of indaziflam used in ecosystem restoration and to identify techniques that afford protection from the herbicide to non-target species so that indaziflam can be used in a single entry approach.

Activated Carbon: A Tool for the Future

Activated carbon (AC) may represent a tool that can be used to overcome the effects of herbicide

damage to seeded species. AC is carbon that has been treated by steam or chemical activation to increase porosity and adsorptive surface area. Surface area of AC is generally between 600-1200 sq m/g³ (Coffey and Warren, 1969). Increased porosity, both macro and micro pores, allow AC to adsorb many different compounds (Hung, 2012; Koehlert, 2017). Adsorption is the process of accumulating materials, either physically or chemically, onto a solid surface (Koehlert, 2017). AC best adsorbs compounds that have an aromatic structure and that are undissociated organic acids (Giusti et al., 1974). Since most herbicides including imazapic (PubChem, 2005*a*), indaziflam (PubChem, 2005*b*), 2,4-D, and MCPA, (Abdel daiem et al., 2015) have an aromatic structure, they adsorb readily to AC. In a study comparing AC to other soil adsorbents for eight different herbicides, AC was the best adsorbent for all but one of the herbicides (Coffey and Warren, 1969).

The use of AC in crop agriculture was mostly initiated in the late 70s and early 80s and various studies have shown that applying AC to the desired crop is effective at limiting herbicide effect. In one study, sweet potato (*Ipomoea batatas*) sprouts were protected when planted in fields previously treated with 2,4-D by dipping their roots in powdered AC (Arle et al., 1948). In direct seeded asparagus (*Asparagus officinalis*), a 3 cm band of AC protected seedlings and improved stand density compared to control (no AC) treatment when three of five herbicides were applied (Ogg, 1978). AC at 300 kg/ha prevented loss of biomass of alfalfa (*Medicago sativa* L.), beans (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), potatoes (*Solanum tuberosum* L.), and sugarbeets (*Beta vulgaris* L.) from soil residues of terbacil (Ogg, 1982). In cotton (*Gossypium birsutum* L. 'Stoneville 213'), AC applied at 83 kg/ha adequately, but not absolutely, protected the crop from diuron applied at 3.55 kg/ha (Chandler et al., 1978).

Various studies have also demonstrated AC's effectiveness when applied to protect grass species. A greenhouse study found that AC applied at seeding stage protected Italian rye-grass

(Lolium multiflorum Lam.) from diuron (Burr et al., 1972). In grass seed crops, including Italian ryegrass (Lolium multiflorum Lam. 'Tetrone'), perennial ryegrass (Lolium perenne L. 'Linn'), tall fescue (Festuca arundinacea Schreb. 'Fawn'), chewings fescue (Festuca rubra var. commutata Gaud.), orchardgrass (Dactylis glomerata L. 'S-143'), Kentucky bluegrass (Poa pratensis L. 'Merion'), and colonial bentgrass (Agrostis tenuis Sibth. 'Highland'), AC applied in a slurry band over the desired grass seed protected grasses from herbicide applications enough to grow a satisfactory grass stand. However, amounts of AC needed to produce satisfactory grass stands varied between grass species, type of herbicide, and amount of herbicide (Lee, 1973). In a wild grass study, AC applied in a 3 cm band effectively protected switchgrass (Panicum virgatum L.) from imazethapyr and streambed bristlegrass [Setaria leucopila (Scribn. & Merr.) K. Schum.] and shortspike windmillgrass (Chloris subdolichostachya Nash) from flumioxazin (Grichar et al., 2012).

While banding AC has shown promise for preventing herbicides from damaging desired species, improvements on application of AC could be made, particularly for use in rangeland settings. The largest issue with banding is that it causes a decrease in weed control within the bands (Lee, 1973; Ogg, 1978). Additionally, protection offered to desired species by banding varies based on soil texture, with sandy loam soils requiring three times more AC in effective band application compared to clay-loam soil due to lateral movement of herbicides (Burr et al., 1972). Coating seeds with AC may provide protection for the seed while weeds remain unprotected (Madsen et al., 2014). However, a thin layer of carbon, of usually around 1-2 mm, if the coating is applied using commercial systems, may not provide enough protection from herbicides as the seed germinates and the radical extends away from the offered protection (Madsen et al., 2014). In a study comparing AC coating and thicker AC pellets around seeds, coating was only effective at protecting seeds at low herbicide rates (Madsen et al., 2014). AC pellets (herbicide protection

pods), providing a thicker layer of AC encompassing the seeds, protected seeded species from herbicide toxicity at much higher rates of herbicide application than AC seed coatings (Madsen et al., 2014). These herbicide protection pods (HPPs) were produced using a pasta extruding machine and a dough containing AC, diatomaceous earth, seed, and water (Madsen et al., 2014).

HPPs may also be more effective than seed coatings due to additional factors. For example, the pelleting process agglomerates seeds. Madsen et al. (2012) found that agglomeration of seeds caused an increase in seedling emergence and an increase in individual plant biomass, despite the potential effects of intraspecific competition. Agglomeration is likely most helpful in clay soils with an abiotic crust because more force is necessary for seedling emergence (Madsen et al., 2012). Pelleting may also be useful for establishing native species with seeds that are small or have low vigor because pellets may create a favorable microclimate during germination. For example, pelleting Wyoming big sagebrush (A. tridentata Nutt. ssp. wyomingensis) seed, which are approximately 0.5 mm long, increased seedling emergence (Madsen et al., 2016*a*). When pellets were applied across an elevation gradient with sagebrush seed, pellets increased success in revegetating sites when combined with bare seed (Davies et al., 2018). This suggests success rate could be increased by applying both techniques because pelleting may be more suited to one site due to micro-variations in environment while the site directly adjacent is more suitable for bare seed (Davies et al., 2018). Pelleting may also provide a concentration of nutrients, including AC to further encourage seedling survival. Amending potting soil with AC has been shown to increase biomass of plants by an average of 33%, however results differ among species (Lau et al., 2008).

Increasing success of rangeland revegetation with pellets could offset the costs of pelleting seeds. It is estimated that establishment of seeded native species in the sagebrush steppe has a very low success rate, resulting in a large amount of funding being expended on repeated seeding
attempts (Madsen et al., 2016). Pelleting may increase costs up front, but if it reduces the number of seed applications it will decrease overall costs (Madsen et al., 2016*a*). Boyd and Davies (2012) estimated costs of successful revegetation ranged from \$247.81 to \$695.44/ha. A small increase in successful revegetation (determined as establishment of > 5 plants m⁻²) could dramatically lower the per hectare costs. In one field study, crested wheatgrass (*Agropyron desertorum* [Fisch.] Schult.) seedling density was 300% greater when seeded in HPPs compared to bare seed when imazapic was being used to control invasive annuals (Davies et al., 2017). A second field study showed that squirreltail (*Elymus elymoides* (Raf.) Swezey) and Siberian wheatgrass (*Agropyron fragile* (Roth) Candargy) plant densities, height, leaf length, and stem densities were greater in a HPP treatment compared to bare seed when applied concurrent with imazapic (Davies, 2018).

