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DOES TIMING OF HERBICIDE USE INFLUENCE RATES OF GERMINATION OR SEEDLING BIOMASS OF NATIVE PLANTS USED FOR RESTORATION?

By

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Thesis

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Does Timing of Herbicide Use Influence Rates of Germination or Seedling Biomass of Native Plants Used For Restoration?

Chairperson: Dr. Cara R. Nelson

Invasive plants can negatively impact native grasslands by changing their species composition, productivity, and function. Managers commonly use herbicides as a control method; however, this practice can lead to secondary invasion by other non-native invasive plants, unless measures are taken to promote natives. Because of this, managers often seed native plants after spraying herbicides. There is evidence, however, that chemical control of invasive plants may reduce the effectiveness of subsequent seed-addition treatments, but there is currently little quantitative information on optimal timing between spraying and seeding or on variation in herbicide sensitivity among native plants commonly used in seed mixes. I conducted an investigation of the magnitude and duration of effects of two commonly used herbicide active ingredients, picloram and aminopyralid, on performance of ten native grassland plants at the seed stage. I separated timing of herbicide applications by 0, 3, 6, 9, and 11 months before seed addition to potted soil in the greenhouse and then recorded rates of germination and germinant biomass after six weeks. Additionally, I installed seventy-two one-m^2 plots at a nearby field site where I tested the effects of fall and spring-treated plots on seed performance after a spring seed addition. In the greenhouse experiment, the effect of timing on seed performance was significant for seven of 10 species, and the effect of herbicide was significant for all species. Four species had a 100% reduction in germination throughout the 11-month greenhouse trial, while there were significant among-time-period differences in germination for six species. In general, the herbicide impact on germination rate and biomass was more severe for picloram than for aminopyralid. In the field experiment, herbicide application significantly reduced seed performance for three of four species in spring-sprayed plots, while the effects of herbicide treatments were not significant in fall-sprayed field plots. Separating herbicide applications and native seed additions by as much time as field conditions allow may improve the germination rates and size of seedlings of some seeded species. Results from the greenhouse and field studies combined indicate that herbicides can have strong adverse effects on germination, but that the actual effects in field settings will be based on complex interactions between species traits, field conditions (including soil type), and management choices (season, herbicide used, and timing of seed addition after spraying). Thus, site-specific trials will ultimately be the best method for making inference to particular target sites.

Introduction

Invasive plants are a pervasive impediment to the rehabilitation, restoration and maintenance of many native plant communities (Barnes 2004; Clout & Williams 2009). To control invading plants and restore native species, many land managers combine herbicide treatments with reseeding of desirable species (Endress et al. 2012). While herbicides can control invasive plants, they also may adversely affect non-target, desirable ones (Cox 1999; Clout & Williams 2009; Rinella et al. 2009) Furthermore, several studies have shown that chemical control can have significant negative impacts on reseeding efforts (Biggerstaff & Beck 2007; Rokich et al. 2009; Wagner & Nelson 2014; Souza & Engel 2016). If seed is sown too soon after chemical application, rates of germination of seeded species may be reduced (Wagner & Nelson 2014); but, if reseeding does not occur soon enough, the site may be reinvaded due to availability of open niches. Thus, there is a need for information that will assist managers in navigating this tradeoff. Towards that end, I investigated the duration and magnitude of effects of chemically treated soil on seedling emergence and biomass of ten native plants commonly used for revegetation of invaded temperate grasslands in the western United States.

Invasive plants have multiple adverse ecological and economic consequences. The persistence of weedy species can result in shifts in ecosystem structure and composition (Vitousek et al. 1997), loss of ecosystem services (Mack et al. 2000; Pejcahr & Mooney, 2009), and the loss or reduction of native plants and wildlife from ecosystems (Vitousek et al. 1997; Wilcove et al. 1998; Mack et al. 2000; Clout & Williams 2009). Invasive-

species-caused environmental damage was estimated to cost the United States alone up to $100 - $200 billion a year (Pimentel et al. 2005). Since invasive species continue to impact ecosystems and economies worldwide, herbicides remain a highly used tool for natural areas management. Although records of the amount of herbicide used specifically for conservation lands management is not available for most countries, a recent survey provided a conservative estimate of 182,344 kg used on roughly half a million hectares of publicly managed natural areas in the United States in 2010 (Wagner et al. 2016). Furthermore, the U.S. EPA estimated that the industrial, commercial and government market sectors in the U.S. spent roughly 896 million dollars on herbicide and plant growth regulators in 2007, a 300-million-dollar increase since 1988 (Grube 2011). As investments in chemical control of weedy species continues to rise, and as land managers struggle to effectively restore desirable species to degraded plant communities, it is becoming increasingly critical that scientists reevaluate the efficacy of available plant management tools.

Recovery of native plant communities in areas where invaders dominate is a major challenge. Past studies have shown that chemical application to reduce invasive forbs and grasses, although initially successful, may fail to result in a significant increase in native plants (Tyser & Asebrook 1998; Sheley & Mangold 2006; Ortega & Person 2011; Pearson et al. 2016). One reason for the lack of success is that invasive plant seeds rather than native plant seeds may make up the majority of soil seedbanks at heavily invaded sites. At heavily invaded sites chemical control of a weedy species makes resources available for other plants and may result in the subsequent invasion of equally or more

undesirable species that replace the controlled invader (DiTomaso 2000; Pearson & Ortega 2009). Managers are, therefore, encouraged to combine herbicide use with revegetation methods. Revegetation can be accomplished by adding seeds, seedlings or adults plants to an invaded system, which may reduce the risk of reinvasion; however, revegetation with adult or seedling plants is often too time-intensive and costly for large restoration projects. Land managers, therefore, commonly rely on seed additions to restore invaded sites, yet little information is available about which native species germinate best in herbicide-treated soils (Wagner & Nelson 2014).

Another reason why native plant communities may not thrive after chemical control of weeds is that the native plant populations, already susceptible to local disturbance and stochasticity in invaded areas, can be significantly damaged or even eradicated by herbicide treatments (Pimentel & Zuniga 2005; Rinella & Maxwell 2009). Germinants are particularly sensitive to growth-regulating herbicides (synthetic auxins), such as aminopyralid and picloram (Fedtke & Duke 2005), because they contain higher relative auxin concentrations than adult plants to allow for a rapid growth stage. Synthetic auxin herbicides have been developed specifically to cause growth deformation, growth inhibition, senescence and ideally death in broadleaf plants; however, several studies have shown that they can cause significant damage to graminoid species at the germination and seedling stages as well (Hsueh & Lou 1947; Huffman & Jacoby Jr. 1984; Tyser & Asebrook 1998; Jacobs 2001; Sheley & Mangold 2006; Douglass & Nissen; Wagner & Nelson 2016). Wagner and Nelson (2014) suggest that herbicide damage to graminods at the seed stage may be due to a lack of morphological features

such as leaf sheaths that protect adult graminods from the negative effect of herbicides. In contrast, there is some evidence that at low concentrations synthetic auxins, including picloram, can have positive effects on some species at certain life stages due to mechanisms such as increased protein synthesis or increased cytoplasmic streaming and stem elongation (Hsueh & Lou 1947; Chang & Foy 1971; Sheley et al. 2002). Since broadleaf-selective herbicides are among the most commonly used for invasive plant control for natural areas management and restoration (Wagner et al. 2017), it is imperative that managers better understand the implications of using these herbicide in areas where recovery of native plant populations is a goal.

Although many studies have investigated the benefits of chemical control and seed additions to manage invasive species, few have addressed how well these two practices work together to improve native plant communities. Only a handful of studies have explored the immediate effect of synthetic auxin herbicides on germination of native and invasive species (but see Hseuh & Lou; Chang & Foy 1971; Huffman & Jacoby Jr. 1984; Wagner & Nelson 2104), and all of these were conducted to test the effects of adding seed immediately after spraying, exclusively in greenhouse or growth chamber settings, limiting inference to responses in the field. This study is the first to explore the duration of synthetic auxin herbicide effects on native seed over a one-year period using standard field application rates in the greenhouse and is one of a few to investigate seed performance in herbicide-treated soil in a field setting. Specifically, I examined the effects of two commonly used herbicides, picloram (chemical formulation: Tordon 22K®)

and aminopyralid (chemical formulation: Milestone®), on germination and establishment of 10 native grasses and forbs, by addressing the following research questions:

(1) How does the timing of herbicide applications and reseeding influence native plant germination and seedling biomass?
(2) Does the duration and magnitude of effects vary by species and type of herbicide?

