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Authors: Greet, Brandon J., Meador, Brian A., and Kniss, Andrew R.

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## Response of *Delphinium occidentale* and Associated Vegetation to Aminocyclopyrachlor<sup>☆</sup>



Brandon J. Greet<sup>a,1</sup>, Brian A. Mealor<sup>b,c,\*</sup>, Andrew R. Kniss<sup>c</sup>

<sup>a</sup> Rancher, Greet Ranch, Ten Sleep, WY 82442, USA

<sup>b</sup> Director, Sheridan Research and Extension Center, Sheridan, WY 82801, USA

<sup>c</sup> Associate Professor, Department of Plant Sciences, University of Wyoming, Laramie, WY 82071, USA

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### ABSTRACT

Duncecap larkspur (*Delphinium occidentale* [Wats.] Wats.) is an important perennial weed on high-elevation rangelands because of significant cattle losses due to toxic alkaloids in the plant. Aminocyclopyrachlor was evaluated at six rates between 17.5 and 315 g ai · ha<sup>-1</sup> for larkspur control alone, and in combination with chlorsulfuron or metsulfuron, at a high-elevation location in Wyoming. Aminocyclopyrachlor-containing treatments were compared with 1120 g ai · ha<sup>-1</sup> picloram and 63 g ai · ha<sup>-1</sup> metsulfuron-methyl. Herbicides were applied to two sites in a randomized complete block design with four replicates each on June 18, 2010 at the first site and June 28, 2011 at the second site. Larkspur mortality, plant species richness, vegetation cover, and grass biomass data were collected 1 yr after treatment. Cover data were used to calculate vegetation diversity and to assess changes in species composition associated with herbicide application. A four-parameter log-logistic model was used to evaluate larkspur mortality, species richness, and vegetation cover in response to aminocyclopyrachlor rate. Ninety-percent larkspur reduction was obtained with aminocyclopyrachlor applied alone at rates of 168–303 g ha<sup>-1</sup>, depending on site. Mixture of aminocyclopyrachlor plus chlorsulfuron at a 2.5:1 ratio required 102–127 g ha<sup>-1</sup> of aminocyclopyrachlor to reduce larkspur 90%. Aminocyclopyrachlor plus metsulfuron was the most effective herbicide combination for larkspur control of those we evaluated, requiring 47 + 15 g ha<sup>-1</sup>, respectively, to reduce larkspur 90%. Species richness and diversity were reduced by herbicide rates required to effectively control larkspur. Graminoid biomass was not significantly impacted by herbicide or rate. Aminocyclopyrachlor may be a useful tool for duncecap larkspur control. Addition of chlorsulfuron or metsulfuron to aminocyclopyrachlor increased larkspur control but had a greater impact on associated nontarget vegetation.

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### Introduction

Larkspur is the one of the most important weeds on high-elevation rangelands in the western United States (Ralphs et al., 1988) due to livestock poisoning by toxic alkaloids. Duncecap larkspur (*Delphinium occidentale* [Wats.] Wats.) is included in the group of tall larkspurs (Dorn, 1992). Fatal livestock poisoning can be as high as 15% but usually ranges from 2% to 5% of cattle that are grazing on larkspur-infested areas (Li et al., 2002). Management of this weed is economically important for ranchers in infested areas (Nielsen et al., 1994).

Duncecap larkspur is a native of subalpine plant communities (Ralphs, 1995). It is a perennial growing 1–2 m in height with hollow stems and large, lobed leaves (Whitson et al., 2002). Duncecap larkspur (hereafter referred to as larkspur) has sparse, short, white hairs on the upper part of the stem and inflorescence. The flowers are small, spur shaped, and blue and can sometimes have a tinge of white (Gardner et al., 2002). There can be as many as 150 stems on a single plant growing from buds on the root crown each year (Ralphs and Gardner, 2003).

Larkspurs are relatively palatable to all livestock (Pfister et al., 1988), but cattle are more susceptible to larkspur poisoning compared with most other livestock (Majak, 1993). Cattle must eat approximately 0.7% of their body weight in actively growing larkspur to be fatally poisoned (Pfister et al., 1994). The amount of larkspur required to cause death depends on stage of growth, location, year, other forages consumed, and the size and condition of the animal. Death is usually attributed to paralysis of respiratory muscles, but there may be complications from bloat or inhalation of digesta that may also cause death in some

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\* Correspondence: Brian Mealor, Sheridan, WY, USA.

E-mail address: [bamealor@uwyo.edu](mailto:bamealor@uwyo.edu) (B.A. Mealor).

<sup>1</sup> At the time of research, Brandon Greet was a Master's Student, Department of Plant Sciences, University of Wyoming, Laramie, WY 82071, USA.

situations (Stegelmeier et al., 2003). Ingestion rate, rate of absorption, metabolism, and the elimination of alkaloids are the primary determining factors of susceptibility (Manners et al., 1992).

Modifications of grazing management can reduce the amount of larkspur poisonings that occur but will not eliminate them altogether (Holecheck, 2002). Larkspur can be managed with fencing and herding (Knight and Walter, 1994), but these practices require large amounts of time and labor. Chemical control is an effective and economically feasible method of controlling larkspur (Nielsen et al., 1994; Ralphs, 1995). Metsulfuron methyl (MSM), picloram, and glyphosate are the standard herbicides used to treat larkspur infestations. MSM and picloram may indirectly contribute to invasion by other undesirable forbs following treatment (Ralphs, 1995). Land managers with multiple-use goals, such as balancing habitat diversity with livestock production, may face a potential tradeoff between reducing livestock poisoning and potentially reducing plant diversity on treated sites when using broad-spectrum broadleaf herbicides to control poisonous plants.