Further research needs to be conducted on the efficacy of utilizing HPPs (pellets containing AC) as an herbicide protectant when revegetating rangeland ecosystems (Davies 2018; Davies et al., 2017; Madsen et al., 2014). Currently there are relatively few studies evaluating the use of HPPs with different pre-emergent herbicides, different species, and under field conditions. In studies using bands of activated carbon slurry, amounts of AC needed varied between grass species, type of herbicide, and amount of herbicide (Lee, 1973). It is likely that different applications of HPPs will similarly require unique concentrations of AC. Some species may be more sensitive to herbicides and will be less protected by HPPs. HPPs will likely also have different adsorptive capacity for different herbicides due to differences in herbicide molecular structure (Giusti et al., 1974). More field studies are necessary to evaluate the effectiveness of HPPs across spatially and temporally heterogeneous landscapes. Additionally, more studies using a greater suite of species are necessary to fully investigate the effectiveness of HPPs in creating a diverse and functional plant community.

This manuscript will attempt to further investigate the use and efficacy of HPPs in rangeland revegetation/restoration. To that end, this manuscript will address the research objectives of 1) determining the effectiveness of HPPs with a new, pre-emergent herbicide, indaziflam, at multiple application rates; 2) identifying if HPPs can be used with sagebrush, a vitally important shrub species in the sagebrush steppe; 3) evaluating the efficacy of HPPs for protecting several important revegetation species from imazapic in a field setting; and 4) determining the effect of HPPs on establishment of seeded species over the course of two years in a field setting.

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CHAPTER 2:

NATIVE SEEDS INCORPORATED INTO ACTIVATED CARBON PODS APPLIED CONCURRENTLY WITH INDAZIFLAM: A NEW STRATEGY FOR RESTORING ANNUAL-INVADED COMMUNITIES?

D.R. Clenet¹, K.W. Davies², D.D. Johnson³, J. Kerby⁴

Authors are: ¹Masters Student at Oregon State University, ²Rangeland Scientist USDA-Agricultural Research Service, Eastern Oregon Agricultural Research Center, Oregon State University, ³Associate Professor of Oregon State University, ⁴Southeast Oregon Sagebrush Steppe Coordinator for the Nature Conservancy

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Abstract

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

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

Implications for Practice

• Activated carbon herbicide protection pods (HPPs) can be used to seed native species simultaneously with indaziflam application to control exotic annual grasses.

- HPPs used with indaziflam increase the likelihood of successful restoration because indaziflam should reduce exotic annual grass competition for extended time frames.
- Shrubs, bunchgrasses, and likely other plant functional groups, can be seeded in HPPs when indaziflam is applied to control exotic annuals.
- HPPs will likely be effective when combined with other pre-emergent herbicides.
- Refinement in the formulation of HPPs tested in this study may be needed to improve establishment of small-seeded species.

Introduction

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

Restoration of invaded rangelands is imperative in order to support native fauna and regain agricultural and recreational services provided by sagebrush (*Artemesia* L.) steppe ecosystems (Davies et al., 2014*b*; Masters et al., 1996). Competition from invasive annual grasses limits

restoration success (Boyd and Davies, 2012; Madsen et al., 2016*a*; Young et al., 1999). Invasive annual grasses need to be controlled to decrease competition with seeded native perennial grass (Huddleston and Young, 2005; Sheley and Krueger-Mangold, 2003; Young et al., 1999) and this is often achieved with pre-emergent herbicides (Kyser et al., 2007; Sheley et al., 2007). However, the decrease in competition afforded by pre-emergent herbicides is difficult to take advantage of while the herbicide is active due to non-target damage to seeded species (Davies et al., 2014*a*; Sheley et al., 2007). To avoid this, a multiple entry method is used wherein the herbicide is applied and a year or more passes before seeds are sown (Huddleston and Young, 2005). While waiting a year to seed limits herbicide damage to seeded species, it also increases the likelihood that invasive species will begin to re-establish (Madsen et al., 2014). A single-entry method, where pre-emergent herbicide and seeds are applied concurrently, has been attempted, but very low herbicide application rates are required to limit damage to non-target species and results in limited control of invasive species (Sheley, 2007; Sheley et al., 2012).

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

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

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

Methods

Experimental design

The study was conducted in a grow room at the Eastern Oregon Agricultural Research Center, in Burns, OR. Soil used in the experiments was collected in eastern Oregon from the Northern Great Basin Experimental Range (43° 27'58.18" N 119° 41'49.11" W). The soil was a Gradon gravelly fine sandy loam and was sandy-clay-loam when textured (USDA NRCS 2018). Soil was sifted to exclude particles above a 6.35 mm. Medusahead seed was collected in Harney County, OR (43° 43.845" N, 118° 22.353" W, 1138 m elevation) and was frozen for 2 days before planting to break dormancy and ensure maximum germination (Young et al., 1968). Wyoming big sagebrush and bluebunch wheatgrass seed were purchased from a commercial dealer.

Treatments were bare seed and seed incorporated into an AC pod (i.e. HPPs). HPPs were composed of 43% Ca Bentonite, 33% AC, 6% worm castings, 14% compost, and 4% seed by dry weight. Dry materials were thoroughly mixed, then water was added so the material could be formed and passed through a pasta extruder (Model TR110, Rosito Bisani, Los Angeles, CA). The AC mixture was extruded through an 8 mm diameter die, resulting in cylindrical strands which were then cut into pods approximately 15 mm long. Pre-emergent herbicide treatments were applied to sagebrush and bluebunch wheatgrass, at four indaziflam (Esplanade 200 SC, Bayer CropScience, Monheim am Rhein, Germany) rates, and replicated five times. The study was conducted in 53 cm x 42 cm x 11.5 cm boxes. Twenty boxes were filled and lightly packed to 2.5 cm below the top with soil. Each box was divided into five 10.6 cm x 42 x 11.5 cm containers with plastic dividers. Each box was randomly assigned one of the indaziflam application rates. The species and seed treatments (bare seed or HPP) were each randomly assigned to one of four containers in each box. One container in each box was planted with medusahead as a bio-indicator of herbicide effectiveness. Seeds were planted at a rate of 50 pure live seeds per container for each species-treatment combination. Seed rate per container for HPP treatments was determined by estimating the number of viable seeds per pod. All pods were pressed gently into the soil and left uncovered. Medusahead and bluebunch wheatgrass bare seed were pressed into the soil and left uncovered while sagebrush bare seed was lightly covered with soil to prevent movement due to small size during watering. This resulted in each box containing one container each of bare seed bluebunch wheatgrass, HPPs bluebunch wheatgrass, bare seed sagebrush, HPPs sagebrush, and bare seed medusahead. Boxes were watered to field capacity the day before planting. Boxes were then weighed to determine weights at 75% field capacity for later watering. After seeding,

