THESIS

EMERGENCE OF SEEDED FORBS IN ESTABLISHED STANDS OF GEYER'S LARKSPUR ON COLORADO RANGELANDS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2013

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ABSTRACT

EMERGENCE OF SEEDED FORBS IN ESTABLISHED STANDS OF GEYER'S LARKSPUR ON COLORADO RANGELANDS

Larkspurs (*Delphinium* spp.) are considered by many to be the most damaging poisonous plants on rangelands in the western United States. Larkspurs are palatable and acutely toxic to cattle resulting in a consistently large number of annual cattle deaths on western rangelands in the United States. Attempts to avoid the toxic effects of larkspurs often result in missed opportunities to harvest considerable amounts of high-quality forage and dictate management of infested rangelands. Herbicide application can effectively reduce larkspur, but also reduces other herbaceous plants expected to compete most directly with larkspur for resources, so recovery of larkspur following herbicide application is common. A field study was conducted in the foothills of northern Colorado to determine whether seeded forbs would emerge after being sown into existing stands of Geyer's larkspur (*Delphinium geyeri*), and whether pre-seeding application of two herbicides at light-rates would reduce initial competition from larkspur and increase emergence of seeded forbs. Seedling emergence of native forbs was compared to introduced forbs in sprayed (2 different herbicides) and unsprayed stands of Geyer's larkspur. Experimental plots were randomly assigned one of nine possible treatment combinations and replicated 3 times in each of 3 locations. The treatments consisted of all possible combinations of seeding (native forb mixture, introduced forb mixture, and unseeded), herbicide (2,4-D LV4, picloram, and unsprayed) and location (1, 2, and 3). Larkspur density was consistently reduced by herbicide at all locations regardless of seed mixture and no difference was detected between the two herbicides. Perennial grasses were unaffected by herbicide and seeding treatments. Treatment effects on larkspur canopy cover were not obvious. At 2 of the 3 locations, larkspur canopy

cover in unsprayed plots was similar to one or both herbicide treatments. At the third location, larkspur canopy cover in plots treated with picloram was less than 2 of the 3 unsprayed plots and less than all three plots treated with 2,4-D. 2,4-D reduced canopy cover of non-target forbs compared to the unsprayed plots at all three locations, and also compared to the picloram treatment at two locations. Canopy cover of non-target forbs in plots treated with picloram was similar to unsprayed plots at 2 of the 3 locations, and reduced at the other. Sub-shrub cover was greatest in unsprayed plots and reduced by picloram and 2,4-D at 2 of 3 locations. Seedling density of seeded forbs was very low and dependent on location, herbicide, and seed mixture but the effects were variable and subtle. Results suggest that both herbicides reduced larkspur and other existing broadleaf species. The picloram treatment seemed to be more effective at reducing larkspur while leaving greater non-target forb cover than 2,4-D. However, there are indications of a slight reduction in seedling density of seeded forbs in the picloram treated plots.

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INTRODUCTION

Larkspurs (*Delphinium* spp.) are considered to be among the most detrimental poisonous plants on rangelands in the western United States. Ralphs et al. (1988) stated, "Larkspur kills more cattle on mountain rangelands in the western United States and Canada than any other plant or disease." Toxic larkspurs are found in the high plains, foothill, and mountain rangelands (Green et al. 2009) and are generally divided into three groups: tall larkspurs, low larkspurs, and plains or foothills larkspurs, based on mature plant height and geographic location (USDA 2012). Larkspurs are palatable, acutely toxic to cattle (Ralphs et al. 1988), and have consequently been responsible for a large number of cattle deaths on western rangelands for many years, making them a very serious concern for land and livestock managers (Pfister et al. 1999). As the livestock industry moved west into the Rocky Mountains in the early 1800's, significant livestock death-loss occurred, much of which was eventually linked to larkspur (Knight and Pfister 1997). Larkspurs were recognized as a serious problem, and in 1885, the USDA Bureau of Animal Industry began investigating poisonous plants (Knight and Pfister 1997). Larkspur research centered in Colorado due to the enormity of the losses there (Knight and Pfister 1997). In 1909, the US Department of Agriculture established a research station near Gunnison, Colorado known as "Camp Delphinium" to study and find solutions to larkspur poisoning (Green et al. 2009). Over a century of larkspur research has been completed and much advancement has been made, however, livestock deaths attributable to larkspurs still amount to millions of dollars each year (Manners et al. 1993) and have remained surprisingly consistent. To this day, many land and livestock managers in the western United States continue to struggle with death losses and grazing management limitations associated with larkspur-infested rangelands.

North American larkspurs contain numerous toxic alkaloids, most of which are derivatives of the norditerpenoid lycoctonine (Dobelis et al. 1999). Some examples include deltaline, nudicauline, 14-deacetylnudicauline (14-DN), and barbinine (Dobelis et al. 1999). Norditerpeniod alkaloids in *Delphinium* spp. make ingestion toxic and potentially fatal to cattle and other livestock (Pfister et al. 1999). The primary site of action for larkspur alkaloids is the neuromuscular junction where they bind to acetylcholine receptor sites and inhibit nerve impulse transmission (Knight and Pfister 1997). Neuromuscular paralysis develops after this blockage has occurred (Knight and Pfister 1997, Pfister et al. 2002). Clinical signs of larkspur toxicity in cattle include muscular weakness, trembling, straddled stance, periodic collapse into sternal recumbency, bloat due to reduction in rumen motility, and finally death from respiratory failure (Pfister et al. 2002, Green et al. 2009, USDA 2012).

Larkspurs begin growth in early spring, often before most perennial grasses start their spring flush of growth (USDA 2012). Due to its early spring growth, larkspur may be consumed by cattle before other green forage is available and cause poisonings (Green et al. 2009). Alkaloid concentrations are highest in the early stages of vegetative growth (Pfister et al. 2002) that occur in spring and early summer (FEIS 2012). Toxicity of tall larkspur usually declines as it matures, but palatability tends to increase (Pfister et al. 2002, FEIS 2012, USDA 2012). In tall larkspur, the period of highest toxicity and greatest danger to cattle is termed the toxic window, which occurs between the flowering and the pod stages (Green et al. 2009). The toxic windows for low and plains larkspurs last much longer because alkaloid concentrations in these plants remain high much later in the season (Pfister et al. 1999, Manners et al. 1993). Therefore, it is crucial that managers know which species of larkspur are present before they can plan to effectively avoid problems.

This study focused on Geyer's larkspur (*Delphinium geyeri* Greene). Geyer's larkspur is an early emerging, poisonous, perennial forb found on the plains and foothills of Colorado, Wyoming, Utah, Montana, and Nebraska (Hyder and Sabatka 1972). It is typically found in desert shrub, mountain brush, sagebrush, and short-grass prairie plant communities at elevations between 1400 and 3000 meters (Panter et al. 2007). The stem is erect (30 to 60 cm tall) and topped with a terminal raceme of many flowers and often has a reddish base (Flora of North America North of Mexico 1997). The seed are unwinged follicles (11-15 mm long) and about 3 times longer than wide. Geyer's larkspur can be distinguished from the closely resembled low larkspur (*Delphinium bicolor* Nutt.) by the presence of dense, minute pubescence and a 2mm cleft in the lower petal blades (Flora of North America North of Mexico 1997).

Presence of larkspur often dictates when and how rangelands are grazed (USDA 2012). Livestock producers using rangelands with populations of larkspur must be able to identify these plants and adjust cattle management to reduce risk of losses (Gardner and Pfister 2007). Managers use several grazing strategies in an attempt to avoid toxic windows and minimize losses, most of which involve simply removing livestock from affected areas during the most dangerous period. Many factors, such as larkspur species, soil moisture, temperature and elevation, can influence the duration of the toxic window (Green et al. 2009). The least dangerous periods for livestock to graze tall larkspur infested rangelands are early spring and summer until larkspur flowers, or late in the season after the follicles have shattered (Green et al. 2009). Another option involves grazing sheep, before cattle are turned out, to reduce the density and acceptability of larkspur to cattle (Green et al. 2009). Pfister and Gardner (1999) also suggest that losses may be reduced by ensuring that grazing pressure and stock density are low. These practices are used successfully on tall larkspurs; however, alkaloid concentrations in low and plains larkspurs remain high for longer periods making the toxic window difficult to avoid (Pfister et al. 1999, Gardner and Pfister 2007). Regardless of the species, attempts to avoid toxic windows usually result in missed opportunities to harvest considerable amounts of high-quality forage for domestic cattle (Green et al. 2009) and loss of nutrients as the forage matures (Ralphs et al. 1988). Both of these problems lead to decreased seasonal gains and increased feeding costs. Ralphs et al. (1988) stated, “No other group of plants has hindered cattle grazing on mountain rangelands and dictated the present use patterns more than larkspurs.”