By conducting experiments both in the greenhouse and the field, I was able to determine potential effects (greenhouse study) as well as realized effects (field study), allowing for broader inference for management applications.

Materials and Methods

Study herbicides: I used two herbicides, picloram and aminopyralid, both of which are commonly used worldwide (Cobb & Reade 2010) to control invasive broadleaf species and are among the top ten herbicides for area treated in the U.S. (Wagner et al 2017). Instruction on the labels for both of these herbicides currently provide little to no guidance for managers interested in how to time herbicide applications and native seed additions for best native plant establishment results (Dow AgroSciences 2009; Dow AgroSciences 2016). For instance, the label for Milestone® gives only a general recommendation to wait 90 days after treating in the summer to seed forbs, but does not provide any species-specific information on seed sensitivity to the herbicide or more detailed advice on the timing of applications and seed additions for species, genus or even plant families. The label for Tordon 22K® does not even address how long to wait

after applying Tordon 22k before seeding native forb species for natural areas management, but does recommend waiting until the fall to seed perennial graminoid species after spraying a site in late spring or early summer.

Study Species*:* I selected ten native species (Table 1) that are commonly used for grassland restoration in the Rocky Mountain region of the western US and can be reliably germinated in a greenhouse. The same species were used for both the greenhouse and field experiment. Seeds were field collected by Missoula Parks and Recreation near Missoula, MT or grown by Granite Seed Company (Denver, CO), Wind River Seed (Manderson, WY) or Native Ideals (Arlee, MT).

Greenhouse Experiment*:* To test the duration and magnitude of effects of herbicides on germination of native plant seeds, I conducted a year-long experiment in University of Montana's greenhouse at Fort Missoula (Missoula, MT). The greenhouse is unheated during the winter months. In summer, wall fans cooled the greenhouse when temperature rose above 30^{o} C, greenhouse doors were left open, and the bottom 0.5 m of greenhouse siding was rolled up to improve airflow and moderate temperatures. To prepare for the experiment, I filled 10.16-cm diameter, hard plastic pots (Novosel Enterprises) with a mixture of one part sand to two parts loamy topsoil, sifted through a 2-mm mesh sieve to remove debris and large particles. The soil mixture was then heat-treated in an oven at 180^{o} C for 1 hour to remove any seeds in the soil.

Beginning in April of 2015, 360 pots were treated with one of the following three treatments and then randomized in the greenhouse on benches: 1) aminopyralid at a recommended rate of 0.52 L/ha, 2) picloram at a recommended rate of 4.78 L/ha or 3) control (water). I repeated the treatment process described above four additional times: 360 new pots were treated and stored in the greenhouse during July of 2015, October of 2015, January of 2016 and April of 2016. For each combination of herbicide treatment, time period and species, I created 12 replicate pots (n=1,800 pots: 3 herbicide treatments × 5 time periods × 10 species × 12 replicates of each combination). Soil in pots were wetted individually (to avoid cross-contamination) on a weekly basis during the summer and fall and a bi-weekly basis in the winter. In addition, pots were periodically re-randomized to account for any minor differences in temperature, light and humidity in the greenhouse. Three weeks after the final herbicide treatment in April 2016, all 1,800 pots were seeded with fifty seeds of one of the 10 study species. To improve seed-soil contact in each pot, I gently tilled the top 1 cm of soil with a toothpick prior to adding seeds. Pots were watered individually to thoroughly wet the soil surface once a day (or twice a day if local temperatures rose above 30 ^{o}C) for six weeks after seeding. At the end of the six-week growing period, the number of live seedlings in each pot was counted, and above-ground biomass of all germinants in each pot was collected. Plant material was dried in an oven for 24 hours at 80^{o}C and weighed before analysis.

In order to confirm that the soil heat treatment, a standard process for greenhouse studies, was not affecting germination, in April of 2016 I tested for differences in germination rates between unsterilized and heat-treated soil for each of my three treatments

(aminopyralid, picloram or the water control). For this test, I randomly selected one study species, *E. compositus* (cutleaf daisy). No significant differences were found for germination rate or biomass of *E. compositus* in unsterilized versus heat-treated soil for any of the three treatments.

Statistical Analysis: I tested for the main effects of herbicide type (3 levels: aminopyralid, picloram and control), and duration of effect (5 levels: 0, 3, 6, 9 and 11 months) using ANOVA models, with separate models for number of germinants and seedling biomass for all species combined, lifeform groups (grasses vs. forbs) and each species individually. I used an alpha level of 0.05 to determine statistical significance and applied a Bonferroni correction for multiple tests (13 tests; corrected alpha level of 0.0038).

Field Experiment: In cooperation with Missoula Parks and Recreation (MPR), I conducted a field test of herbicide effects on native plant germination at the MPR-managed Ft. Missoula "Triangle" property (Figure 1). The property is a 0-2% sloped grassland, dominated by noxious weeds; *Bromus tectorum, Centaurea maculosa*, *Euphorbia esula*, *Poa bulbosa*, and *Poa pratensis* are the most abundant, but several other weedy species are also present including *Hyoscyamus niger, Linaria dalmatica*, and *Tanacetum vulgare*. There are a few scattered patches of native-dominated plant communities, with *Boechera holboellii and Festuca idahoensis*. Soil on the site is a moiese gravelly loam (NRCS, 2016). The property was last treated with chemicals in 2010 and has since been managed only through biannual mowing.

In fall 2015, I located 72 1-m^2 plots in 12 study blocks, with six plots per block. Blocks, and plots within blocks, were located in areas dominated by bare ground and exotic species (to minimize impacts to existing native plant populations) and with good drainage. Plots within blocks were separated by at least a 2-m buffer. All plots were tilled and raked to clear plant materials in mid-November. Removing all plant material from plots allowed me to single out herbicide and timing impacts on seed success. I randomly assigned plots to one of six treatments within each block, using a split-plot design to reduce chances of cross-contamination between herbicide applications: 1. aminopyralid - fall application, 2. picloram - fall application, 3. water control – fall application, 4. aminopyralid – spring application, 5. picloram – spring application and 6. water control – spring application. On November 22nd and 23rd, half of the plots in each block were sprayed, using a 10-liter backpack sprayer, with one of the following treatments: aminopyralid applied at a rate of 0.52 L/ha, picloram applied at a rate of 4.78 L/ha or water control. Herbicide treatments were mixed with 0.05 oz./liter of a non-ionic agricultural surfactant to improve herbicide contact with soil and 0.025 oz./liter of blue marker dye mix to improve spray visibility for the applicator. The control treatment did contain marker dye but did not contain surfactant. A 2-m buffer surrounding each plot was also treated, in order to reduce edge effects of spray treatments in each plot. In early spring, germinating or re-sprouting plant material was hand-pulled from the plots that were sprayed in the fall and the plots designated for spring spraying.

In late March of 2016, the remaining plots designated for spring treatment were sprayed, using the same 10-liter backpack sprayer as used in the fall. Seven weeks later, in mid-April, I hand-seeded all plots (those treated in both fall and spring) with a mix of native forbs and grasses (Table 1) that included 100 seeds per species (a total of 1000 seeds per plot). After seeding, plots were hand-patted to improve seed contact with the soil. Non-study species were hand-pulled from plots as soon as they were identified to reduce competitive effects after the seeding occurred.

Seven weeks after seeding both the fall and spring treated plots, I recorded the number of live seedlings that had emerged and survived on each plot using a gridded 1 m × 1 m plot marker. I also collected, dried in an oven for 24 hours at 80^{o} C, and weighed above-ground biomass of all live seedlings of study species.