indaziflam was applied at the following rates 1) 46.7 g ai·ha⁻¹(low), 2) 66.7 g ai·ha⁻¹ (mid), 3) 93.4 g ai·ha⁻¹ (high), and 4) zero (the control). Indaziflam was applied using a hand operated backpack sprayer (Solo, Newport News, VA). After indaziflam application, the boxes were placed 61 cm below PlatinumLED P1200 lights (PlatinumLED, Kailua, HI) using a randomized design. The LED lights were set to a cycle of 12 hours of light (5:00-17:00) followed by 12 hours of darkness, per manufacturer specifications for germination and seedling growth. The grow room was set to 22°C temperature and 50% relative humidity. Boxes were watered daily to 75% field capacity by weight for 2 weeks, then every other day for the remainder of the experiment.

Measurements

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

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

Statistical analysis

Mixed model analysis of variance (ANOVA) was used to compare seeds incorporated into HPPs with bare seed at different levels of indaziflam application (SAS ver. 9.4). Treatment (i.e., HPPs or bare seed) and rate were fixed variables, while replicate and treatment by replicate were random variables in the models. Data were analyzed individually by species. Effects and differences in treatment means were considered significant if P values were ≤ 0.05 and means are reported with standard errors (mean ± standard error). Treatment means were separated using the LS means procedure in SAS. All data reported were original data (non-transformed).

Results

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

Sagebrush height, diameter, and container biomass were affected by treatment, herbicide rate, and the interaction between treatment and herbicide rate (P<0.05; Fig. 2B-D). Sagebrush

density was influenced by herbicide rate and the interaction between herbicide rate and treatment (P<0.05) but was not affected by treatment alone (P=0.10; Fig. 2A). When indaziflam was not applied, sagebrush density and biomass were greater in the bare seed compared to HPPs treatment (Fig. 2A, D). When indaziflam was applied, HPPs had greater density, height, diameter, and biomass at all rates (P<0.05; Fig. 2A-D). Sagebrush bare seed container biomass was more than four times greater than the biomass in the HPPs container without indaziflam application (Fig. 2D). Density of medusahead, the bio-indicator of indaziflam effectiveness, varied by herbicide application rate (P<0.001). Density was lower in low, medium, and high herbicide application rates compared to the control (P<0.001; Fig. 3). However, there was no difference between the low, medium, and high rates (P>0.05; Fig. 3).

Discussion

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

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

Our results show that HPPs are effective when used with indaziflam. Although not currently registered for use on grazing lands (Bayer, 2018), our results indicate that indaziflam may be a promising restoration tool for annual grass-invaded communities. Indaziflam has longer soil activity compared to other common pre-emergent herbicides (Sebastien et al., 2016*a*; 2016*b*; 2017*a*; 2017*b*) that affords seeded restoration species a longer establishment window before experiencing competition from re-invading annual grass. However, land managers cannot seed until indaziflam soil activity significantly diminishes in order to avoid desired-species damage. The delay in seeding after indaziflam would therefore be longer than the delay after application of other, common pre-emergent herbicides. Integrating HPPs with indaziflam also increases the time that seeded species have to grow when competition from annual grasses is limited. This may lead to greater establishment and growth of seeded species, increasing the likelihood that they would

limit re-invasion by exotic annuals.

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

Growth characteristics and abundance for both species were generally greater for bare seed than for seed incorporated into HPPs when indaziflam was not applied. This indicates that HPPs may hinder the emergence and growth of plants. This trend was more pronounced in sagebrush compared to bluebunch wheatgrass. It is possible that sagebrush was more inhibited by HPPs because sagebrush seed is very small, only has the ability to emerge from a depth of ~5 mm, and can be easily restricted by soil crusts (Jacobson and Welch, 1987; Madsen et al., 2012; Madsen et al., 2016*b*). The clay and powdered AC used in the HPPs may have compacted when compressed through the die and thus presented a physical barrier to seedling emergence similar to a soil crust.

Additionally, since HPPs have a diameter of 8 mm, some sagebrush seed may have been too deep to emerge. Further research is needed to refine the HPP formulation to reduce its inhibition of small-seeded species emergence. This may include reducing the clay component of the formula or by adding a fibrous component to help limit compaction. The HPPs used in this study also had a similar, though smaller, effect on the emergence of bluebunch wheatgrass, a much larger seeded species. Despite the limits to seedling density and growth, the benefits of HPPs could outweigh their costs when used in combination with pre-emergent herbicides because they increase potential seedling establishment.

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

Future research in the field to validate the results of this study are necessary because grow room experiments generally limit the amount of stress that seedlings experience. In contrast,

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rangelands have high annual climactic variability and heterogeneous landscapes. Additionally, field experiments evaluating long-term survival of seedlings established within HPPs are crucial. Soil organic matter content, soil volumetric water content, soil texture, indaziflam application rate, and rooting depth of plants all affect the amount of injury caused by indaziflam to post-germinative establishment of grass species (Jeffries and Gannon, 2016; Jones et al., 2013; Gomez de Barreda et al., 2013; Schneider et al., 2015). The long-term effects of HPPs have not been studied and it is unknown if they will limit indaziflam injury beyond early seedling growth.

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

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



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



Figure 3. Bare seed medusahead density (mean \pm S.E) across increasing indaziflam application rates

CHAPTER 3: ACTIVATED CARBON PODS FACILITATE SAGEBRUSH AND BUNCHGRASS ESTABLISHMENT UNDER IMAZAPIC CONTROL OF EXOTIC ANNUAL GRASSES

Danielle R. Clenet¹, Kirk W. Davies², Dustin D. Johnson³, and Jay D. Kerby⁴

Authors are: ¹Masters Student at Oregon State University, ²Rangeland Scientist USDA-Agricultural Research Service, Eastern Oregon Agricultural Research Center, Oregon State University, ³Professor of Animal and Rangeland Sciences, Oregon State University, ⁴Southeast Oregon Sagebrush Steppe Coordinator for the Nature Conservancy

Rangeland Ecology and Management

Society for Range Management International Headquarters 6901 S Pierce St Suite 230 Littleton, CO 80128

IN REVIEW

Abstract

Revegetation of annual grass-invaded rangelands is a primary objective of land managers following wildfires. Controlling invasive annual grasses is essential to increasing revegetation success; however, pre-emergent herbicides used to control annual grasses limit immediate seeding due to non-target herbicide damage. Thus, seeding is often delayed one year following herbicide application. This delay frequently allows for re-invasion of annual grasses, decreasing the success of revegetation efforts. Incorporating seeds into herbicide protection pods (HPPs) containing activated carbon (AC) permits concurrent high herbicide application and seeding because AC deactivates herbicides. While HPPs have, largely in green-house studies, facilitated perennial bunchgrass emergence and early growth, their effectiveness in improving establishment of multiple species and functional groups in the field has not been assessed. We seeded five bunchgrass species and two shrub species as bare seed and seed incorporated into HPPs at two field sites with high imazapic application rates to control annual grasses. HPPs significantly improved establishment of sagebrush (Artemesia tridentata Nutt. Spp. wyomingensis Beetle & Young), and crested wheatgrass (Agropyron cristatum (L.) Gaertn.) over the two-year study. Three native perennial grass species were protected from herbicide damage by HPPs but had low establishment in both treatments. The two remaining shrub and grass species did not establish sufficiently to determine treatment effects. While establishment of native perennial bunchgrasses was low, this study demonstrates that HPPs can be used to protect seeded bunchgrasses and sagebrush from imazapic, prolonging establishment time in the absence of competition with annual grasses.