Herbicides have been used with partial success for decades to reduce larkspur. Ralphs et al. (1991) suggest using picloram (Tordon®) or 2,4-D for Geyer’s larkspur. Picloram has been reported to reduce Geyer’s larkspur as much as 95% when combined with phenoxy herbicides (Hyder and Sabatka 1972). Studies show 2,4-D can also be an effective herbicide for reducing Geyer’s larkspur (Hyder and Sabatka 1972, Hyder 1971), however, they also discovered that results can vary greatly depending on timing of application and growing conditions. There are some underlying risks that should be considered before applying herbicides to larkspur-infested rangelands. A study by Green et al. (2009) showed that cattle losses to larkspur poisoning could be reduced if herbicide applications decreased larkspur density. However, increased palatability to cattle has been observed in chemically treated larkspur (USDA 2012, Green et al. 2009) and toxicity remains high (USDA 2012). Hyder and Sabatka (1972) also showed that if a complete root kill is not obtained, rootstock buds can grow new foliage which can minimize the long-term effectiveness of the treatment. Other problems can arise because herbicides commonly used on larkspur are broadleaf selective. Application of these herbicides creates an inherent risk of killing important broadleaf perennial forbs that may compete directly with larkspur for resources and provide important forage. This might render herbicide treated areas more prone to future larkspur problems due to a lack of forb diversity. In order to obtain successful larkspur reduction, management efforts must be multifaceted and consider both short and long-term treatment options. Similarly, consideration should be given not only to the plants targeted for reduction, but also to the desired future composition of the area.

Increasing forb diversity is another potential management strategy for reducing larkspur. Studies show that species and functional group diversity within a plant community can be important factors related to invasion resistance and productivity (Levine 2000, Pokorny et al. 2005, Hooper et al. 2005, Lehman and Tilman 2000, Fargione et al. 2003). Increased forb diversity has several potential benefits. It may be a more sustainable long-term larkspur management tool and is considered by many to be safer and more acceptable than repeated herbicide applications. A study by Kedzie-Webb et al. (2001) reported that species richness and diversity were inversely related to cover and biomass of undesirable forb species such as spotted knapweed (*Centaurea stoebe* L.). Competition for resources exists to some extent in most plant communities, and often plays a major role in community structure (Fowler 1986). Direct competition for resources can potentially be obtained by increasing the number of species with similar characteristics (Drenovsky et al. 2008), which could lead to a reduction of an undesirable species such as larkspur. Functional group diversity may also be related to increased productivity and stability (Pokorny et al. 2004, Lehman and Tilman 2000) and decreased invasibility through niche differentiation and resource acquisition (Pokorny et al. 2004). Benz et al. (1999) showed that planting perennial grasses following herbicide application was much more effective at controlling Russian knapweed [*Acroptilon repens* (L.) DC] than the herbicide application alone, suggesting that competition may be an important factor in reducing unwanted plant species. Studies such as Benz et al. (1999) and Kedzie-Webb et al. (2001) suggest that competition and diversity may play important roles in the management of invasive, exotic species such as knapweed, which are often very difficult to control. This suggests that similar benefits may also be applicable to situations where native species such as larkspur need to be reduced. Overlap in nutrient consumption can occur because plants in a community often have similar resource demands and consequently might extract resources from similar nutrient pools in the soil (Pokorny et al. 2005). Therefore, management efforts should focus on maintaining forb diversity which could increase the potential for effective resource competition with larkspur.

Considering both native and introduced forb mixtures provides increased opportunity to identify effective potential competitors with larkspur. This requires careful attention to avoid planting species with any known history of aggressive spread or toxicity to livestock or wildlife. Including some carefully picked introduced species is partially supported by some common theories such as enemy release and novel weapons. The enemy release hypothesis (Wolfe 2002), suggests that introduced plants might experience decreased pressure in their introduced range due to a lack of predators or competition present in their native range. The novel weapons hypothesis (Callaway and Ridenour 2004) suggests that some introduced plants have adaptations that increase their success in their introduced range. Increased success of the introduced species due to enemy release or novel weapons could provide an establishment advantage and improve chances for successful larkspur reduction. Manipulation of community composition has become an important aspect of successful integrated management of undesirable species. In this case, successful seeding might reduce larkspur through targeted competition as well as an increased availability of palatable forbs that could potentially decrease larkspur consumption. The species included in the introduced mixture have been used successfully in North America for various applications and have not shown evidence of aggressive spread.

Determining if an initial herbicide treatment will increase seedling success is important due to the commonly recognized difficulty of seeding into stands of established plants. Bakker et al. (2003) showed that herbicide treatments aimed at controlling crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] led to an increase in establishment and survivorship of seeded native grasses. Washburn and Barnes (2000) also showed that pre-emergent herbicide applications resulted in better establishment of native warm season grasses and forbs, suggesting that reducing competition with herbicides can increase seeding success. Seedling success and establishment is crucial in obtaining the potential long-term benefits of larkspur reduction.

The overall goal of this study was to explore the possibility of combining herbicide application and subsequent forb seeding as a long-term integrated management strategy for reducing Geyer's larkspur. Our primary objectives were to determine: 1) whether native or introduced forbs would emerge after being seeded into established stands of Geyer's larkspur and, 2) whether pre-seeding application of herbicides at light rates could be used to increase native and introduced forb seedling emergence in Geyer's larkspur stands and minimize impacts on non-target broadleaf plants. We are also interested in whether or not treatment effects would be consistent across 3 different locations at the study area. Due to the evidence of the effects of competition and the possibility of residual effects of picloram on planted seedlings, it was hypothesized that seedling emergence of both forb seed mixtures would be greatest in plots treated with 2,4-D LV4. However, it was also hypothesized that initial larkspur reduction would be greater in plots treated with picloram than those treated with 2,4-D, but both herbicides would reduce larkspur compared to the controls. The introduced seed mixture was hypothesized to produce greater seedling densities than the native seed mixture. Finally, it was hypothesized that treatment effects would be consistent across 3 locations at the study site. Applying all possible combinations of the herbicide and seed treatments provides information on the best management option for Geyer's larkspur reduction. Following herbicide treatments with native and introduced seed mixtures could help managers utilize the relatively rapid benefits of larkspur reduction provided by herbicides, while potentially providing long-term reduction benefits by maintaining diversity within the community. Identification of effective larkspur reduction techniques could be extremely beneficial to land and animal managers in the western United States.

MATERIALS AND METHODS

Study area:

The study was located in the foothills approximately 48 kilometers north of Fort Collins, CO at an elevation of about 2149 meters above sea level. Mean annual precipitation ranges from 36 to 45 centimeters. The soil is a well-drained, shallow, gravelly sandy loam that formed in material weathered from granite (USDA 1980). Average soil depth is between 25.4 and 50.8 centimeters. The mean annual air temperature ranges from 6.7 to 7.8 degrees Celsius and the frost-free period ranges from 75 to 100 days (USDA 1980). Plant nomenclature follows the USDA PLANTS Database (USDA PLANTS Database 2012). The most abundant plant species in the community include western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve], blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], needle and thread [*Hesperostipa comata* (Trin. & Rupr.) Barkworth], threadleaf sedge (*Carex filifolia* Nutt.), wooly groundsel [*Packera cana* (Hook.) W.A. Weber & Á. Löve] and Geyer's larkspur.

Plots were located and marked during the second week of May 2011 and targeted areas with the highest and most consistent larkspur abundance. Three sets of plots were established, each containing three complete blocks comprised of one plot for each of nine treatments. Plots were 3 x 10-m in size and were randomly assigned to one of nine treatments within each block. A 0.5-m buffer was left between neighboring plots to minimize treatment overlap.

Treatments:

The treatments consisted of all possible combinations of seeding (native forb mixture, introduced forb mixture, and unseeded), herbicide (2,4-D LV4, picloram, and unsprayed) and location (1, 2 and 3). The native forb seed mixture was comprised of 11 species and the introduced forb seed mixture included 10 species. 2,4-D LV4 was applied at a rate of 2.96 kg a.i. ha^{-1} (2 quarts of product per acre) and picloram was applied at a rate of 0.28 kg a.i. ha^{-1} (1 pint of product per acre). The herbicides were applied the third week of June 2011 when approximately half the larkspur plants had bolted. A small tractor-mounted boom sprayer was used to apply the herbicides. The sprayer was adjusted to have a 3 meter swath when the boom was 91 cm (36 inches) above the ground to obtain even and complete coverage of each sprayed plot. Adjacent plots were covered with plastic during herbicide application to minimize the effects of spray drift.