Statistical Analysis - I tested for the main effects of herbicide type (3 levels: aminopyralid, picloram and control) and season of application/duration (2 levels: spring /0.75 months and fall/5 months) using ANOVA models, with separate models for each response variable (number of seedlings and seedling biomass) and each species. I used an alpha level of 0.05 to determine statistical significance and used Bonferroni corrections for multiple tests (5 tests; corrected alpha level of 0.001).

I had to exclude six species from analysis of the field experiment due to low germination in control plots: C*erastium arvense* (field chickweed), *Clarkia pulchella* (deerhorn

clarkia), *Erigeron compositus*, *Festuca idahoensis* (Idaho fescue), *Koeleria macrantha* (prairie junegrass) and *Procerus penstemon* (littleflower penstemon) all had on average less than one seedling in the control group. Differences in how I seeded pots in the greenhouse and plots in the field may have influenced difference in how species performed in both settings regardless of treatments. Seeding method plays an important role in establishment of species and that germination of grass species may be higher when methods are used to incorporate seeds more fully into soil, such as drill-seeding (Montalvo et al. 2002). Smaller seeded species from the study, which included many forbs, have been found to germinate at higher densities when sown using a broadcast and imprint method (Montalvo et al. 2002). Therefore, my seeding method in the greenhouse study, which involved incorporating seeds below the soil surface, may have favored graiminoids while my broadcast and hand-patting method of seeding onto field plots may have favored forb species.

Results

Greenhouse- The main effect of herbicide treatment was significant for both germination rate and average biomass for all species combined (Table 2, Figure 1). The effect of timing (time between spraying and seeding) was significant for germination rate, but not for biomass, for all species combined. In addition, the interaction between timing × herbicide was significant for germination rate for all species combined.

The main effect of herbicide type was significant for all grasses combined while the effects of timing and the timing × herbicide interaction were nonsignificant. For all grass

species combined, aminopyralid-treated pots had on average 22-100% fewer seedlings, depending on time period, and these seedlings had on average 58-99% less biomass than in controls, while picloram-treated pots had on average 76-96% fewer seedlings and these seedlings had 76-96% less biomass than in controls. Forb species as a group showed even more extreme effects when exposed to herbicides than did grasses. The effects of timing, herbicide type, and the interaction between timing and herbicide type were all significant for all forbs combined. For grouped forbs, aminopyralid-treated pots had on average 83-100% fewer seedlings, depending on the time period, and these seedlings had 80-99% less biomass than in controls, while picloram-treated pots had on average of 98-100% fewer seedlings and these seedlings had 98-99% less biomass than in controls.

Nine of 10 individual species germinated at lower rates in herbicide-treated pots relative to the control for both herbicides at all time periods (Table 3, Figures 2 and 3). The one species that differed from this trend was *F. idahoensis,* which responded to picloram treatments in the same way as other species (significantly lower germination than in controls throughout the study period), but in aminopyralid-treated pots at the 11-month time period had an insignificant difference in germination rate compared to the control group. Individual grass species varied in germination rate among time periods (Table 3, Figure 2). For instance, in aminopyralid-treated pots, *K. macrantha* had nearly 100% lower germination in treated pots than in controls at the 3, 6 and 9 month time periods, but only 52 and 59% fewer seedlings at the 0 and 11 month time periods, respectively. In picloram treated pots, *K. macrantha* had 89-100% fewer seedlings across all time periods. In aminopyralid-treated pots, *Pseudoroegneria spicata* (bluebunch wheatgrass)

ranged from 7-99% fewer seedlings compared to controls, depending on time period, while in picloram-treated pots, the difference ranged from 55-96% fewer seedlings. In contrast to the observed among-species variability in germination rate, all grass species had significantly lower seedling biomass at all treatment × timing combinations.

Of the seven forb species, four (*Artemisia frigida* (fringed sage), *C. arvense*, *E. compositus*, *P. penstemon*) had nearly 100% fewer germinants in herbicide-treated pots compared to the controls regardless of the time period between treatment and seed addition or type of herbicide (Table 3, Figure 3). Two other forbs, G*aillardia aristata* (blanketflower) and *Boechera holboellii* (Holboell's rockcress), followed a similar pattern for the first four time periods (up through 9 months), but at the 11-month time-period there were smaller differences in number of germinants and their biomass compared to control groups. *C. pulchella*, the only annual forb studied, did not germinate in aminopyralid-treated pots at any time period and only germinated in picloram-treated pots at the 11-month time-period. However, at the 11-month time-period it exhibited significantly greater rates of germination than the control (9% more seedlings).

Field Experiment- The main effects of herbicide and season/timing on both germination rate and germinant biomass was significant for all species combined (Table 4). The interaction between season × herbicide on germination rate and germinant biomass was also significant for all species combined; however, the effects of block and the herbicide × block, season × block and season × herbicide × block interactions were not significant for either variable. The main effect of season/timing was significant for germination rate

for one of four individual species in the field study (*G. aristata*) while all other effects and interactions on germination rate were nonsignificant for all individual species. The main effect of season/timing on germinant biomass was significant for two of the four species, *B. holboellii* and *G. aristata*, and the effects of herbicide and season/timing × herbicide interaction were significant for only *G. aristata*.

Fall treatments –Germination rates for all species combined were 33% higher in aminopyralid-treated plots and 5 % lower in picloram-treated plots than in untreated control plots ($df = 2$, $F = 13.83$, and $p < 0.000$). Biomass of germinants also tended to be greater in plots treated with either of the herbicides relative to controls (17 and 8% larger in aminopyralid and picloram-treated plots, respectively), for all species combined ($df = 2$, $F = 9.77$, and $p < 0.000$). The mean rate of germination and biomass of seedlings for *A. frigida*, *B. holboellii* and *G. aristata* were higher in aminopyralid-treated versus control plots (and picloram-treated plots for *G. aristata*); however, these trends were only significant for *G. aristata* ($df = 2$, $F = 5.61$, and $p = 0.009$).

Spring treatments - For all species combined, germination rates were 56% lower in aminopyralid-treated plots and 96 % lower in picloram-treated plots than in untreated control plots (Table 5, Figure 4). In addition, seedling biomass was significantly smaller in treated plots relative to untreated controls (48 and 93% smaller in aminopyralid and picloram-treated plots, respectively). Three of the four species tested had significantly lower germination rates and smaller seedlings in plots treated with either herbicide than in untreated control plots ($df =2$, $F = 6.62$ and $p = 0.004$ for *G. aristata*; $df =2$, $F = 4.11$

and p = 0.026 for *A. frigida*; df = 2, F = 4.24 and p = 0.024 for *B. holboellii* and df =2, F = 6.62 and p = 0.004 for *G. aristata*). For the fourth species, *P. spicata,* trends were not significant (df = 2, F= 2.22, and p = 0.127).

Discussion

Given widespread use of herbicides and reseeding to control invasive species in areas where restoring native plant communities is a goal, it is important to understand the impact of herbicides on native species at the seed stage. Although a small number of previous studies have investigated how synthetic auxin herbicides impact germination of different species (Hsueh & Lou 1947; Chang & Foy 1971; Huffman & Jacoby Jr. 1984; Wagner & Nelson 2014), this is, to my knowledge, the first study to investigate the duration of herbicide impacts over a significant amount of time (greater than 2 months). Additionally, this is one of a small but growing number of investigations on herbicide impacts on seed additions in a field setting.

Findings from my greenhouse experiment provide strong evidence that germination of the study species is significantly negatively impacted by both types of herbicides when seeding and spraying are not separated by enough time. These results also support findings of previous greenhouse studies that assessed immediate responses to management-relevant herbicide dosages, including Wagner and Nelson (2014), Biggerstaff and Beck (2007) and Rokich et al. (2009). Although some previous investigators have found enhanced germination after application of synthetic auxin herbicides in greenhouse experiments, these findings were limited to experiments that

used lower doses of the active ingredient than are generally used for management (Hsueh & Lou 1974; Chang & Foy, 1971). In addition, I found that the effects persisted over long periods of time (more than 11 months for most species) and tended to be less severe for grasses than forbs and for aminopyralid than for picloram. Results from the greenhouse suggest that combining seeding with spraying in order to fill empty niches before secondary invasion may not be effective, as 11 months is likely too long a delay.