Keywords: Wyoming big sagebrush, herbicide protection pod, revegetation, restoration, post-fire, seed enhancement technology

Introduction

Seeding of desired species following wildfires is a crucial tool used by managers to mitigate ecological damage from fires on rangelands (Pyke et al., 2013; James and Svejcar, 2010; Eiswerth and Shonkwiler, 2006). Revegetation efforts are intended to decrease post-fire erosion and limit positive feedback of the annual grass-fire cycle (Pyke et al., 2013; Eiswerth and Shonkwiler, 2006). However, rapid post-fire increases in exotic annual grasses often limit the success of seeding attempts because they are competitive with perennial grasses at the seedling stage (Clausnitzer et al., 1999; Humphrey and Schupp, 2004; James and Svejcar, 2010).

In order to increase revegetation success post-fire, pre-emergent herbicides are often used to control invasive annual grasses (Sheley and Krueger-Mangold, 2003; Sheley et al., 2007). Perennial bunchgrasses are usually seeded one year after pre-emergent herbicide application to avoid non-target species damage (Huddleston and Young, 2005; Davies et al., 2014a). Once established, mature perennial bunchgrasses are able to limit exotic annual grass dominance, decreasing the risk of catastrophic wildfire, and providing habitat and forage for wildlife and livestock (Davies and Johnson, 2017; Davies and Nafus, 2013; Madsen et al., 2016a; Duncan et al., 2004; D'Antonio and Vitousek, 1992). Waiting a year following herbicide application reduces risk of non-target damage to seeded species but may also allow for the re-invasion and dominance of annual grasses (Huddleston and Young, 2005; Sheley et al., 2012; Madsen et al., 2014). Sheley et al. (2012) evaluated a single entry approach for medusahead (Taeniatherum caput-medusae (L.) Nevski)-invaded rangelands in which herbicide is applied concurrently with seeding of desired species. A single-entry approach is more cost efficient and allows seeded species the opportunity to establish while competition from invasive annual grasses is limited, but necessitates a low herbicide rate which may not sufficiently control invasive annual grass enough for successful revegetation (Sheley, 2007; Sheley et al., 2007; Sheley et al., 2012; Davies et al., 2014a).

Therefore, use of higher herbicide application rates to achieve more complete, longer-lasting control of annuals may be necessary for the single entry approach to be a practical option in annual grass-invaded rangelands (Monaco et al., 2005; Sheley et al., 2012; Kyser et al., 2007).

Herbicide protection pods (HPPs) are a recent seed enhancement technology that employs activated carbon (AC) to adsorb and deactivate herbicide to protect desired seed from damage (Madsen et al., 2014). HPPs allow a more effective single entry approach because desired seed can be protected from higher rates of herbicide application, which are necessary for lasting, effective control of annual grasses (Madsen et al., 2014; Davies, 2018; Clenet et al., 2019). In combination with pre-emergent herbicides, HPPs can prolong the length of time when seeded species can establish in the absence of competition from exotic annual grasses (Madsen et al., 2014).

Research has shown that HPPs are capable of protecting perennial bunchgrasses from preemergent herbicides (Davies et al., 2017; Davies, 2018; Clenet et al., 2019). However, only a few species have been tested thus far and the use of HPPs with native bunchgrass and shrub species in the field has not yet been fully explored (Davies, 2018; Clenet et al., 2019; Davies et al., 2017). Sagebrush is an essential component of the sagebrush-steppe and provide multiple ecosystem services as well as providing forage, shelter, and other habitat services for wildlife (Vander Haegen et al., 2000; Holthuijzen and Veblen, 2015; Longland and Bateman, 2002; Reynolds and Trost 1980). Sagebrush is difficult to establish from seed, and any seed enhancement technology that improves sagebrush establishment would be a valuable tool in sagebrush-steppe restoration (Ott et al., 2017; Knutson et al., 2014; Davies and Bates, 2017). A recent lab study showed that HPPs can protect sagebrush from pre-emergent herbicide toxicity, but they may limit sagebrush emergence and growth (Clenet et al., 2019). While controlled laboratory studies are valuable, they do not provide the full range of environmental conditions that will ultimately determine the efficacy of HPPs (Clenet et al., 2019). Information from field studies evaluating efficacy of HPPs for promoting establishment of shrubs and multiple species of native grasses are therefore essential to determining if HPPs are a viable technology for use in rangeland revegetation efforts (Davies et al., 2017).

To address this, we performed a two-year, two-site study with the purpose of evaluating the effectiveness of HPPs for protecting five species of perennial bunchgrass and two shrub species from a high rate of imazapic application post-fire, in invasive grass invaded sagebrush-steppe. We hypothesized that emergence of seeds protected within HPPs would result in greater density, cover, and size of established plants compared to bare seed.

Materials and Methods

Study Sites

The study was conducted at two sites (Wagontire and Gap Ranch) that were burned by the 2017 Cinder Butte Fire in southeastern Oregon. Both sites were formerly big sagebrush (*Artemesia tridentata* Nutt.)-bunchgrass communities that had been invaded by exotic annual grasses. The Wagontire site is located at 43° 19.749' N, 119° 50.341' W; 1511 m elevation and the Gap Ranch site is located at 43° 26.74' N, 119° 50.258' W; 1439 m elevation. Wagontire has a slope of 12% and a northeastern aspect with a Pernty gravelly silt loam soil (USDA NRCS 2019). Gap Ranch is relatively flat (slope = 2%) with a Gradon gravelly fine sandy loam soil (USDA NRCS 2019). Both sites are a Droughty Loam 11-13 PZ Ecological site (R023XY316OR).