The seed mixtures were planted in the fall to allow cold stratification and take advantage of early moisture for successful seedling establishment. Seeds were planted using a Truax Flex-II 86 rangeland drill. Ten samples of 100 seeds were weighed for all 21 species to estimate seed mass. The mass of the mixes for a given area was calculated and used to insure proper seed drill calibration. Both seed mixtures were divided into 2 groups based on seed size. The large and small seeds were separated in the drill using boxes designed to accommodate each size. The large and small seed boxes in the drill were calibrated separately on site before each mix was drilled. Each mixture was seeded a rate of 1000 pure live seed (PLS) per square meter and all species were equally represented within each mixture. Individual species were seeded at a rate of 91 PLS per square meter in the native mixture and 100 PLS per square meter in the introduced mixture. All plots were seeded in late November 2011.

Table 1. Plant species used in the native and introduced seed mixture treatments.

Seed mix	Scientific name	common name
Native	*Achillea millefolium* L. var. *occidentalis* DC. ′Eagle′	western yarrow
	Astragalus canadensis L.	Canadian milkvetch
	Balsamorhiza sagittata (Pursh) Nutt.	arrowleaf balsamroot
	Coreopsis lanceolata L.	lanceleaf tickseed
	Dalea candida Michx. ex Willd. ′Antelope′	white prairie clover
	Eriogonum umbellatum Torr.	sulfur-flower buckwheat
	Hedysarum boreale Nutt. ′Timp′	northern sweetvetch
	Linum lewisii (Pursh) ′maple grove′	Lewis blue flax
	Penstemon bradburii	largeflowered penstemon
	Penstemon palmeri A.Gray	Palmer's penstemon
	Penstemon rydbergii A. Nelson	Rydberg's penstemon
Introduced	*Astragalus cicer* L. ′Monarch′	Cicer milkvetch
	Bassia prostrata (L.) A.J. Scott	forage kochia
	Linum lewisii Pursh ′Apar′	Lewis blue flax
	Lobularia maritima (L.) Desv. ′Carpet/Snow′	sweet alyssum
	Medicago sativa L. ssp. *falcate* (L.) Arcang. ′Don′	yellow alfalfa
	Melilotus officinalis (L.) Lam.	yellow sweetclover
	Onobrychis viciifolia Scop. ′Eski′	sainfoin
	Papaver rhoeas L. ′Red′	corn poppy
	Sanguisorba minor Scop. ′Delar′	small burnet
	Vicia villosa Roth ssp . *varia* (Host) Corb.	winter vetch

Data collection:

Data were collected in May and June of 2011 and May and August of 2012. Larkspur density was estimated using a rectangular 0.5 m^{-2} frame (50 X 100 cm). Six frame readings were taken in each of the 81 plots, ensuring that 3 frames were located in each half of each plot. Six random numbers between 1 and 5 were generated and used to determine frame locations. Sampling of a plot began on one plot edge. The first random number was used to determine the number of steps between the plot edge and the first frame location roughly centered in the observer's right-hand side of the plot. The second and third random numbers determined the number of steps to the second and third frame locations on that side of the plot. Sampling resumed on the opposite edge of the plot and random numbers 4-6 were used to determine 3 frame locations in the other half of each plot as described above. Percent canopy cover by species was estimated using a 0.5 m^{-2} extended Daubenmire frame (Bonham et al. 2004) during the last 2 weeks of June 2011 and third week of August 2012. Again, 6 frame readings were taken in each of the 81 plots. Frame placement followed the same procedure described for larkspur density. The step sequence was the same for all plots for both years. The edges of the plots were avoided when placing frames to help insure that data were collected in areas with consistent and thorough treatment application. Seedling densities were estimated in July of 2012 to quantify seedling emergence. All seedlings were counted by species for the entire length in 6 of the 12 drill rows in each seeded plot. The totals were then used to obtain an estimate seedlings m^{-2}. Comparison of the 2012 larkspur density across all treatments allowed determination of the effectiveness of herbicide and seed treatments in reducing larkspur. The 2012 canopy cover data were also analyzed to investigate changes in community composition caused by treatments. Treatments were administered after the density and canopy cover data were gathered in 2011. Air and soil temperature data were gathered at 2 hour intervals using an Onset Hobo data logger (Hobo 4-channel external data logger - U12-008, http://www.onsetcomp.com) placed at a central location among the 3 locations. Mean air and soil temperatures for each month from April through September 2012 are presented in Table 5. A rain gauge was also installed on site to collect growing season precipitation during 2012. The weather station data were used to describe growing conditions of interest and help us better understand patterns observed in the vegetation data.

Experimental design and data analysis:

The study was a randomized complete block design. Each of the 3 locations contained 3 blocks comprised of 9 randomly assigned plots representing each of the possible treatment combinations. All data (canopy cover, larkspur density, and seedling density) were square root transformed to meet assumptions of analysis of variance. An Arc-sin square root transformation was also performed on all cover data but examination of residual plots confirmed that the square root transformation was as effective as the arc-sin square root transformation. Analysis of variance and Type 3 tests of fixed effects were used to investigate effects of treatment (herbicide and seed mixture) and location on canopy cover and density of larkspur, canopy cover of other established plants, and seedling density of seeded plants. Analyses were conducted using the "mixed" procedure in SAS 9.3 (2010). An α level of 0.05 was determined to be sufficient and was used for all analyses. When main effect or interaction terms had significant F-tests, treatment means were compared using Fischer's least significant difference method.

RESULTS

Larkspur density:

Larkspur density was affected only by herbicide treatment ($P=0.0006$). Location, seed mixture and all 2- and 3-way interaction terms had non-significant F-tests ($0.1990<P<0.8018$). Both the 2,4-D ($P=0.0230$) and picloram ($P=0.0001$) herbicide treatments resulted in lower larkspur density in 2012 compared to unsprayed plots, but there was no difference in larkspur density between the two herbicide treatments ($P=0.0774$). Mean larkspur densities (± SE, n=27) in plots treated with picloram, 2,4-D and the unsprayed plots were, 2.3 ± 0.4, 3.0 ± 0.4 and 4.0 ± 0.4 plants m^{-2}, respectively. Mean larkspur density across all locations in 2011, prior to herbicide application, was 7.5 ± 0.3 plants m^{-2}, n=81.

Canopy cover:

Of the 24 species encountered in 2012, 12 had enough non-zero data to attempt analysis using the square root transformation. The others did not have sufficient non-zero data to meet assumptions of analysis of variance. Therefore, individual species were combined into groups including C3 grasses, C3 grass-likes, C4 grasses, forbs, sub-shrubs and larkspur for analysis. Combining the species into groups allowed inclusion of all species and minimized problems of zeros in the data set thereby meeting the assumptions of analysis of variance.

C3 grasses in decreasing order of abundance based on canopy cover included needle and thread, western wheatgrass, squirreltail *[Elymus elymoides* (Raf.) Swezey], prairie Junegrass [*Koeleria macrantha* (Ledeb.) Schult.] and Sandberg bluegrass [*Poa secunda* J. Presl]. C3 grass cover was affected only by location ($P=0.0381$). Herbicide, seed mixture and all 2- and 3-way interaction terms had non-significant F-tests ($0.0633<P<0.3014$). The mean percent canopy cover of C3 grasses (± SE, n=27) at location 2 (21.1 ± 1.7%), was greater than location 3 (12.5 ± 1.7%, P=0.0155). Canopy cover of C3 grasses at location 1 was intermediate (15.1 ± 1.7%) and not different from location 2 (P=0.0555) or location 3 (P=0.3679).

Threadleaf sedge was the only C3 grass-like plant. Threadleaf sedge cover was affected only by herbicide treatment (P=0.0019). Location, seed mixture and all 2- and 3-way interaction terms had non-significant F-tests (0.1674<P<0.4094). Canopy cover of threadleaf sedge was lower in plots treated with picloram (6.4 ± 0.7%; mean ± SE, n=27) compared to unsprayed plots (9.4 ± 0.7%, P=0.0005) as well as plots treated with 2,4-D (8.3 ± 0.7%, P=0.0162). There was not a significant difference between the 2,4-D treatment and unsprayed plots (P=0.2318).

C4 grasses included blue grama and mountain muhly [*Muhlenbergia montana* (Nutt.) Hitchc.]. Blue grama was far more abundant than mountain muhly. There was no treatment effect on C4 grass canopy cover. All F-tests were non-significant (0.0704<P<0.9975). Mean canopy cover of C4 grasses (± SE, n=81) was 35.9 ± 1.7%.

Larkspur cover was affected by location (P=0.0061), and herbicide (P=0.0069), but the effects of herbicide varied by location (P=0.0003) and the three-way interaction was also significant (P=0.0103). Other F-test results were not significant (0.1327<P<0.7354). Effects of herbicide and seed mixture are summarized by location in Table 2. There were no differences among means for location 3 (0.0880<P<1.0).