Although the greenhouse study yielded consistently adverse effects and corroborates findings of Wagner and Nelson (2014), there was much more variability in seed response to herbicide and season/timing treatments in my field trial. Although I had expected to see a similar pattern of sensitivity to herbicide use between both the greenhouse and field investigations, some species that exhibited strong adverse effects for the 11-month duration of the greenhouse experiment outperformed the control group in the fall-treated field plots. In particular, all of the forb species seeded onto aminopyralid-treated plots in the fall (with 5 months between spraying and seeding) outperformed control groups. In contrast, in the greenhouse all of these species had significantly lower germination in treated compared to control pots, even when seeded into soil that had been treated nearly a year before seeding. Results of previous studies of the short-term effects of herbicides on germination in field settings have been variable: some investigators have found that herbicide treatments did not significantly impact the germination of subsequent seed additions (Sheley et al. 2002; Douglass et al. 2016), while others reported negative effects (Lym & Messersmith 1994; Ortega & Pearson 2011; Endress et al. 2012). This difference in results between my greenhouse and field environments, coupled with variable findings

from other field studies, suggests that results from greenhouse experiments may represent a maximum potential negative impact of herbicides at the germination stage, while field results may represent realized and more variable effects of those herbicides.

There is some evidence that soil type, which affects differential rates of herbicide degradation, may be driving observed variation in response of seeded species to herbicide treatment in field settings (Douglass et al. 2016). For instance, the half-life of both aminopyralid and picloram are reported to be highly variable (from 1 month-1.5 years and from 5.5 months-1.5 years respectively) depending on soil type (EPA 1995). Soil texture and organic matter play a role in herbicide activity, mobility and residual time in soil, with degradation of herbicides often occurring more quickly and mobility potentially decreasing in soils with a finer texture and relatively high organic matter (Ogle & Warren 1954; Farmer & Aochi 1974; Bukun et al. 2010; Douglass et al. 2016). I did not track the concentration of herbicide residuals in either the field plots or greenhouse pots over time, and can only speculate that differences in soil composition and structure contributed to variable degradation of herbicide in soil and thus seed responses in the field and greenhouse. Additionally, heterogeneity in moisture availability, distribution of microsites, leaching, and biological activity — all factors beyond the scope of my investigation — may have also played a role in observed differences in greenhouse and field results, by altering herbicide residual time in field plots.

Factors related to implementation of my treatments also could have impacted seed performance in the field. My removal of plant material from field plots, and the resulting

increased exposure of bare mineral soil, likely played a role in seed performance by changing herbicide residual time and activity. Although it was necessary to remove competing vegetation to isolate the effect of timing and herbicide treatments on seed performance, the increased bare mineral soil in the plots does not reflect the full reality of field conditions for invaded restoration sites.

In order to improve inference from future studies of herbicide impacts to native species, investigators should analyze herbicide degradation in soil over time, as well as the effects of soil structure, chemistry, and biota. Temporal replication of future testing of seed responses to timing and herbicide treatment combinations can also tie in the influence of variation in seasonal weather patterns and soil conditions on seed sensitivity to herbicide-treated soil. Future studies also should attempt to integrate the effects of vegetation in combination with management (herbicide treatments and timing) to better understand the realized impacts of spraying and seeding strategies for seed mix success.

In my study, the fall field plots that were sprayed 5 months before seeding did not show significant differences in germination from the controls, and even tended to have higher mean germination rates (although the trend was not significant). In contrast, the plots treated 0.75 months before seeding in spring showed significantly lower germination than did the controls. Although given my study design it is not possible to separate the effects of season of spraying from time between spraying and seeding, the lag between spraying and seed additions could have played a role. To my knowledge, the only other study that addressed timing of herbicide treatments and seeding of native species in a natural area

also found that timing was important for seedling establishment. Sheley et al. (2002), in a 44 day study of effects of herbicides on grass species, reported that delaying seeding by 24 or 44 days after herbicide application improved the vigor of several desirable grass species by 40% in comparison to seeding immediately after herbicide application. The positive effect of seeding delay on seed performance of some of the species found in my study add to a small but growing body of evidence that suggest that the timing of integrated management strategies may be a critical component of the successful establishment and productivity of species seeded for restoration in invaded areas.

In addition, the type of active ingredient can also change the impact of chemical treatments on native species and thus the success of revegetation after invasive plant control. Picloram, which is known to have a longer half-life than aminopyralid, had stronger negative impacts on study species than did aminopyralid in both the greenhouse and field trial, particularly for forbs (see Wagner & Nelson 2014 for similar results). This significant difference between two herbicides that have different soil residue times corroborates the work of Douglass et al. (2016), who found that field application rates of triclopyr, a synthetic auxin herbicide with short-lived soil activity, had less of an effect on the productivity of seeded grass species than did the herbicide imazypr, which has a much longer half-life in soil.

Secondary invasion, an often unanticipated and unwanted consequence of chemical control of invaders, also remains a real challenge for land managers (Smith et al 2006; Pearson & Ortega 2009; Ortega & Pearson 2011; Pearson et al. 2016). Thus, finding seed

mixes that can resist secondary invaders should be a high priority. The range of seed performance of different species in this study and others suggests that establishing a functionally diverse and resilient native plant community that can reduce secondary invasion will be highly dependent on complex interactions among the herbicide used, the specific sensitivity of native species, and the environmental conditions present at the target site (Sheley et al. 2002; Douglass et al. 2016). Although the effects of herbicides persisted for long periods of time for all species in the greenhouse, there was some variation in species response to treatment indicating variation in species sensitivity. Several species germinated more consistently and produced more biomass relative to other study species in both the greenhouse and the field experiments including *P. spicata*, *G. aristata* and *B. holboellii*. Other researchers have found that seeded *P. spicata* established relatively well on aminopyralid (Mangold et al. 2015) or picloram-treated soils (Sheley et al. 2002). Although information on the relative tolerance of *G. aristata* and *B. holboellii* seed to aminopyralid or picloram is limited, Rice & Toney (1998) and Mikkelson & Lym (2012) found adult *G. aristata* to be moderately tolerant to synthetic auxin herbicide treatments while Dow AgroSciences (2013) found other brassica species, *Alyssum alyssoides* (alyssum) and *Arabis nuttallii* (Nuttall's rockcress), to be tolerant to aminopyralid. *B. holboellii*, *G. aristata* and *P. spicata* might make appropriate additions to seed mixes for invaded areas where chemical control, particularly with aminopyralid, is necessary and seeding needs to occur relatively quickly to prevent reinvasion. However, the specific conditions at a site and management choices will play a large role in the actual tolerance of any particular species to being sown on herbicide-treated soil. Future studies that investigate the relationship between seed and seedling traits, such as

seed size or seed coating, and response to herbicides are needed to determine species-specific responses to herbicide effects.

Labels for both of the herbicide products (Milestone® and Tordon 22K®) used in this investigation recommend the use of soil bioassays as a tool to detect potential herbicide impacts to crops species in agricultural settings (Dow AgroSciences 2016). Given the range of native species' responses to herbicide-treated soil, and the relative gap in knowledge between what is known about herbicide impacts to crop species compared to native species, it is clear that soil bioassays are also needed as a follow up to herbicide applications in natural areas management. The success of native plant community restoration projects could be improved by using the soil bioassay or field testing to explore species tolerance to different herbicides and specific applications at the seed stage. Additionally, spot spraying in place of a broadcast application of herbicide when applicable can minimize the negative impact of herbicides on germination of seeded species and native germinants from the soil seedbank (Crone et al. 2009; Rinella et al. 2009; Pearson & Ortega 2009; Ortega & Pearson 2011). Another option for managers interested in increasing the efficacy of seed additions after herbicide use may be to use newer seed treatment technologies to artificially improve the resistance of native seed to damage by chemical treatments intended for invasive plant control. A recent review of seed enhancement technologies highlighted the potential effectiveness of the "herbicide protection pod" (HPP) an amendment that mixes and dries seeds, water sensitive binders and activated carbon into an herbicide resistant strip that can be sown directly after herbicide applications (Madsen et al. 2016). HPPs were shown to increase the

establishment of *P. spicata* by 4.8 times compared to controls when sown into *B. tectorum* invaded pots shortly after imazipic treatments in a laboratory setting (Madsen, et al. 2013).