Long term (1979-2018) mean annual precipitation was 290 mm for Wagontire and this site received 212 mm in 2018; 73% of the mean (Great Basin Weather Applications, 2019). Long term mean annual precipitation was 298 mm for Gap Ranch and in 2018 it received 198 mm; 67% of the annual mean (Great Basin Weather Applications, 2019). During the growing season (April-July) of 2018 Wagontire and Gap Ranch received 86% and 67% respectively, and in 2019, 141% and 142%

respectively of long-term mean growing season precipitation (Great Basin Weather Applications, 2019). Test sites were 20 x 30 meters in size and fenced to exclude livestock.

Experimental Design and Measurements

At each site, seven species were planted: bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve), basin wild rye (*Leymus cinereus* Scribn. & Merr.) Á. Löve), Sandberg bluegrass (*Poa secunda* J. Presl), squirreltail (*Elymus elymoides* (Raf.) Swezey), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), Wyoming big sagebrush (*Artemisia tridentata* Nutt. Spp. *wyomingensis* Beetle & Young), and antelope bitterbrush (*Purshia tridentata* (Pursh) DC.). Species were planted using two seeding treatments, bare seed (BS) and herbicide protection pods (HPP) replicated four times in a randomized design. Each replicate included two 5 m imitated drill rows (seeded by hand in a furrow) of each seeding treatment. The two rows of the same treatment were parallel to and 40 cm apart from each other while rows of contrasting treatments were separated by 1 meter. End to end the rows had a buffer of 2 meters.

In each 5-m row, 200 pure live seed (pls) \cdot m⁻¹ for each species were planted, except bitterbrush where 100 pls \cdot m⁻¹ were seeded. This resulted in 500 pls \cdot row⁻¹ for bitterbrush and 1000 pls \cdot row⁻¹ for all other species. HPPs were composed of 43% Calcium Bentonite, 33% activated carbon, 6% worm castings, 14% compost, and 4% seed by dry weight. Dry materials were mixed thoroughly then water was added to achieve a doughy consistency that could be passed through a pasta extruder (Model TR110, Rosito Bisani, Los Angeles, CA). Dough was pushed through an 8 mm diameter circular die for all species except for bitterbrush, which had larger seeds and was extruded through a 16 x 8 mm die. All extruded pods were cut into pods ~15 mm in length.

Species were planted by hand in rows to imitate drill seeding on 16 September 2017. The
sites were sprayed one day after planting with imazapic (Panoramic 2 SL, Alligare, Opelika, AL) at 210 g ai·ha⁻¹ using a hand operated backpack sprayer (Solo, Newport News, VA). During application, temperature was 18.3° C and 16.6° C, max wind speed was 11.4 km·hr⁻¹ and 12.9 km·hr⁻¹, and relative humidity was 17% and 18% for Wagontire and Gap Ranch, respectively.

Density of seeded species was determined by counting all seedlings in rows in late June 2018 and 2019. Plant height was measured for five randomly selected grass plants and five randomly selected shrub plants per row. Plant height was estimated by measuring the height of the tallest vegetative structure present on the plant. Canopy diameter was measured for five randomly selected shrub plants per row. If fewer than five plants were present, all plants were measured. Cover of seeded species was visually estimated using 0.2 m^2 quadrats at 1.5, 2.5, and 3.5 meters centered over each row. Cover and density measurements of invasive annual grass species were taken at ten random points within the sites using 0.2 m^2 quadrats. Another ten random points were measured outside of the sites to evaluate the effectiveness of imazapic control by comparing annual grass density and cover between herbicide treated and untreated areas.

Statistical Analysis

All analyses were performed using R Software version 3.5.2 (R Core Team, 2018). Analysis of variance (ANOVA) using linear mixed effects models was used to compare seeds of six of the seeded species incorporated into HPPs with bare seed when imazapic was applied to control invasive annual grasses using the {nlme} R package (Pinheiro et al., 2017). Each species was analyzed separately. Fixed effects of the perennial bunchgrasses were seeding treatment, site, year, and their interactions, with replication as a random effect. Each species had four replicates. Site was included as a fixed effect because the two sites had differing environmental conditions and making it possible to test for a treatment by site interaction. The compound symmetry model was

selected as the correlation structure used within the model based on Akaike information criterion to account for potential correlation within a year. Applying a cautionary approach, and as determined by visual analysis of error residuals, variances between years were allowed to vary within the model when necessary. Antelope bitterbrush was not analyzed because most seed was consumed by rodents within a few weeks of planting and any emergent seedlings were lost to herbivory.

Wyoming big sagebrush response was only analyzed in the second year of the study because no plants emerged the first year and there was a treatment by year interaction (p < 0.001). The fixed effects for the sagebrush mixed model were treatment and site and their interaction, while the random effect was replicate. Site was included as a fixed effect for the same reasons as above. Normality of model residuals was supported by visual analysis of graphs and thus all data were untransformed. Differences were considered significant at $P \le 0.05$. Means and standard errors are reported in text and figures.

Results

Exotic annual grass control at Wagontire was 93% in the first year and dropped to 46% in the second year. Control of exotic annual grasses was greater at Gap Ranch with 100% and 96% control in the first and second year, respectively.

In the second year (2019), sagebrush density was influenced by treatment (P < 0.001) and was almost seven times greater in the HPP compared to bare seed (BS) treatment (Fig. 4A). Sagebrush cover was influenced by treatment (P = 0.007) and was about three times greater in HPP compared to the BS treatment (Fig. 4B). Sagebrush diameter and height were not influenced by treatment (P > 0.05).

Bluebunch wheatgrass density was influenced by treatment and year (P = 0.009 and P = 0.001, respectively). Bluebunch wheatgrass density was greater in the HPP treatment compared to

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the BS treatment in both years and by 2019, bluebunch wheatgrass density was about four times greater in the HPP compared to BS treatment (Fig. 5A). Basin wildrye density was influenced by treatment, year, with a treatment by year interaction effect (Fig. 5B; P = 0.018, P = 0.002, and P =0.042, respectively). In both years, basin wildrye density was greater in the HPP treatment. However, the difference between treatments the second year decreased due to poor survival of plants (HPP = 0.2 ± 0.1 plants \cdot m row, BS = 0.1 ± 0.1 plants \cdot m row). Sandberg bluegrass density was influenced by treatment, year, and the treatment by year interaction (Fig. 5C; P = 0.012, P < 0.0120.001, and P = 0.027, respectively). Sandberg bluegrass density was greater in the HPP treatment compared to BS in both years, with a greater magnitude of difference in 2019. Crested wheatgrass density was significantly affected by seeding treatment, with a treatment by year interaction (Fig. 5D; P < 0.001 and P = 0.003 respectively). Crested wheatgrass density was greater in the HPP treatment compared to BS for both years. However, this difference narrowed in the second year of the study. Squirreltail density did not differ between seeding treatments (P = 0.855) and was low by the second year at 0.38 ± 0.1 plant \cdot m row and 0.51 ± 0.1 plants \cdot m row for bare seed and HPP treatments, respectively.