The forb category was comprised of tarragon [*Artemisia dracunculus* L.], western tansymustard [*Descurainia pinnata* (Walter) Britton], hairy false goldenaster [*Heterotheca villosa* (Pursh) Shinners var. *minor* (Hook.) Semple], lambsquarters [*Chenopodium album* L.], sulphur-flower buckwheat [*Eriogonum umbellatum* Torr.], dotted gayfeather [*Liatris punctata* Hook.], Northern Idaho biscuitroot [*Lomatium orientale* J.M. Coult. & Rose], wooly groundsel, sainfoin [*Onobrychis viciifolia* Scop.], little sunflower [*Helianthus pumilus* Nutt.] and whiskbroom parsley [*Harbouria trachypleura* (A. Gray) J.M. Coult. & Rose]. Forb cover was

Table 2. The effects of herbicide treatment and seed mixture in each of 3 locations on larkspur canopy cover (mean and SE, n=3) in foothills rangelands of north central Colorado. Larkspur cover at location 3 was consistently low and did not differ among treatments. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means within a location containing the same letter are not different (α=0.05).

Location	Seed Treatment	Herbicide Treatment	Larkspur Canopy Cover (%)		Standard Error
1	Native	2,4-D	1.61	A	0.44
1	Introduced	No Herbicide	1.28	AB	0.43
1	Introduced	2,4-D	1.06	AB	0.38
1	No Seed	2,4-D	0.78	B	0.29
1	No Seed	No Herbicide	0.67	B	0.27
1	Native	No Herbicide	0.44	C	0.23
1	Native	picloram	0.33	C	0.18
1	No Seed	picloram	0.28	C	0.11
1	Introduced	picloram	0.17	C	0.09
2	No Seed	2,4-D	0.28	A	0.19
2	Introduced	No Herbicide	0.22	AB	0.13
2	Native	No Herbicide	0.11	AB	0.08
2	No Seed	No Herbicide	0.11	AB	0.08
2	No Seed	picloram	0.06	AB	0.06
2	Introduced	2,4-D	Trace	B	0.0
2	Native	2,4-D	Trace	B	0.0
2	Native	picloram	Trace	B	0.0
2	Introduced	picloram	Trace	B	0.0
3	Introduced	picloram	0.17	A	0.12
3	No Seed	No Herbicide	0.17	A	0.09
3	Introduced	2,4-D	0.06	A	0.06
3	Native	picloram	0.06	A	0.06
3	No Seed	2,4-D	0.06	A	0.06
3	No Seed	picloram	0.06	A	0.06
3	Native	2,4-D	Trace	A	0.0
3	Native	No Herbicide	Trace	A	0.0
3	Introduced	No Herbicide	Trace	A	0.0

affected by herbicide treatment (P=0.0001) and the location by herbicide interaction (P=0.0001). Other F-test results were non-significant (0.2512<P<0.9391). Effects of herbicide on forb canopy cover are summarized by location in Table 3.

Table 3. The effects of herbicide treatment on forb canopy cover (mean and SE, n=18) in each of 3 locations of study plots in foothills rangelands of north central Colorado. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means within a location containing the same letter are not different (α=0.05).

Location	**Herbicide Treatment**	**Forb Canopy Cover (%)**		**Standard Error**
1	No Herbicide	1.94	A	0.37
1	picloram	1.52	AB	0.33
1	2,4-D	0.79	B	0.22
2	No Herbicide	6.85	A	0.65
2	picloram	2.93	B	0.43
2	2,4-D	0.20	C	0.07
3	No Herbicide	3.07	A	0.51
3	picloram	1.93	A	0.37
3	2,4-D	0.33	B	0.11

Prairie sagewort [*Artemisia frigida* Willd.] was the only sub-shrub encountered. The canopy cover of sub-shrubs was affected by location (P=0.0051), herbicide (P=0.0001) and the two factors simultaneously (P=0.0005). Other F-test results were non-significant (0.3510<P<0.8848). Effects of herbicide treatment on canopy cover of sub-shrubs are summarized by location in Table 4.

Table 4. The effects of herbicide treatment on sub-shrub cover (mean and SE, n=18) in each of 3 locations of study plots in foothills rangelands of north central Colorado. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means within a location containing the same letter are not different (α =0.05).

Location	Herbicide Treatment	Sub-Shrub Canopy Cover (%)		Standard Error
1	No Herbicide	0.28	A	0.13
1	picloram	Trace	B	0.0
1	2,4-D	Trace	B	0.0
2	No Herbicide	1.02	A	0.22
2	picloram	0.02	B	0.02
2	2,4-D	Trace	B	0.0
3	No Herbicide	0.20	A	0.10
3	picloram	0.04	A	0.0
3	2,4-D	Trace	A	0.0

Seedling density of seeded forbs:

Seedling density was affected by location (P=0.0485), seed mixture (P=0.0001), herbicide (P=0.0021) and the three factors simultaneously (P=0.0379). All three 2-way interactions had non-significant F-tests (0.1014<P<0.9801). Figures 1, 2 and 3 depict how each seed mixture performed within each location and how they reacted to the herbicide treatments.

For location 1, all treatment combinations were similar except the native seed + picloram combination (Figure 1), which produced the lowest seedling density and differed from all three treatment groups with the introduced seed mix (0.0004<P<0.0139) as well as the other two treatments with the native seed mix (0.0045<P<0.0049).

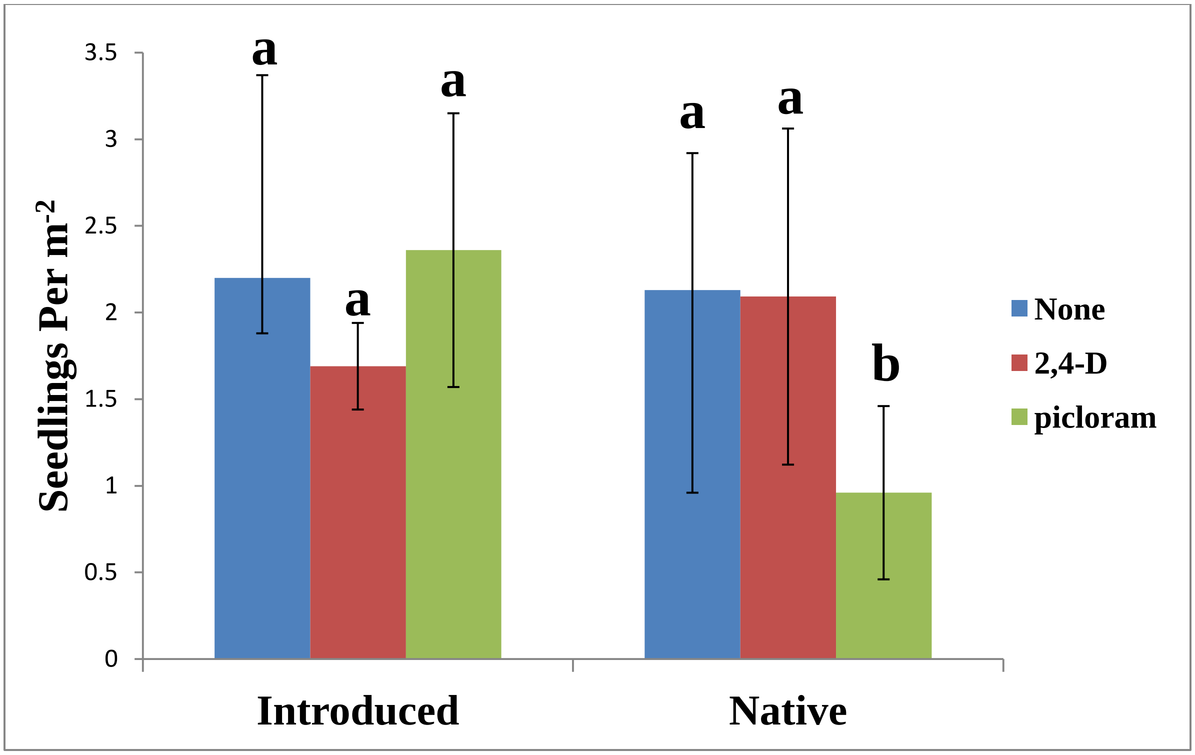

Figure 1. The effects of herbicide treatment and seed mixture for location 1 on the mean number of planted native and introduced seedlings m^{-2} (n=3) in foothills rangelands of north central Colorado. Error bars represent the standard error of the mean. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means labeled with the same letter are not different (α =0.05).