Conclusion

The addition of native seeds could facilitate the restoration of native plant communities after herbicide use but benefits may be hampered if the seeding occurs too soon after herbicide application. Managers should consider how the timing of herbicide applications may impact the reestablishment and continued existence of native vegetation and plan control of invasive species accordingly. Delaying seed addition of sensitive species after applying aminopyralid or picloram may improve the efficacy of herbicide and revegetation measures, especially in cases where the potential for secondary invasion is low. In areas where spraying is scheduled, managers can select species for seeding that exhibit lower sensitivity to herbicide-treated soil. Of the ten species we studied, three (*B. holboellii*, *G. aristata* and *P. spicata*) showed greater tolerance to herbicide exposure.

The variation that I observed between the greenhouse and field study suggest that greenhouse trials may provide managers with information on the maximum impact of herbicides (in my case, aminopyralid and picloram) on germination and seedling growth, but soil bioassays or field trials may be the only way to reliably determine the magnitude of effects at project sites. The success of seed additions after herbicide treatments will vary based on herbicide application rate, soil type and conditions, seeded species, and management decisions such as timing between spraying and seeding. The complicated

nature of these interactions demonstrates that managers concerned about herbicide impacts to seed additions will need to examine species-specific responses to local conditions and management choices. Furthermore, as Crone et al. (2009) have shown, there is a need to quantify herbicide impacts to population fitness and vital rates if we are to understand the effects on native plants over the long-term.

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Literature Cited

Barnes TG (2004) Strategies to Convert Exotic Grass Pastures to Tall Grass Prairie Communities. Weed Technology 18:1364-1370

Biggerstaff MS, Beck CW (2007) Effects of method of English ivy removal and seed addition on regeneration of vegetation in a southeastern piedmont forest. The American Midland Naturalist 158:206-220.

Bukun B, Shaner DL, Nissen SJ, Westra P, Brunk G (2010). Comparison of the interactions of aminopyralid vs. clopyralid with soil. Weed science, *58*(4): 473-477

Chang I, Foy CL (1971) Effect of picloram on germination and seedling development of four species. Weed Science 19:58-64

Clout MN, Williams P (2009) Invasive species management: a handbook of techniques. Oxford University Press, New York, New York, USA

Cobb AH, Reade JP (2010) Auxin-type herbicides. Pages 133-156 In: Herbicides and Plant Physiology, Second Edition, Wiley-Blackwell, Chichester, West Sussex, UK.

Cox GW (1999) Alien Species in North America and Hawaii. Island Press, Washington, DC, USA.

Crone EE, Marler M, Pearson DE (2009) Non-target effects of broadleaf herbicide on a native perennial forb: a demographic framework for assessing and minimizing impacts. Journal of Applied Ecology 46:673-682.

DiTomaso JM (2000) Invasive weeds in rangelands: species, impacts, and management. Weed Science 48:255-265.

Douglass C, Nissen S, Meiman P, and Kniss A (2016) Impacts of Imazapyr and Triclopyr Soil Residues on the Growth of Several Restoration Species.Rangeland Ecology and Management 69:199-205.

Dow AgroSciences (2009) Speciemen label: Tordon® 22K http://www.dowag ro. com/en-us/range/products/tordon-22k (accessed on 16 March 2017)

Dow AgroSciences (2013) Invasive plant management with Milestone® and other herbicides. http://msdssearch.dow.com/PublishedLiteratureDAS/dh_0948/0901b803809 4860b.pdf?filepath=ivm/pdfs/noreg/010-50904.pdf&fromPage=GetDoc (accessed 5 April 2017)

Dow AgroSciences (2016) Specimen label: Milestone® http://www.dowagro. co m/en-us/vm/products/milestone (accessed on 16 March 2017)

Endress BA, Parks CG, Naylor BJ, Radosevich SR, Porter M (2012) Grassland response to herbicides and seeding of native grasses 6 years posttreatment. Invasive Plant Science and Management 5:311-316.

Farmer WJ, Aochi Y (1974) Picloram sorption by soils. Soil Science Society of America Journal 38(3): 418-423.

Grube A, Donaldson D, Kiely T, and Wu L (2011) Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. U.S. Environmental Protection Agency.

HsuehYL, Lou CH (1947) Effects of 2,4-D on Seed Germination and Respiration. Science 105:283-285.

Huffman AH, Jacoby Jr. PW (1984) Effects of herbicides on germination and seedling development of three native grasses. Journal of Range Management 37:40-43.

Lym RG, Messersmith CG (1994) Leafy spurge (Euphorbia esula) control, forage production, and economic return with fall-applied herbicides. Weed Technology 8:824-829.

Mack RN, Simberloff D, Mark Lonsdale W, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecological applications 10(3): 689-710.

Madsen MD, Davies KW, Mummey DL, Svejcar TJ. 2013. Improving imazipic selectivity through activated carbon seed enhancement technologies. Rangeland Ecology & Management. 67: 61-67.

Madsen, MD, Davies KW, Boyd CS, Kerby JD, Svejcar TJ (2016) Emerging seed enhancement technologies for overcoming barriers to restoration. Restoration Ecology. 24: 77-84.

Montalvo AM, McMillan PA, Allen EB (2002) The relative importance of seeding method, soil ripping, and soil variables on seeding success. Restoration Ecology 10:52-67.

Natural Resources Conservation Service. (2016). Web Soil Survey https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (accessed on 8 December 2016)

Ogle RE, Warren G F (1954) Fate and activity of herbicides in soils. Weeds, 257-273.

Ortega YK, Pearson DE (2011) Long-term effects of weed control with picloram along a gradient of spotted knapweed invasion. Rangeland Ecology & Management 64:67-77.

Pearson DE, Ortega YK (2009) Managing invasive plants in natural areas: moving beyond weed control. Pages 1-21 In: Kingley RV (ed) Weeds: Management, Economic Impacts and Biology. Nova Science Publishers, Inc. Hauppauge, NY, USA.

Pearson DE, Ortega YK, Runyon, JB, Butler JL (2016) Secondary invasion: The bane of weed management. Biological Conservation, 197: 8-17.

Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends in Ecology & Evolution 24: 497-504.

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Rice PM, Toney JC, Bedunah DJ, Carlson CE. (1997) Plant community diversity and growth form responses to herbicide applications for control of Centaurea maculosa. Journal of Applied Ecology 34:1397-1412.

Rice PM, Toney JC. (1998) Exotic weed control Treatments for conservation of fescue grassland in Montana. Biological Conservation 85: 83-95.

Rinella MJ, Maxwell BD, Fay PK, Weaver T, Sheley RL (2009) Control effort exacerbates invasive-species problem. Ecological Applications 19:155-162.

Rokich DP, Harma J, Turner SR, Sadler RJ, Tan BH (2009) Fluazifop-p-butyl herbicide: Implications for germination, emergence and growth of Australian plant species. Biological Conservation 142:850-869.

Sheley RL , Jacobs JS, Lucas DE (2001) Revegetating spotted knapweed infested rangeland in a single entry. Journal of Range Management. 54: 144-151

Sheley RL, Mangold JM, Anderson JL.(2006) Potential for successional theory to guide restoration of invasive-plant-dominated rangeland. Ecological Monographs 76:365-379.

Sheley RL, Whitson TD, Carrithers V, Jacobs JS, Gehrett J (2002) Grass Response to Picloram and Clopyralid Applied Before Seeding1. Weed Technology 16:645-650.

Smith RG, Maxwell BD, Menalled FD, Rew LJ (2006) Lessons from Agriculture May Improve the Management of Invasive Plants in Wildland Systems. Frontiers in Ecology and the Environment 4:428-434.

Souza DC, Engel V L (2016) Pre-emergence herbicides affect seedling emergence of tropical forest tree species. Journal of Forestry Research 1-7.