Of the native perennial bunchgrasses, only bluebunch wheatgrass cover differed between seeding treatments (P = 0.032). Bluebunch wheatgrass cover was greater in the HPP treatment in both years and was approximately five times greater in the HPP compared to the BS treatment in the second year (Fig. 6A). Crested wheatgrass cover was influenced by treatment, year, with a treatment by site interaction (P < 0.001, P = 0.004 and P = 0.05 respectively). The treatment by site interaction was due to a difference of magnitude of the effect between the two treatments but the trend of HPP being greater than BS remained consistent across sites and years (Fig. 6B). Additionally, in 2019, crested wheatgrass cover was more than two times greater in the HPP treatment compared to the bare seed treatment. Squirreltail, basin wildrye, and Sandberg bluegrass

cover were not influenced by treatment (P = 0.227, P = 0.685, and P = 0.757 respectively). Squirreltail, basin wildrye, and Sandberg bluegrass cover were low and quite variable for both the HPP treatment (1.3 ± 0.9 , 1.3 ± 1.2 , and 0.7 ± 0.3 , respectively) and the BS treatment (0.4 ± 0.3 , 3.3 ± 3.2 , and 1.7 ± 0.5 , respectively) in 2019.

Bluebunch wheatgrass height was influenced by treatment and year (P = 0.007 and P = 0.015, respectively). Bluebunch wheatgrass height was greater in the HPP treatment (Fig. 7A). Crested wheatgrass height was influenced by treatment and year (P = 0.016 and P < 0.001 respectively). Crested wheatgrass height was greater in the HPP treatment compared to BS (Fig. 7B). Squirreltail, basin wildrye, and Sandberg bluegrass height were not influenced by treatment (P = 0.778, P = 0.441, and P = 0.894 respectively).

Discussion

Our results show that herbicide protection pods can decrease herbicide effects on seeded perennial bunchgrasses and sagebrush when imazapic is applied to control invasive annual grasses. By the second year of this study, Wyoming big sagebrush density was seven times greater and bluebunch wheatgrass density was four times greater when incorporated into HPPs compared to bare seed applications. Our results, and other studies, demonstrate that HPPs can be effective at protecting multiple species from different functional groups from pre-emergent herbicides in a variety of sites invaded by medusahead or cheatgrass (Davies et al., 2017; Davies, 2018; Clenet et al., 2019). HPPs likely have benefits over bare seed when high rates of imazapic are applied because activated carbon adsorbs and deactivates the pre-emergent herbicide around the seeds, allowing them to grow while invasive annual grasses are controlled (Madsen et al., 2014; Davies et al., 2017).

Wyoming big sagebrush is often difficult to establish from seed (Ott et al., 2017; Knutson et al., 2014; Lysne and Pellant, 2004). This is the first study to show that HPPs can be used with

sagebrush in the field due to failure of the shrub to establish in past studies (e.g. Davies, 2018). The results of this study also show that, in the field when in conjunction with pre-emergent herbicide control of exotic annual grasses, the benefit of the HPPs outweighs any potential limitation to sagebrush emergence. This study found that HPPs had no effect on sagebrush seedlings after emergence and contrasts the results that HPPs limit early sagebrush growth found by a prior lab study (Clenet et al. 2019). This may have been because HPPs broke down following multiple freeze-thaw events in the field (personal observation).

In this study, the density of sagebrush seedlings was 1.2 ± 0.2 plants \cdot m row⁻¹ in the HPP treatment. These rows were planted in imitation of a rangeland drill which generally spaces rows ~12 in (30.5 cm) apart. Extrapolating to a density \cdot m⁻² based off of a rangeland drill, this study resulted in 4.8 ± 0.8 plants \cdot m⁻². A study which had similar favorable precipitation conditions found that broadcast seeding resulted in less than 0.1 plants \cdot m⁻² (Boyd and Obradovich, 2014). The HPP treatment of this study resulted in plant density far exceeding the average density of mature sagebrush plants in relatively intact Wyoming big sagebrush plant communities (Anderson and Inouye, 2001; Davies and Bates, 2010) . However, sagebrush seedlings often experience significant mortality in their first few years of life (Boyd and Obradovich, 2014). While the sagebrush seedlings in this study may experience significant mortality in the following years, beginning with a greater number of sagebrush increases the probability of a sufficient number of seedlings surviving to maturity.

Sagebrush were only detected in the second year of the study and this is probably because more optimal springtime conditions occurred in 2019 (Great Basin Weather Applications, 2019). Wyoming big sagebrush and other native plants tend to establish more reliably in years with average or above average growing season precipitation (Davies et al., 2018; Hardegree et al., 2011) and a proportion of sown Wyoming big sagebrush seeds have been shown to stay viable, especially when buried, for at least two years (Wijayratne and Pyke, 2012). Since Wyoming big sagebrush is notoriously difficult to establish reliably from seed, any technology which facilitates establishment could have wide applicability for managers across the sagebrush steppe (Ott et al., 2017; Knutson et al., 2014; Davies and Bates, 2017).

In this study, overall establishment was more limited with native bunchgrasses compared to the introduced bunchgrass, crested wheatgrass. Bluebunch wheatgrass had the best establishment among the large native perennial bunchgrasses and when scaled to m² in the same way as sagebrush density, resulted in 1.6 ± 0.44 plants \cdot m⁻² and 0.4 ± 0.24 plants \cdot m⁻² in the HPP and BS treatments respectively by 2019. In contrast, scaled crested wheatgrass density by 2019 were 23.8 \pm 7.12 plants \cdot m⁻² and 18.12 \pm 6.36 plants \cdot m⁻² in the HPP and BS treatments respectively. The limited establishment of the native perennial bunchgrasses compared to the introduced bunchgrass used is similar to results reported by other authors (Boyd and Davies, 2010; Davies et al. 2015; Hull, 1974). Native perennial bunchgrasses generally establish infrequently and do not establish as well as introduced species in years with unfavorable precipitation patterns, thus limited success in the first year of the study was not unexpected (James and Svejcar, 2010; Davies et al. 2015; Boyd and Davies 2010). When conditions were more conducive in the second year of the study to native bunchgrass establishment, it is likely that few viable seeds remained. James et al. (2011) found that by the second year, less than 1% of ungerminated native perennial grass seeds were still viable. While low establishment rates made it difficult to detect treatment effects for all native bunchgrasses, HPPs were effective as a technology used to protect seeded species from damage by imazapic. Sandberg's bluegrass may have also had greater seed treatment differences than was captured, due to the re-sprouting of pre-fire survivors within the plots which made it difficult to identify planted individuals. Additionally, the common HPP formulation used may have induced differing degrees of establishment rates, and different species may require slightly different HPP

mixtures. Refinement of HPPs to meet individual restoration species' needs may further improve the effectiveness of HPPs with native perennial bunchgrass species. For example, small seeded species may need smaller pod diameters to facilitate emergence.