Location 2 (Figure 2) displays a similar pattern where the native seed + picloram treatment produced the lowest seedling density. However for location 2, this value differed only from the introduced seed + 2,4-D treatment (P=0.0354)

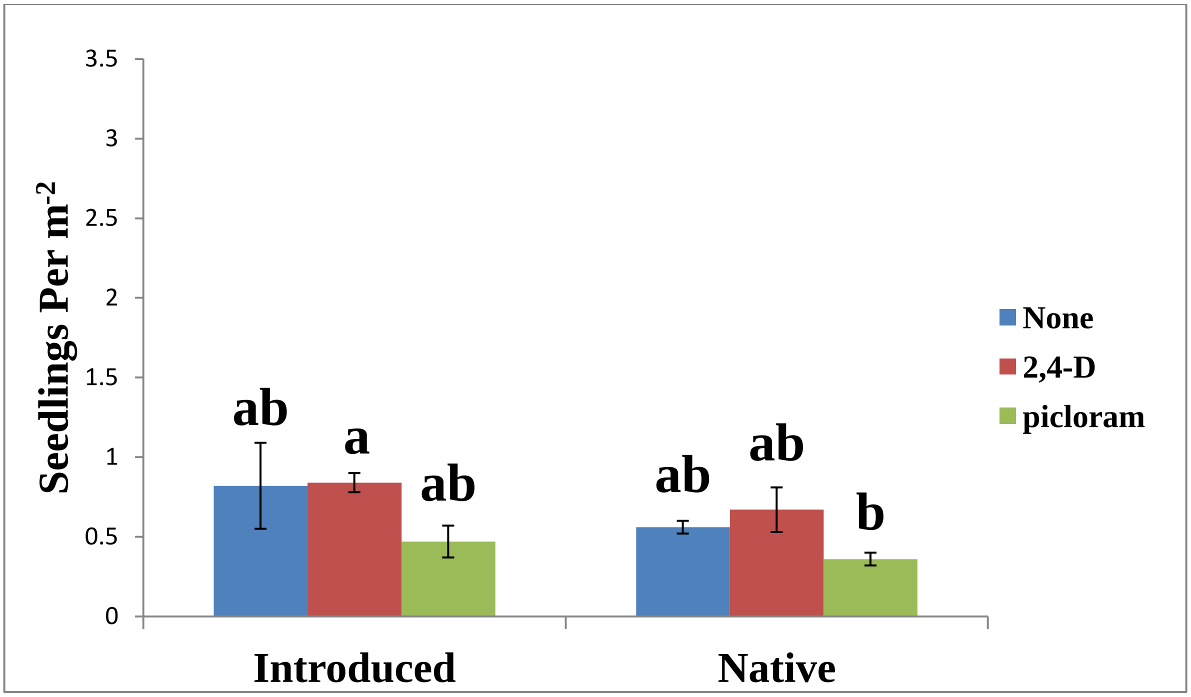

Figure 2. The effects of herbicide treatment and seed mixture for location 2 on the mean number of planted native and introduced seedlings m^{-2} (n=3) in foothills rangelands of north central Colorado. Error bars represent the standard error of the mean. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means labeled with the same letter are not different (α =0.05).

For location 3 (Figure 3), the native seed + picloram treatment again had the lowest seedling density and was different from the introduced seed + 2,4-D (P=0.0062) and introduced seed + no herbicide (P=0.0023) treatments. Unlike locations 1 and 2, seedling density in the introduced seed + picloram treatment was significantly lower than the introduced seed + no herbicide treatment (P=0.0325). Interestingly, the density of seeded forbs in the native seed + picloram plots was similar to the native seed + no herbicide (P=0.6059), the native seed + 2,4-D (P=0.1795) and the introduced seed + picloram treatments (P=0.2850).

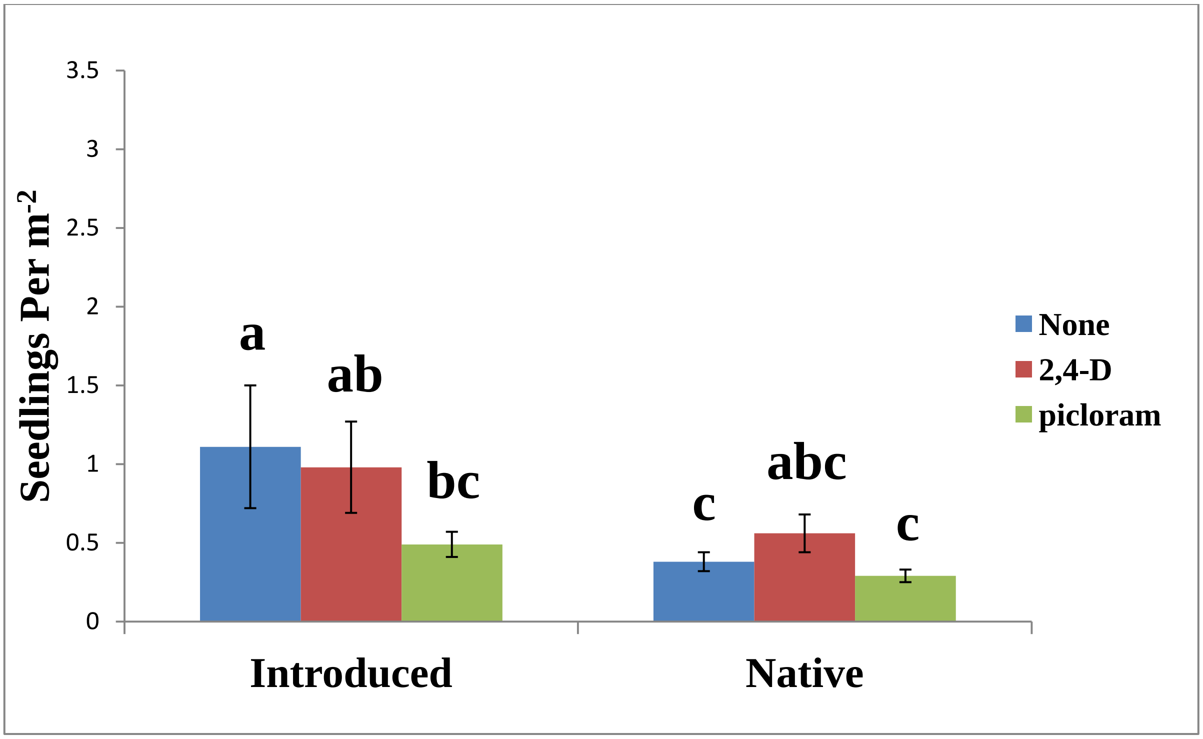

Figure 3. The effects of herbicide treatment and seed mixture for location 3 on the mean number of planted native and introduced seedlings m^{-2} (n=3) in foothills rangelands of north central Colorado. Error bars represent the standard error of the mean. Fischer's LSD method and SAS 9.3 were used to perform mean separations. Means labeled with the same letter are not different (α =0.05).

Weather conditions:

Table 5. Mean daytime and nighttime air temperatures and overall soil temperature from April 2012 to September 2012 near UTM 13 480923E, 4529324N. The means were calculated from data collected on site at 2 hour intervals.

Month	**Mean Soil Temperature ^{o}C**	**Mean Daytime Air Temperature ^{o}C (6AM-6PM)**	**Mean Nighttime Air Temperature ^{o}C (6PM-6AM)**
April	10.6	10.9	4.3
May	16.9	13.8	9.3
June	23.2	21.5	17.1
July	23.9	22.5	18.2
August	22.6	21.4	16.7
September	18.7	17.5	12.8

Numerous small precipitation events totaling 2.79 cm occurred from 29 March, 2012 to 9 June, 2012. During the first week of July 2012, 5.4 cm of rain fell in one storm. The last three weeks of July 2012 produced 3.1 cm of rain at the site, and 0.63 cm of rain was collected during the month of August 2012. The total collected rainfall from 29 March, 2012 to 1 September, 2012 was 11.9 cm.

DISCUSSION

Mean larkspur plant density of unsprayed plots in 2012 was roughly half of the 2011 mean, which was likely due to the unusually dry and warm spring and summer conditions. Even with this reduction in larkspur density on untreated plots between 2011 and 2012, we still detected a significant effect of herbicide treatments. Both herbicides reduced larkspur density compared to the control, which partially supports the second hypothesis. Picloram was not more effective at reducing larkspur density than 2,4-D, which was not hypothesized. Perhaps differences in herbicide performance between picloram and 2,4-D would have been evident if growing conditions had been more favorable. A greater number of larkspur plants would likely have been encountered under better growing conditions, which might have reduced variability within treatments. This speculation is supported by the larkspur cover results (Table 2). Larkspur cover was greatest for location 1 and there was a significant reduction of larkspur cover by picloram compared to all plots sprayed with 2,4-D and all but one unsprayed plot that had low initial larkspur cover. Location 2 had intermediate larkspur cover and the results are less clear than for location 1. For location 2, the two lowest larkspur cover values are picloram treatments, however they are only significantly different from one other treatment. Location 3 had the lowest larkspur cover and showed no differences among herbicides. Therefore, it appears that if growing conditions had been more conductive for larkspur growth in 2012, resulting in greater larkspur cover values, our ability to detect differences in the efficacy between the two herbicides would have been improved.