Tyser RW, Asebrook JM, Potter RW, Kurth LL (1998) Roadside revegetation in Glacier National Park, USA: effects of herbicide and seeding treatments. Restoration Ecology 6:197-206.

U.S. Environmental Protection Agency (EPA). 2005. Pesticide Fact Sheet: aminopyralid [Fact sheet] https://www3.epa.gov/pesticides/chem_search/reg_ actions/reregistration/fs_PC-005101_1-Aug-95.pdf (accessed 25 March 2016)

U.S. Environmental Protection Agency (EPA). 1995. R.E.D Facts: Picloram [Fact sheet]. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-005100_10-Aug-05.pdf (accessed 25 March 2016)

Vitousek PM, D'antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology, 1-16.

Wagner V, Antunes PM, Irvine M, Nelson CR (2016) Herbicide usage for invasive non-native plant management in wildland areas of North America. Journal of Applied Ecology 54:198-204.

Wagner V, Nelson CR (2014) Herbicides Can Negatively Affect Seed Performance in Native Plants. Restoration Ecology 22:288-291.

Wilcove DS, Rothstein D, Dubow DJ, Phillips A, Losos E 1998. Quantifying Threats to Imperiled Species in the United States Assessing the relative importance of habitat destruction, alien species, pollution, overexploitation, and disease. Bioscience 48:607-615.

Tables and Figures

Table 1. Scientific and (common) names, plant family, growth habit and seed source of species used in greenhouse and field investigations.

SPECIES	FAMILY	GROWTH HABIT	SOURCE
Artemisia frigida (fringe sage)	Asteraceae	Perennial forb	Wind River Seed
Boechera holboellii (Holboell's rockcress)	Brassicaceae	Perennial forb	Wind River Seed
Cerastium arvense (field chickweed) *	Caryophyllaceae	Perennial forb	Native Ideals
Clarkia pulchella (deerhorn clarkia) *	Onagraceae	Annual forb	Field collection
Erigeron compositus (cutleaf daisy) *	Asteraceae	Perennial forb	Native Ideals
Festuca idahoensis (Idaho fescue) *	Poaceae	Perennial graminoid	Granite Seed
Gaillardia aristata (blanketflower)	Asteraceae	Perennial forb	Native Ideals
Koeleria macrantha (prairie junegrass) *	Poaceae	Perennial graminoid	Granite Seed
Penstemon procerus (litteflower penstemon) *	Plantaginaceae	Perennial forb	Wind River Seed
Pseudoroegneria spicata (bluebunch wheatgrass)	Poaceae	Perennial graminoid	Granite Seed

Note: * indicates species that were excluded from analysis for the field experiment due to poor germination in control pots.

Table 2. Results of ANOVA-tests of the effects of timing, herbicide type, and their interaction on germination rate and germinant biomass in the greenhouse experiment. **Bold** font indicates significant tests at the Bonferroni-corrected $p < 0.0038$). See Table 1 for full species names.

GREENHOUSE

	Timing			Herbicide			Timing × Herbicide		
	df	F	p	df	F	p	df	F	p
Germination Rate									
All forbs	4	20.52	**0.000**	2	225.80	**0.000**	8	17.57	**0.000**
A. frigida	4	2.28	0.063	2	98.76	**0.000**	8	2.50	0.014
B. holboellii	4	15.20	**0.000**	2	60.58	**0.000**	8	13.14	**0.000**
C. arvense	4	6.79	**0.000**	2	18.00	**0.000**	8	79.00	**0.000**
C. pulchella	4	4.49	**0.002**	2	54.94	**0.000**	8	1.84	0.073
E. compositus	4	2.72	0.031	2	19.61	**0.000**	8	2.72	0.008
G. aristata	4	3.20	0.015	2	323.10	**0.000**	8	1.06	0.396
P. procerus	4	19.29	**0.000**	2	21.54	**0.000**	8	17.86	**0.000**
All grasses	4	3.77	0.005	2	70.07	**0.000**	8	2.79	0.005
F. idahoensis	4	10.85	**0.000**	2	193.95	**0.000**	8	9.43	**0.000**
K. macrantha	4	4.49	**0.002**	2	54.94	**0.000**	8	1.84	0.073
P. spicata	4	9.83	**0.000**	2	243.04	**0.000**	8	15.95	**0.000**
All species	4	14.18	**0.000**	2	206.35	**0.000**	8	9.51	**0.000**
Germinant Biomass									
All forbs	4	7.76	**0.000**	2	173.17	**0.000**	8	7.05	**0.000**
A. frigida	4	2.89	0.024	2	41.08	**0.000**	8	2.92	0.005
B. holboellii	4	1.28	0.282	2	37.72	**0.000**	8	1.39	0.206
E. compositus	4	3.40	0.011	2	18.87	**0.000**	8	3.40	**0.001**
C. arvense	4	6.21	**0.000**	2	17.88	**0.000**	8	4.55	**0.000**
C. pulchella	4	1.95	0.105	2	15.76	**0.000**	8	2.00	0.050
G. aristata	4	2.61	0.038	2	579.40	**0.000**	8	8.37	**0.000**
P. procerus	4	17.96	**0.000**	2	20.40	**0.000**	8	16.79	**0.000**
All grasses	4	0.38	0.825	2	51.73	**0.000**	8	1.12	0.350
F. idahoensis	4	5.37	**0.000**	2	38.58	**0.000**	8	2.73	0.007
K. macrantha	4	9.99	**0.000**	2	28.72	**0.000**	8	4.38	**0.000**
P. spicata	4	4.14	**0.003**	2	138.69	**0.000**	8	5.582	**0.000**
All species	4	0.42	0.792	2	10.25	**0.000**	8	0.54	0.826

Table 3. Mean number of germinants per pot for each treatment × time period × species combination in the greenhouse study (n= 12 replicates). See Table 1 for full species names.

GREENHOUSE

	Control					Aminopyralid					Picloram				
	Time (months)					Time (months)					Time (months)				
	0	3	6	9	11	0	3	6	9	11	0	3	6	9	11
No. Germinants															
A. frigida	6.5	8.6	6.5	6.5	2.7	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
B. holboellii	18.3	2.3	3.3	6.6	2.3	1.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
C. arvense	12.0	3.2	0.3	2.9	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. pulchella	3.0	0.6	0.6	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
E. compositus	1.2	1.0	0.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F. idahoensis	32.6	24.0	23.8	25.9	19.8	10.3	2.5	0.0	3.9	20.1	8.4	6.8	2.7	0.5	4.4
G. aristata	16.5	12.0	13.7	13.5	14.0	3.0	0.5	0.0	0.2	2.6	0.0	0.1	0.0	0.0	0.8
K. macrantha	8.5	4.7	5.3	6.7	3.8	4.1	0.3	0.0	0.1	1.6	0.3	0.0	0.3	0.5	0.4
P. spicata	35.8	33.6	34.3	32.9	31.3	21.1	17.5	0.5	18.5	29.0	5.2	15.1	10.8	1.3	9.7
P. procerus	9.8	0.5	0.1	0.2	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4. Results of ANOVA tests on the effect of herbicide type, block and their interaction on germination rate and germinant biomass in spring and fall field plots. **Bold** font indicates significant tests at Bonferroni-corrected $p < 0.01$)