While the results of the study suggest that HPPs increased cover and density of crested wheatgrass compared to bare seed, by the second year of the study the difference had decreased between the two treatments (Fig. 6A and Fig. 5A respectively). This is probably, to some degree, an artifact of the study design instead of an accurate representation of treatment effects over time. BS and HPP treatment rows were planted only one meter apart, which may have allowed seed from HPP-established crested wheatgrass, which produced seed in the first year of the study, to disperse into the bare seed treatment, inflating the density and cover of crested wheatgrass in the bare seed treatment. Further field studies in which treatments are applied to larger areas and are spread further apart to diminish edge effect are probably necessary to determine the long-term effects of using bare seed versus HPPs.

The success of HPP technology at protecting perennial bunchgrass and sagebrush seed from damage by imazapic indicates that this technology is effective with multiple functional groups. The increased establishment of sagebrush, crested wheatgrass and bluebunch wheatgrass incorporated into HPPs indicates that HPPs may improve restoration success in annual grass-invaded sagebrush-steppe rangelands. While HPP are more costly than bare seed, the tradeoff of increased establishment success and decreased necessity for repeated application of herbicide and seeding attempts could make this technology valuable (Madsen et al., 2016*a*). Additionally, increased establishment of perennial bunchgrass species, which are competitive with invasive annual grasses when mature, can decrease the redominance of invasive annual grasses (Davies and Johnson 2017; Sheley and James 2010). Decreased cover of invasive annual grasses decreases the continuity of

the fuel bed, potentially leading to a reduction in the extent of wildfires and future cost of restoration (Davies and Nafus 2013; Reed-Dustin et al., 2016; Epanchin-Niell et al., 2009). HPPs are a tool which has the potential to increase revegetation success in areas where annual grasses are treated with pre-emergent herbicides. Greater revegetation success will be even more critical in the face of predicted climate changes and increasing atmospheric CO_2 levels which are likely to favor invasive annual grass growth and distribution and alter wildfire characteristics and regimes (Bradley, 2009; Abatzoglou and Kolden, 2011; Creutzberg et al., 2015; Smith et al., 1987).

Management Implications

Herbicide protection pods (HPPs) limited herbicide toxicity to seeded species and thus allowed bunchgrass species and sagebrush to be seeded concurrently with imazapic application to control exotic annual grasses. This suggests that HPPs can be used effectively with multiple plant functional groups when exotic annual species are controlled with a pre-emergent herbicide. HPPs allow seedlings a longer establishment window prior to experiencing substantial competition from exotic annuals and could facilitate establishing perennial dominated communities that will be resistant to redominance by exotic annual species. Further refinements of HPPs are warranted, with respect to tailoring size and matrix formulation to specific individual or groups of revegetation species and with other pre-emergent herbicides. More importantly, however, will be scaling up the production of HPPs to decrease their cost and make them readily available for restoration projects. It is important to note that this studied included sites between 1439 and 1511 m (4721 and 4957 ft) in elevation and encompassed a year with above-average precipitation. Seedings at hotter and drier sites not followed by a year with adequate precipitation may not perform as well as seen in this study. Regardless, HPPs are a promising tool for managing exotic annual grass invasions and improving post-fire restoration and revegetation.

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Figures



Figure 4. Wyoming big sagebrush density (A; mean + SE) and cover (B; mean + SE) for two treatments (Herbicide protection pods and Bare seed) averaged over site in 2019. Asterisk (*) indicates significance ($P \le 0.05$).



Figure 5. Bluebunch wheatgrass (A), basin wildrye (B), Sandberg bluegrass (C), and crested wheatgrass density (mean + SE) for two treatments (Herbicide protection pods and Bare seed) averaged over sites in 2018 and 2019. Asterisk (*) indicates significance at $P \le 0.05$. Note different y-axis scales.



Figure 6. Bluebunch wheatgrass (A) and crested wheatgrass (B) cover (mean + SE) for two treatments (Herbicide protection pods and Bare seed) averaged over sites in 2018 and 2019. Asterisk (*) indicates significance at $P \le 0.05$. Note different y-axis scales.



Figure 7. Bluebunch wheatgrass (A) and crested wheatgrass (B) height (mean + SE) for two treatments (Herbicide protection pods and Bare seed) averaged over sites in 2018 and 2019. Asterisk (*) indicates significance at $P \le 0.05$. Note different y-axis scales.

CHAPTER 4: OVERARCHING CONCLUSIONS AND FUTURE RESEARCH

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Multiple studies support the effectiveness of HPPs as a seed enhancement technology that allows desired species to be seeded concurrently with application of pre-emergent herbicide (Madsen et al., 2014; Davies et al., 2017; Davies, 2018; Clenet et al., 2019). HPPs act as an herbicide protectant because they contain activated carbon which adsorbs and deactivates herbicide around the desired seed (Madsen et al., 2014). In addition to offering protection from herbicides, HPPs may also benefit seeds within them through agglomeration of seeds, by neutralizing allelopathy present, by providing nutrients to the seed, by limiting small seeded species movement from areas they were planted, and by increasing seed ability to persist until more optimal conditions for growth are present (Madsen et al., 2012; Madsen et al., 2016*a*; Lau et al., 2008; Kulmatiski and Beard, 2005). HPPs in combination with pre-emergent herbicides enable seeded species increased time to establish without competition from other plants, like invasive annual grasses (Madsen et al., 2014). This jump start in establishment allows desired species to grow past the point when interspecific competition would normally cause them to fail.

HPPs are likely able to adsorb and deactivate many herbicides due to the chemical and physical structure of activated carbon (Koehlert, 2017; Hung, 2012; Coffey and Warren, 1969). Recent studies support this idea and have shown that HPPs are effective when used with both imazapic and indaziflam (Davies et al., 2017; Davies, 2018; Clenet et al., 2019). Imazapic is a commonly used pre-emergent herbicide in rangelands but is only effective at providing control for 1 to 2 years (Kyser et al., 2013; Monaco et al., 2005; Sheley et al., 2007; Kyser et al., 2007). Indaziflam is a new pre-emergent herbicide that has a different mode of action and is more hydrophobic than imazapic (Brabham et al., 2014; Sebastian et al., 2017*a*). Most importantly, is that indaziflam appears to have greater control of invasive annual grasses at lower rates and exhibits longer lasting control than imazapic (Sebastian et al., 2016; 2017*b*; Clark et al., 2019).

While currently in the process of becoming approved for grazing lands, indaziflam has great potential in combination with HPPs for revegetating rangelands. HPPs applied concurrently with indaziflam application may further extend the amount of time that desired species have to establish and mature with limited competition, increasing the probability of establishing a plant community which provides desired ecosystem services and resists future dominance of exotic annual grasses (Clenet et al., 2019). In addition to showing that HPPs can be used with two different pre-emergent herbicides, recent research has also proven that HPPs are useful at highest recommended herbicide rates for both, though at the highest rate of indaziflam, protection offered by HPPs decreases (Davies, 2018; Clenet et al., 2019). This means that, in cases where greater control of the target species needs to be achieved, high rates of herbicide can be used.