The inability to detect differences in larkspur reduction between 2,4-D and picloram may also be affected by other factors such as the light application rates, the similar mode of action between the herbicides and the unusually dry growing conditions in 2012. First, light application rates were used intentionally to decrease larkspur abundance while minimizing effects on other broadleaf species and the planted species. The light application rates of picloram and 2,4-D used in this study in combination with the dry conditions likely made it difficult to detect differences between the two herbicides in their effectiveness to reduce larkspur. Duncan and McDaniel (2009) and Hyder and Sabatka (1972) report using rates of both herbicides that were approximately 2 times the rates we used. Second, 2,4-D and picloram come from different herbicide families (Phenoxy and Pyridine, respectively), however, they share the same mode of action (Tu et al. 2001). Both are auxin-like herbicides, meaning they mimic the plant growth hormone auxin, causing uncontrolled and disorganized plant growth that can cause plant death (Tu et al. 2001). The similar mode of action may have prevented any further differentiation in performance because the unusually dry conditions likely had similar effects on the lethality of each herbicide. Last, the dry conditions likely reduced the number and vigor of larkspur plants which may have dampened the herbicide effects. Hyder (1971) treated a similar rangeland with 2.2 kg ha^{-1} of 2,4-D and noted a decrease in aboveground biomass of Geyer's larkspur without a large decrease in plant density the year following treatment. Hyder and Sabatka (1972) reported using 2,4-D at a much higher rate of 4.5 kg ha^{-1} and noted a similar pattern of decreased plant size (measured in number of leaves per plant) without a large reduction in density. It is possible that we too, reduced the biomass or cover of larkspur plants without killing the treated plants.

However, differences in larkspur cover could have been dampened in 2012 because much of the larkspur that grew early that year had died by the time the cover data were collected due to the dry conditions. Given that we detected a reduction in larkspur density using both 2,4-D and picloram and also saw some reduction in larkspur cover with the picloram treatment, it is likely that better growing conditions in 2012 could have allowed detection of more significant reductions of larkspur density and cover from both herbicide treatments. Further differentiation of herbicide effectiveness may also be apparent after another growing season if a pattern similar to that reported by Hyder and Sabatka (1972) is observed. This would indicate the possibility of a residual effect from picloram leading to slower larkspur recovery compared to 2,4-D.

Differences in canopy cover of C3 grasses among the 3 locations were most likely driven by geographical differences between the locations that resulted in slightly different growing conditions (Burke et al.1989). All three locations were established in drainages that were within approximately 600 meters from one another and where larkspur density was the greatest and most consistent; however, there were differences between locations. Location 2 was established in the deepest drainage of the three, which could have resulted in more available moisture from drifting snow, run-off and underground water movement (Hawley et al. 1983). Location 2 also may have experienced longer shaded periods during the morning and evening, which may have produced slightly more favorable growing conditions for C3 plants than the other locations. Location 3 was established in a drainage that was shallower than Location 2 and had a steeper slope than locations 1 or 2. Location 3 also had more of a south-facing aspect than locations 1 or 2. The combination of characteristics for location 3 likely resulted in warmer and dryer conditions which would be less favorable to C3 plants than the other two locations (Bennie et al. 2008, Hawley et al. 1983). Location 1 had intermediate slope and depth compared to locations 2 and 3 and thus had intermediate C3 grass cover. The soil at locations 1 and 2 was similar in texture, color and depth. Location 3 appeared to have less fine material resulting in a courser texture and lighter color than the other two locations.

There have been reports of some damage to cool season grasses after picloram application. However, a study by Rice and Toney (1998) showed very little or no decrease in grass cover (mostly cool season species) after spraying a similar rate of picloram (0.28 kg a.i. ha^{-1}), and showed a significant increase in standing grass cover 3 years post treatment. The light rate of herbicides used in this study made severe damage unlikely. Perhaps, the dry conditions in our study masked differences that may have been apparent under more suitable growing conditions as reported by Bork et al. (2007).

The reduction of threadleaf sedge in plots treated with picloram was unanticipated because we used light application rates of both herbicides. The Tordon® label states that a reduction in some grass species is possible, but release from competition of weedy species often results in a long-term increase in grass production in the years following treatment (Tordon® 22K Specialty Herbicide Specimen Label 2012). Given similarities between C3 grasses and C3 grass-likes, it is reasonable that a similar response may occur in the grass-likes in the following growing seasons. It is also possible that any injury caused by spraying in 2011 could have made threadleaf sedge more vulnerable to drought stress during the dry growing season of 2012. However, there is the potential for a beneficial outcome of a reduction in threadleaf sedge if it results in an increase of more palatable grass species that may compete with larkspur directly for resources (Aerts 1999, Fowler 1986). The 2,4-D label states nothing about possible injury to threadleaf sedge and it specifically mentions monocots that may be susceptible at a given rate

(2,4-D LV4 Specimen Label 2012). It was not surprising that we did not see a reduction in C3 grass-likes by the 2,4-D treatment.

No treatment effects were detected in the C4 grasses. This was expected because both herbicides used are broadleaf selective and rarely result in reduction of warm season grass growth. The Tordon® 22k label states that short-term reduction of blue grama can occur (Tordon® 22K Specialty Herbicide Specimen Label 2012). However, Bork et al. (2007) reported a significant increase in grass biomass the first year after treatment with 2,4-D and picloram+2,4-D, but the second year after herbicide application was dry and no differences in grass biomass were detected. In this study, the light application rate of both herbicides made considerable damage to warm season grasses unlikely, especially in combination with exceptionally dry growing conditions.

The location by herbicide interaction in the forb community revealed some differences in forb cover between locations in the unsprayed plots. Location 2 appeared to have the greatest forb cover in the unsprayed plots, which may be explained by the geographic characteristics that potentially create slightly cooler and wetter growing conditions. Location 2 was also the only location where both herbicide treatments differed from the control. Perhaps the greater forb cover or the composition of forb species at location 2 allowed for further differentiation of the treatments. However, we were unable to analyze the forbs by species due to zeros in the data. For all three locations, the 2,4-D treatment significantly reduced forb cover relative to the unsprayed treatments while only one showed a significant reduction of forbs due to picloram. However, the plots treated with picloram had the lowest larkspur density, which indicates that picloram is at least as effective as 2,4-D at reducing larkspur while leaving greater non-target forb cover in 2 of 3 locations. The management implications of these findings could prove quite useful as they suggest light rates of picloram could potentially be used to successfully reduce larkspur while damaging fewer native broadleaf species than 2,4-D. Maximizing the cover of desirable forbs and diversity in the community increases the potential for multiple benefits including stability, competition with undesirable species and available variety for livestock and wildlife. Studies have shown species and functional group diversity within a plant community can create stability by increasing benefits such as niche differentiation and competition for resources which can decrease the likelihood of undesirable plant invasion (Levine 2000, Pokorny 2005, Lehman and Tilman 2000, Fargione et al. 2003, Kedzie-Webb et al. 2001, Hooper et al. 2005). Species diversity has also been shown to increase resistance to stressors such as drought by increasing carbon stored in aboveground vegetation and improved ability to take advantage of increased nutrient availability following drought conditions (Bloor and Bardgett 2012). Another study by Richardson et al. 2009, found that plots with greater species richness suffered less plant abundance loss than plots with fewer species under drought conditions. Maximizing the diversity and abundance of palatable herbaceous species could also decrease the likelihood of livestock and wildlife eating harmful species such as larkspur while also creating the potential for competition for resources.