FIELD

	Season			Herbicide			Block			Season × Herbicide			Season × Block			Herbicide × Block			Season × Herbicide × Block		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Germination Rate																					
All species	1	12.62	**0.001**	2	5.51	**0.005**	1	1.80	0.181	2	7.01	**0.001**	1	0.62	0.432	2	0.19	0.831	2	0.18	0.835
A. frigida	1	2.29	0.136	2	2.44	0.096	1	1.34	0.251	2	3.30	0.044	1	0.10	0.748	2	0.13	0.875	2	0.15	0.862
B. holboellii	1	3.06	0.085	2	2.72	0.074	1	3.71	0.059	2	3.33	0.043	1	0.06	0.801	2	0.39	0.681	2	0.86	0.430
G. aristata	1	22.83	**0.000**	2	2.59	0.084	1	0.10	0.749	2	2.71	0.075	1	0.05	0.830	2	0.42	0.662	2	0.02	0.980
P. spicata	1	2.12	0.151	2	1.30	0.280	1	2.87	0.096	2	0.58	0.564	1	0.66	0.418	2	0.12	0.887	2	0.44	0.649
Germinant Biomass																					
All species	1	6.92	**0.009**	2	2.40	0.093	1	0.58	0.448	2	5.07	**0.007**	1	1.20	0.275	2	0.37	0.693	2	0.05	0.948
A. frigida	1	4.42	0.040	2	1.09	0.344	1	2.19	0.145	2	4.03	0.023	1	0.07	0.797	2	0.07	0.937	2	0.84	0.437
B. holboellii	1	10.52	**0.002**	2	2.22	0.118	1	1.33	0.566	2	1.61	0.209	1	0.22	0.638	2	0.23	0.792	2	0.42	0.660
G. aristata	1	28.29	**0.000**	2	6.20	**0.004**	1	0.82	0.368	2	8.95	**0.000**	1	0.81	0.658	2	0.30	0.739	2	1.20	0.307
P. spicata	1	0.85	0.848	2	0.85	0.432	1	2.85	0.097	2	2.26	0.113	1	1.23	0.272	2	0.57	0.567	2	1.77	0.180

Table 5. Mean number of germinants per plot for each treatment × time period × species combination in the field study (n=12).

FIELD

	Control		Aminopyralid		Picloram	
	Spring 0.75 Mo.	Fall 5 mo.	Spring 0.75 Mo.	Fall 5 mo.	Spring 0.75 Mo.	Fall 5 mo.
No. Germinants						
A. frigida	4.9	2.9	0.1	4.3	0.0	2.4
B. holboellii	8.7	5.5	2.7	10.3	0.0	4.8
C. arvense	0.1	0.0	0.0	0.1	0.0	0.5
C. pulchella	0.9	0.3	0.0	1.8	0.0	0.7
E. compositus	1.1	0.5	0.3	1.2	0.0	0.2
G. aristata	9.8	12.5	6.1	14.3	1.0	12.9
F. idahoensis	0.8	0.9	1.2	0.8	0.3	1.1
K. macrantha	0.0	0.0	0.1	0.3	0.0	0.3
P. spicata	2.0	2.7	2.2	2.5	0.1	2.2
P. procerus	0.4	0.5	0.1	0.0	0.0	0.0

Figure 1. Mean relative difference (treated *vs* control) in number and biomass of germinants compared to controls for all species in the greenhouse. Error bars are bootstrapped standard error for mean relative difference.

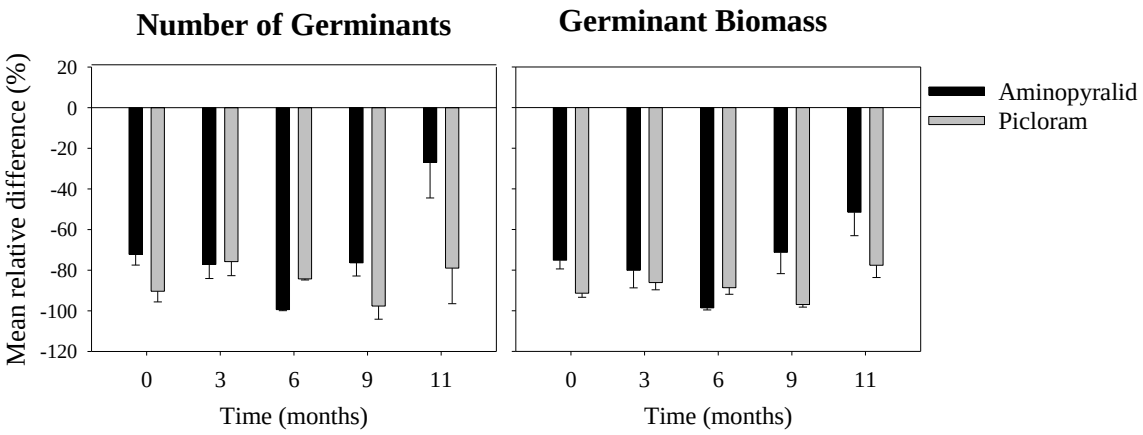


Figure 2. Mean relative difference (treated vs control) in number (a-c) and biomass (d-f) of germinants for graminoid species: *F. idahoensis* (a. d), *K. macrantha* (b, e) and *P. spicata* (c, f). Error bars are bootstrapped standard error for mean relative difference.

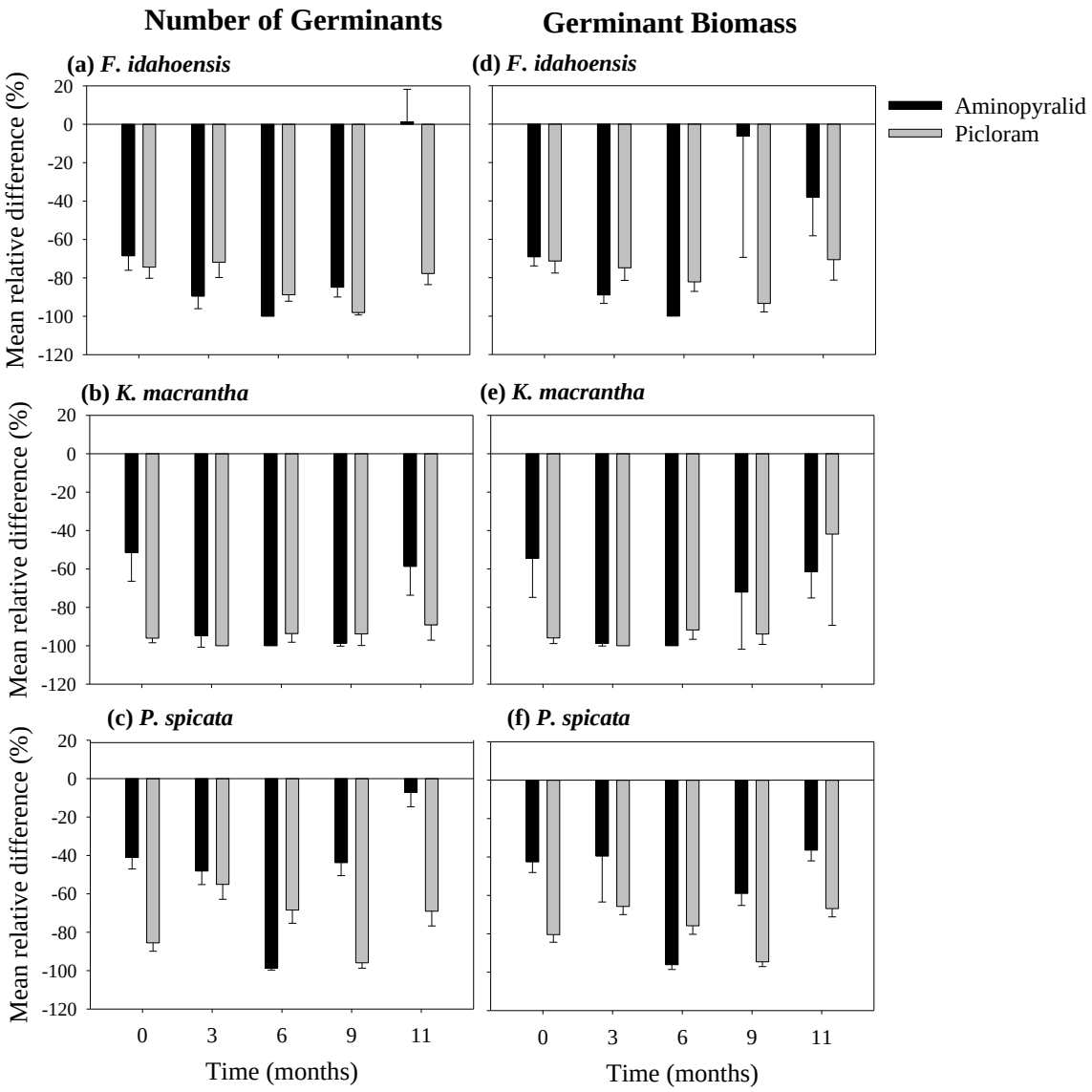
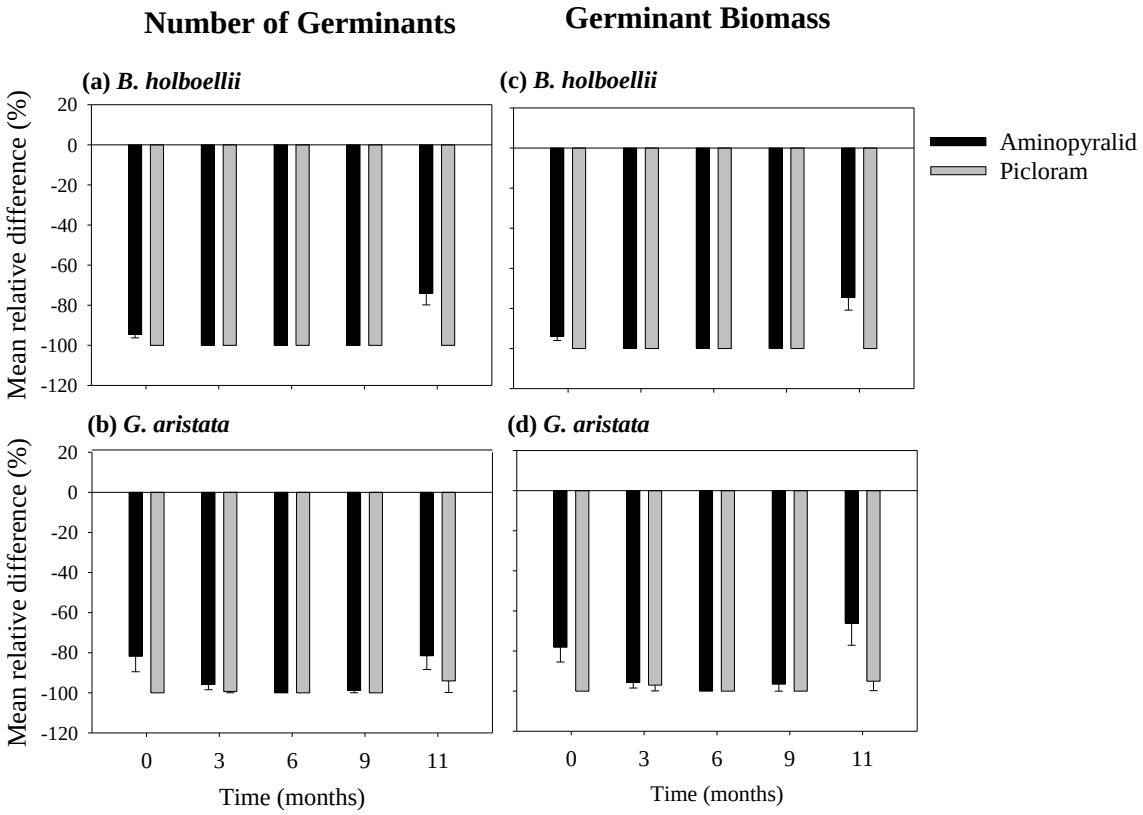