Research shows that HPPs can also be used with a variety of species including crested wheatgrass, Siberian wheatgrass, squirreltail, bluebunch wheatgrass, basin wildrye, Sandberg bluegrass and Wyoming big sagebrush. In sagebrush-steppe rangelands, it is important to be able to use a technology with both introduced species and native species to meet different management goals. HPPs have proven to be a strong candidate as one such seed enhancement technology. As with bare seed, native perennial bunchgrasses in HPPs are still unlikely to establish in all years due to their episodic nature of recruitment which depends on years with average or above average precipitation (Asay et al., 2001; Knutson et al., 2014; Robins et al., 2013; Davies et al., 2015). However, HPPs still provide a considerable benefit because, during years in which native species can establish, the concurrent application of a pre-emergent herbicide and HPPs will extend the amount of time that natives have to establish without competition from invasive species (Madsen et al., 2014).

In the Northern Great Basin, sagebrush species are a critical component of the plant community and provide a wide variety of ecosystem services (Prevey et al., 2010; Davies et al., 2011) including serving as refuge and dispersal islands following disturbances (Longland and Bateman, 2002) and providing forage and habitat for at least 170 different vertebrate species (Connelly et al., 2000; Shipley et al., 2006; Donnelly et al., 2017; Rottler et al., 2015). Success of Wyoming big sagebrush incorporated in HPPs is an important finding of this manuscript. In part, because this success shows that HPPs can be used with multiple functional groups, because previous results with sagebrush in labs were unfavorable (Clenet et al., 2019), and because sagebrush are a species of restoration interest (Boyd and Obradovich, 2014; Meinke et al., 2009). Additionally, the establishment of sagebrush seedlings in HPPs in the field study was much higher than the expected establishment of bare seed planted conventionally in a year with similar precipitation (Boyd and Obradovich, 2014). The results of HPPs with sagebrush are very promising and validate further research to investigate more thoroughly.

Recent research provides evidence that HPPs are a potential useful technology for managers to have in their toolbox in the Great Basin and Intermountain West but, HPPs also have potential in rangelands worldwide (Davies et al., 2017; Davies, 2018; Clenet et al., 2019). Invasive weeds are problematic worldwide and thus there is a widespread need for technologies which improve restoration efforts (D'Antonio et al. 2011; D'Antonio & Vitousek 1992). For example, on the Qinghai-Tibetan Plateau of China, planted perennial grass mixtures have limited success because of competition with annual weeds (Dong et al., 2005). Attempts in Australia to restore and revegetate degraded grasslands have limited success (Prober and Thiele 2005) and could be helped through the use of HPPs with herbicides. HPPs could also be used with perennial grasses that are first controlled with a contact herbicide and then a pre-emergent herbicide is used to control reestablishment from seed, such as *Aristida junciformis* in Africa and *Megathyru maximus* in Hawaii (Wiseman et al. 2002; Ammondt et al., 2013). Essentially anywhere where a pre-emergent herbicide is used to control a species prior to planting of desired species, HPPs could be useful.

Further research and refinement is necessary to determine the optimum HPP formulation and size for different species. Before large-scale management use, it is also necessary to determine the costs associated with HPPs. Current small scale production is very costly, however, efforts to scale-up production and decrease costs are currently being made. Though HPP technologies will be more costly than application of bare seed, increased successful establishment of perennial bunchgrasses could result in considerable savings (Madsen et al., 2016a). When perennial bunchgrasses are established successfully they can competitively exclude invasive annual grasses, decreasing the potential for wildfire and therefore helping to reduce the cost of wildfire suppression and repeated revegetation and annual grass control attempts (Davies and Johnson, 2017; Meyer et al., 2014; Davies and Nafus, 2013; Madsen et al., 2016a; Duncan et al., 2004). Current estimates suggest that rangeland restoration success is at most 10% and thus one area may need to be seeded 10 or more times to have successful establishment (Madsen et al., 2016a). If HPPs can increase restoration success to just 20%, the costs associated with repeated restoration attempts drops to half of what it costs to seed without using seed enhancements. One model's simulations predicted that effective revegetation costs can be up to 1.9 times more expensive than fire suppression costs and still break-even financially over 50 years because of the high cost of wildfire suppression (Epanchin-Niell et al., 2009). In addition, due to cost, research to identify when and where HPPs should be used to have the greatest success, would be valuable.

With an uncertain future ahead and climate change predictions indicating increased spread of invasive species and greater need for successful revegetation of rangelands, HPPs, while not a silver bullet, provide us with one more promising tool to increase restoration success of culturally, ecologically, and socially important ecosystems (Bradley, 2009; Abatzoglou and Kolden, 2011; Creutzburg et al., 2015).

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APPENDICES

Appendix A: Relevant ANOVA tables for Chapter 3

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	18	2.5255	0.1294
Treatment	1	18	8.3808	0.0096 *
Site	1	6	5.8656	0.0517 *
Year	1	18	14.8153	0.0012 *
Treatment:Site	1	18	0.0008	0.9777
Treatment:Year	1	18	0.0656	0.8008
Site:Year	1	18	13.6364	0.0017 *
Treatment:Site:Year	1	18	0.2339	0.6344

Table 1. ANOVA output for bluebunch wheatgrass density linear mixed effects model.

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	6	35.8696	0.0010
Treatment	1	6	54.2257	0.0003 *
Site	1	6	2.0039	0.2066
Treatment:Site	1	6	3.9032	0.0956

Table 2. ANOVA output for Wyoming big sagebrush density linear mixed effects model.

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	18	5.6188	0.0291
Treatment	1	18	5.3721	0.0324 *
Site	1	6	0.6829	0.4402
Year	1	18	0.3820	0.5443
Treatment:Site	1	18	1.2840	0.2720
Treatment: Year	1	18	0.8595	0.3661
Site:Year	1	18	9.8715	0.0056 *
Treatment:Site:Year	1	18	3.2498	0.0882

Table 3. ANOVA output for bluebunch wheatgrass cover linear mixed effects model.

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	18	1574.7952	< 0.0001
Treatment	1	18	7.0396	0.0162 *
Site	1	6	1.1418	0.3264
Year	1	18	218.8825	< 0.0001 *
Treatment:Site	1	18	3.0234	0.0991
Treatment: Year	1	18	2.4709	0.1334
Site:Year	1	18	20.9358	0.0002 *
Treatment:Site:Year	1	18	2.6200	0.1229

Table 4. ANOVA output for crested wheatgrass height linear mixed effects model.