Location 2 also appeared to have the highest sub-shrub (prairie sagewort) cover in unsprayed plots, again indicating it may be more suitable for cool season species because prairie sagewort is a cool season plant (Burke et al.1989). Sub-shrub cover was reduced at locations 1 and 2 by both herbicide treatments, but there was no difference between picloram and 2,4-D. Some reduction of this species was expected as The Weed Management Handbook for Wyoming, Utah and Montana (Dewey et al. 2007) suggests picloram (0.28 kg a.i. ha^{-1}) and 2,4-D ester (0.73 kg a.i. ha^{-1}) or Grazon® (picloram+2,4-D at 2.24kg a.i. ha^{-1}) for treatment of prairie sagewort. The Tordon® label also includes prairie sagewort in the list of controlled species. Wilson and Stubbendieck (1981) reported reduction of prairie sagewort using both picloram and 2,4-D but noted that seedling re-infestation was likely to occur the first year after 2,4-D treatment. Reduction efforts targeting prairie sagewort are not uncommon, especially on land used for livestock production. Our results imply that lighter rates of picloram or 2,4-D have the potential to reduce prairie sagewort, which could potentially reduce herbicide treatment costs for large applications. Any injury caused by either herbicide treatment in 2011 could have made prairie sagewort plants more vulnerable to drought conditions in 2012, potentially causing death of treated plants that may have survived in more favorable conditions. Sub-shrub cover was quite low, so any difference in reduction between herbicides would be difficult to detect because prairie sagewort cover would rapidly approach 0. Further differentiation between herbicide treatments may also become more apparent in the following growing seasons if a more rapid recovery is observed in plots treated with 2,4-D compared to those treated with picloram.

Densities of seeded forbs were much lower than expected. This is most likely due to the unusually warm and dry conditions during the 2012 spring and summer (Fay and Schultz 2009). Mean annual precipitation ranges from 36 to 45 centimeters (USDA 1980), most of which typically falls during the growing season. The average precipitation from April 1 to September 30 in is approximately 28 cm (HPRCC 2012). Only 2.79 cm of precipitation were recorded at the site from 29 March 2012 to 9 June 2012 and it fell in numerous small events. By the end of June 2012 there were very few visible seedlings. Approximately 8.50 cm of rain fell at the site during the month of July, which resulted in most of the plant growth for the season. However, soil and air temperatures remained quite high, leaving little surface moisture available for the shallow-rooted seedlings. Mean seedling density ranged from about 0.29 to 2.36 seedlings per

m^2. The mixes were planted at a rate of 1000 PLS per m^2, making seedling emergence about 0.03% to 0.24%. Drilling broadleaf species into native pasture under more favorable growing conditions can produce higher seedling densities and emergence (Guretzky et al. 2004, Kunelius and Cambell 1984). When considering herbicide effect on density of seeded species, it is interesting to note that in all cases but one, picloram treatments yielded the lowest densities (Figures 1, 2 and 3). Though some of the means are not different (α=0.05), the pattern is quite consistent, indicating that picloram may have had a negative effect on seedling emergence as mentioned in the first hypothesis. The light application rate of picloram was used, in part, to avoid injuring planted seedlings the following year. It is possible that the unusually dry winter and spring conditions slowed the degradation process of the picloram, leaving enough residual in the soil to have a negative impact on seedling emergence. The Tordon® 22K label states that adequate soil moisture and temperature are important in reducing the risk of injury to planted species after spraying (Tordon® 22K Specialty Herbicide Specimen Label 2012). It also suggests waiting 60 to 90 days between spraying and planting grasses and waiting as much as 36 months before planting sensitive broadleaf crop species to avoid the possibility of decreasing crop yield (Tordon® 22K Specialty Herbicide Specimen Label 2012). Managers should carefully consider the use and application timing of a picloram treatment prior to seeding susceptible species.

CONCLUSION

Our research suggests that light rates of both 2,4-D LV4 and picloram were successful at reducing larkspur density. There is reason to believe that light rates of picloram might reduce larkspur abundance more effectively than light rates of 2,4-D LV4, especially considering growing conditions during this study and the larkspur cover results. Our picloram treatment reduced larkspur cover more effectively than our 2,4-D treatment at 1 of the 3 locations. Plots treated with a light rate of picloram had greater canopy cover of existing, non-target forbs at 2 of 3 locations compared to plots treated with a light rate of 2,4-D LV4, indicating that the light rate of picloram was a more selective treatment in this study. This is an important finding, given the potential benefits of maintaining forb diversity in rangeland plant communities. One note of caution is warranted regarding the use of light rates of picloram prior to seeding forbs. At 1 of the 3 locations, the density of seeded native or introduced forbs in plots treated with light rates of picloram were lower than the unsprayed plots, plots treated with light rates of 2,4-D LV4, or both. This provides evidence of a slight residual effect of picloram on the density of seeded forbs, however, further investigation is needed given the very low emergence values (much less than 1%) observed in unsprayed plots in this study.

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APPENDIX

Table 6. 2012 larkspur density means (in plants m^{-2}) and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The “means” procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The “Larkspur density data 2012- data in plants per sq meter” Excel spreadsheet was used to create Table 6. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean Larkspur Density (plants m^{-2})	Standard Deviation
1	Introduced	2,4-D	3	4.00	1.76
1	Introduced	picloram	3	1.56	0.51
1	Introduced	No Herbicide	3	4.56	0.51
1	Native	2,4-D	3	3.89	2.01
1	Native	picloram	3	2.67	0.67
1	Native	No Herbicide	3	3.00	0.88
1	No Seed	2,4-D	3	1.44	0.19
1	No Seed	picloram	3	3.56	1.26
1	No Seed	No Herbicide	3	4.67	4.09
2	Introduced	2,4-D	3	3.33	2.60
2	Introduced	picloram	3	2.56	1.17
2	Introduced	No Herbicide	3	4.44	1.54
2	Native	2,4-D	3	4.11	3.15
2	Native	picloram	3	2.78	2.36
2	Native	No Herbicide	3	5.78	4.11
2	No Seed	2,4-D	3	3.22	0.51
2	No Seed	picloram	3	2.00	0.33
2	No Seed	No Herbicide	3	5.78	1.90
3	Introduced	2,4-D	3	2.78	0.51
3	Introduced	picloram	3	2.22	1.39
3	Introduced	No Herbicide	3	3.00	0.58
3	Native	2,4-D	3	2.56	2.12
3	Native	picloram	3	1.78	0.77
3	Native	No Herbicide	3	2.56	1.17
3	No Seed	2,4-D	3	1.78	1.02
3	No Seed	picloram	3	1.56	1.58
3	No Seed	No Herbicide	3	2.44	0.77

Table 7. 2012 C3 grass % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Canopy cover 2012 compressed raw data- cover by species" Excel spreadsheet was used to create Table 7. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean C3 Grass Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	19.00	1.64
1	Introduced	No Herbicide	3	18.50	7.55
1	Introduced	picloram	3	10.67	4.42
1	Native	2,4-D	3	16.11	5.95
1	Native	No Herbicide	3	11.78	2.56
1	Native	picloram	3	10.44	10.38
1	No Seed	2,4-D	3	13.83	7.34
1	No Seed	No Herbicide	3	15.67	0.73
1	No Seed	picloram	3	19.78	6.73
2	Introduced	2,4-D	3	20.33	6.30
2	Introduced	No Herbicide	3	16.44	2.99
2	Introduced	picloram	3	17.83	2.40
2	Native	2,4-D	3	25.61	3.75
2	Native	No Herbicide	3	18.89	4.13
2	Native	picloram	3	22.39	2.59
2	No Seed	2,4-D	3	26.17	2.19
2	No Seed	No Herbicide	3	22.11	4.26
2	No Seed	picloram	3	20.44	5.43
3	Introduced	2,4-D	3	11.50	2.95
3	Introduced	No Herbicide	3	12.94	3.00
3	Introduced	picloram	3	13.94	2.99
3	Native	2,4-D	3	13.17	2.75
3	Native	No Herbicide	3	11.11	7.19
3	Native	picloram	3	11.67	1.73
3	No Seed	2,4-D	3	13.72	4.15
3	No Seed	No Herbicide	3	8.61	3.97
3	No Seed	picloram	3	14.50	2.59

Table 8. 2012 C3 grass-likes % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m^{-2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Canopy cover 2012 compressed raw data- cover by species" Excel spreadsheet was used to create Table 8. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean C3 Grass-likes Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	8.50	1.61
1	Introduced	No Herbicide	3	7.11	1.23
1	Introduced	picloram	3	7.89	2.22
1	Native	2,4-D	3	8.72	5.80
1	Native	No Herbicide	3	15.33	8.02
1	Native	picloram	3	8.11	1.83
1	No Seed	2,4-D	3	10.61	2.24
1	No Seed	No Herbicide	3	9.89	1.17
1	No Seed	picloram	3	7.44	1.51
2	Introduced	2,4-D	3	8.17	2.40
2	Introduced	No Herbicide	3	7.44	3.34
2	Introduced	picloram	3	5.33	3.37
2	Native	2,4-D	3	7.00	1.33
2	Native	No Herbicide	3	6.72	3.13
2	Native	picloram	3	6.22	1.90
2	No Seed	2,4-D	3	7.22	1.84
2	No Seed	No Herbicide	3	5.89	2.43
2	No Seed	picloram	3	5.06	1.83
3	Introduced	2,4-D	3	9.44	3.15
3	Introduced	No Herbicide	3	8.83	1.64
3	Introduced	picloram	3	8.06	1.40
3	Native	2,4-D	3	9.22	4.61
3	Native	No Herbicide	3	11.67	5.35
3	Native	picloram	3	6.944	2.37
3	No Seed	2,4-D	3	6.06	1.07
3	No Seed	No Herbicide	3	11.61	2.61
3	No Seed	picloram	3	4.83	1.45