Figure 3. Mean relative difference (treated *vs* control) in number (a-b) and biomass (c-d) of germinants for select forb species*: B. holboellii* (a, c) and *G. aristata* (b, d). Graphs for *C. arvense*, *P. penstemon*, *A. frigida*, and *C. pulchella* are not show here. Error bars are bootstrapped standard error for mean relative difference.



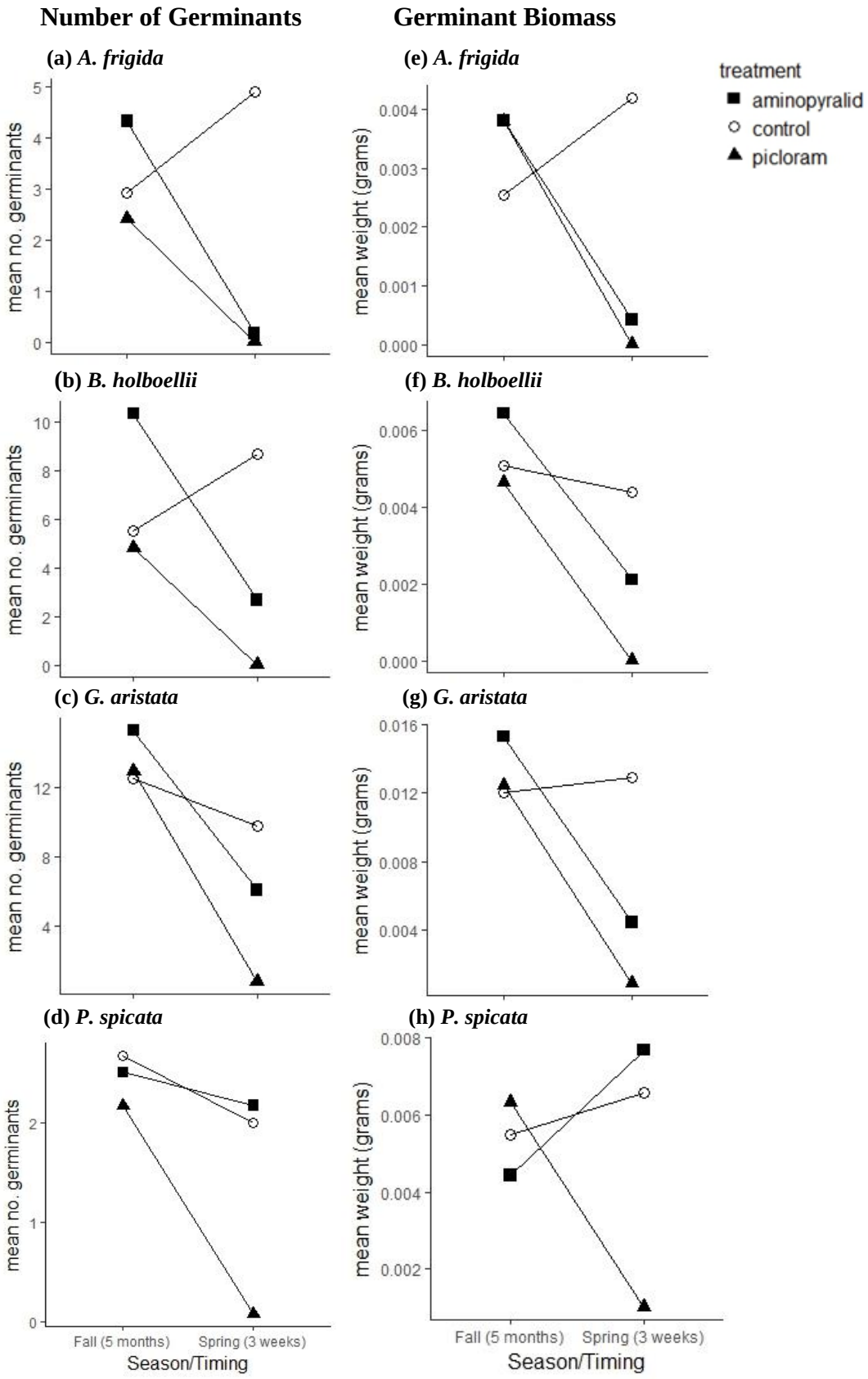

Number of Germinants
Germinant Biomass
(a) A. frigida
(e) A. frigida
(b) B. holboellii
(f) B. holboellii
(c) G. aristata
(g) G. aristata
(d) P. spicata
(h) P. spicata
mean no. germinants
mean weight (grams)
treatment
aminopyralid
control
picloram
Fall (5 months)
Spring (3 weeks)
Season/Timing

Figure 4. Mean number (a-d) and biomass (b-h) of germinants for spring application (0.75 months prior to seeding) and fall application (5 months prior to seeding field plots for *A. frigida* (a, e), *B. holboellii* (b, f), *G. aristata* (c, g) and *P. spicata* (d, h). (n=72)