Table 9. 2012 C4 grass % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Canopy cover 2012 compressed raw data- cover by species" Excel spreadsheet was used to create Table 9. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean C4 Grass Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	36.17	2.68
1	Introduced	No Herbicide	3	39.17	7.29
1	Introduced	picloram	3	46.00	9.96
1	Native	2,4-D	3	38.28	10.50
1	Native	No Herbicide	3	36.33	8.95
1	Native	picloram	3	45.33	14.87
1	No Seed	2,4-D	3	38.72	7.94
1	No Seed	No Herbicide	3	40.50	5.36
1	No Seed	picloram	3	41.61	4.51
2	Introduced	2,4-D	3	30.00	4.87
2	Introduced	No Herbicide	3	29.61	4.81
2	Introduced	picloram	3	31.67	3.49
2	Native	2,4-D	3	32.83	2.09
2	Native	No Herbicide	3	28.94	9.17
2	Native	picloram	3	32.00	3.91
2	No Seed	2,4-D	3	29.89	2.15
2	No Seed	No Herbicide	3	28.11	5.68
2	No Seed	picloram	3	32.61	9.58
3	Introduced	2,4-D	3	36.39	2.50
3	Introduced	No Herbicide	3	33.33	7.76
3	Introduced	picloram	3	35.22	4.22
3	Native	2,4-D	3	35.01	7.38
3	Native	No Herbicide	3	36.89	2.43
3	Native	picloram	3	34.94	4.35
3	No Seed	2,4-D	3	39.44	8.80
3	No Seed	No Herbicide	3	37.22	6.90
3	No Seed	picloram	3	42.78	10.06

Table 10. 2012 larkspur % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Canopy cover 2012 compressed raw data- cover by species" Excel spreadsheet was used to create Table 10. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean Larkspur Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	1.06	0.82
1	Introduced	No Herbicide	3	1.28	0.77
1	Introduced	picloram	3	0.17	0.17
1	Native	2,4-D	3	1.61	0.10
1	Native	No Herbicide	3	0.44	0.77
1	Native	picloram	3	0.33	0.44
1	No Seed	2,4-D	3	0.78	0.35
1	No Seed	No Herbicide	3	0.67	0.17
1	No Seed	picloram	3	0.28	0.19
2	Introduced	2,4-D	3	0	0
2	Introduced	No Herbicide	3	0.22	0.25
2	Introduced	picloram	3	0	0
2	Native	2,4-D	3	0	0
2	Native	No Herbicide	3	0.11	0.19
2	Native	picloram	3	0	0
2	No Seed	2,4-D	3	0.28	0.25
2	No Seed	No Herbicide	3	0.11	0.19
2	No Seed	picloram	3	0.06	0.10
3	Introduced	2,4-D	3	0.06	0.10
3	Introduced	No Herbicide	3	0	0
3	Introduced	picloram	3	0.17	0.17
3	Native	2,4-D	3	0	0
3	Native	No Herbicide	3	0	0
3	Native	picloram	3	0.06	0.10
3	No Seed	2,4-D	3	0.06	0.10
3	No Seed	No Herbicide	3	0.17	0.17
3	No Seed	picloram	3	0.06	0.10

Table 11. 2012 forb % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The “means” procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}.Footnote: The “Canopy cover 2012 compressed raw data- cover by species” Excel spreadsheet was used to create Table 11. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean Forb Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	0.78	1.06
1	Introduced	No Herbicide	3	2.44	0.82
1	Introduced	picloram	3	2.06	1.78
1	Native	2,4-D	3	0.83	0.58
1	Native	No Herbicide	3	1.72	1.60
1	Native	picloram	3	1.94	0.54
1	No Seed	2,4-D	3	0.78	0.48
1	No Seed	No Herbicide	3	1.67	1.00
1	No Seed	picloram	3	0.56	0.42
2	Introduced	2,4-D	3	0.33	0.17
2	Introduced	No Herbicide	3	6.11	1.36
2	Introduced	picloram	3	3.06	0.67
2	Native	2,4-D	3	0.11	0.10
2	Native	No Herbicide	3	8.94	4.24
2	Native	picloram	3	2.67	0.60
2	No Seed	2,4-D	3	0.17	0.17
2	No Seed	No Herbicide	3	5.50	1.48
2	No Seed	picloram	3	3.06	2.38
3	Introduced	2,4-D	3	0.50	0.50
3	Introduced	No Herbicide	3	2.06	1.17
3	Introduced	picloram	3	1.56	0.92
3	Native	2,4-D	3	0.33	0.44
3	Native	No Herbicide	3	3.33	2.68
3	Native	picloram	3	1.56	1.08
3	No Seed	2,4-D	3	0.17	0.29
3	No Seed	No Herbicide	3	3.83	2.96
3	No Seed	picloram	3	2.67	2.32

Table 12. 2012 sub-shrub % cover (absolute) means and standard deviations by treatment and location. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS $m-^{2}$. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Canopy cover 2012 compressed raw data- cover by species" Excel spreadsheet was used to create Table 12. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean Sub-Shrub Cover (%)	Standard Deviation
1	Introduced	2,4-D	3	0	0
1	Introduced	No Herbicide	3	0.28	0.35
1	Introduced	picloram	3	0	0
1	Native	2,4-D	3	0	0
1	Native	No Herbicide	3	0.39	0.54
1	Native	picloram	3	0	0
1	No Seed	2,4-D	3	0	0
1	No Seed	No Herbicide	3	0.17	0.17
1	No Seed	picloram	3	0	0
2	Introduced	2,4-D	3	0	0
2	Introduced	No Herbicide	3	0.67	0.67
2	Introduced	picloram	3	0.06	0.10
2	Native	2,4-D	3	0	0
2	Native	No Herbicide	3	1.33	0.50
2	Native	picloram	3	0	0
2	No Seed	2,4-D	3	0	0
2	No Seed	No Herbicide	3	1.06	0.98
2	No Seed	picloram	3	0	0
3	Introduced	2,4-D	3	0.11	0.19
3	Introduced	No Herbicide	3	0.11	0.19
3	Introduced	picloram	3	0	0
3	Native	2,4-D	3	0	0
3	Native	No Herbicide	3	0.50	0.87
3	Native	picloram	3	0	0
3	No Seed	2,4-D	3	0	0
3	No Seed	No Herbicide	3	0	0
3	No Seed	picloram	3	0	0

Table 13. 2012 seedling density means (seedlings plot^{-1}) and standard deviations of planted forbs by treatment and location. All plots were 3 X 10 meters. The plot values are averages of six sub-samples. The number of samples (N = 3) comes from having 3 blocks for each of 3 locations. The blocks were comprised of 9 plots (one for each treatment). The "means" procedure in SAS 9.3 was used to estimate means and standard deviations. The picloram was applied at 0.28 kg ha^{-1} a.i. and the 2,4-D at 2.96 kg ha^{-1} a.i. The native seed mixture was comprise of 11 species and sown at 1000 PLS m-^{2}. The introduced seed mixture was comprised of 10 species and sown at 1000 PLS m^{-2}. Footnote: The "Seedling density 2012 raw data- Import data" Excel spreadsheet was used to create Table 13 and density values include all species planted. All data were collected at the CSU Maxwell Ranch in northern Colorado.

Location	Seed Mixture	Herbicide	N	Mean Seedling Density of Planted Forbs (seedlings plot^{-1})	Standard Deviation
1	Introduced	2,4-D	3	50.67	12.86
1	Introduced	No Herbicide	3	66.00	16.37
1	Introduced	picloram	3	70.67	41.30
1	Native	2,4-D	3	80.50	54.42
1	Native	No Herbicide	3	29.00	1.42
1	Native	picloram	3	28.67	26.10
2	Introduced	2,4-D	3	25.33	3.06
2	Introduced	No Herbicide	3	24.67	14.19
2	Introduced	picloram	3	14.00	5.29
2	Native	2,4-D	3	20.00	7.21
2	Native	No Herbicide	3	16.67	2.31
2	Native	picloram	3	10.67	2.31
3	Introduced	2,4-D	3	29.33	15.28
3	Introduced	No Herbicide	3	33.33	20.03
3	Introduced	picloram	3	14.67	4.16
3	Native	2,4-D	3	16.67	6.11
3	Native	No Herbicide	3	11.33	3.06
3	Native	picloram	3	8.67	2.31