Aminocyclopyrachlor (AMCP) is a new synthetic auxin herbicide that effectively controls many perennial broadleaf weeds. It is expected that commercial formulations of AMCP mixed with chlorsulfuron (CHLR) and MSM will soon be labeled for rangeland and pasture use. The ability of AMCP to control larkspur could provide an additional weed control tool to land managers. Success does not solely depend on larkspur control, however, but also on the impacts to associated nontarget vegetation. To address these issues, we initiated a field experiment at two locations in 2 different years. The objectives of this study were to evaluate AMCP alone and then in combination with CHLR and MSM for larkspur control and effects on nontarget plants. More specifically, this research sought to address the following questions: 1) How effective is AMCP, alone and in combination with other herbicides, for controlling larkspur? 2) What are the impacts of AMCP, applied alone or in combination with other herbicides, on nontarget vegetation characteristics? and 3) Is there an optimum herbicide rate that provides acceptable larkspur control with minimal impacts on nontarget vegetation?

## Methods

AMCP<sup>2</sup> was evaluated for larkspur control at two high-elevation (2432 m) sites in the Big Horn Mountains of Wyoming. Both sites had diverse vegetation associated with larkspur (Table 1). AMCP was used alone at rates of 18, 35, 70, 140, and 315 g ai · ha<sup>-1</sup>, applied at the same rate plus CHLR<sup>3</sup> at 7, 14, 28, 56, and 126 g ai · ha<sup>-1</sup> and at the same rate plus MSM<sup>4</sup> at 6, 11, 22, 45, and 101 g ai · ha<sup>-1</sup>. Herbicide ratios were chosen to match precommercial formulations of AMCP + CHLR and AMCP + MSM (DuPont, 2011a; DuPont, 2011b). These treatments were compared with the recommended rates of picloram (1120 g ai · ha<sup>-1</sup>) and MSM (63 g ai · ha<sup>-1</sup>) because these are the two herbicides generally used for the control of tall larkspur (Whitson et al., 1992; Ralphs et al., 2003). A nontreated control was also included.

All treatments contained a nonionic surfactant at 0.25% v · v<sup>-1</sup>, with the exception of picloram, to which no surfactant was added. Treatments were replicated four times in 3 × 12 m plots set in a randomized complete block design. Herbicides were applied in a total volume of 187 L ha<sup>-1</sup> with a CO<sub>2</sub>-pressurized sprayer and 3-m boom with six 8002 nozzles. Herbicides were applied on June 18, 2010 at the first site (Site 1) and June 28, 2011 at the second site (Site 2), when larkspur plants were in the vegetative stage ranging from newly emerged to approximately 20 cm in height.

Weather data for both sites were taken from a nearby weather station (lat: 43.8111, long: -107.3652). The year of 2010 had a total precipitation of 280 mm, with 159 mm of this falling in the first

**Table 1**

List of primary species associated with Duncceap larkspur (*Delphinium occidentale*) on two field sites in the Bighorn Mountains of north central Wyoming

Common name	Species	Growth form
Mountain brome	<i>Bromus carinatus</i> H. & A. <i>Achnatherum nelsonii</i> (Scribn.)	Grass
Subalpine needlegrass	Barkworth subsp. <i>Nelsonii</i>	Grass
Smooth brome	<i>Bromus inermis</i> var. <i>inermis</i> <i>Pascopyrum smithii</i> (Rydb.)	Grass
Western wheatgrass	Barkworth & D.R. Dewey	Grass
Common timothy	<i>Phleum pratense</i> L.	Grass
Alpine timothy	<i>Phleum alpinum</i> L.	Grass
Kentucky bluegrass	<i>Poa pratensis</i> L.	Grass
Oniongrass	<i>Melica bulbosa</i> Geyer ex Port. & Coult.	Grass
Idaho fescue	<i>Festuca idahoensis</i> Elmer	Grass
Sedges	<i>Carex</i> spp.	Grass-like
Rushes	<i>Juncus</i> spp.	Grass-like
Big sagebrush	<i>Artemisia tridentata</i> Nutt.	Shrub
False dandelion	<i>Agoseris glauca</i> (Pursh) Raf.	Forb
Common dandelion	<i>Taraxacum officinale</i> Weber	Forb
Subalpine fleabane	<i>Erigeron peregrinus</i> (Banks ex Pursh) Greene	Forb
Yellow owl's-clover	<i>Orthocarpus luteus</i> Nutt.	Forb
Silver lupine	<i>Lupinus argenteus</i> Pursh	Forb
Tower mustard	<i>Turritis glabra</i> L.	Forb
Slender cinquefoil	<i>Potentilla gracilis</i> Douglas ex Hook.	Forb
Smallflower columbine	<i>Aquilegia brevistyla</i> Hook.	Forb
Bluebell bellflower	<i>Campanula rotundifolia</i> L.	Forb
Northern bedstraw	<i>Galium boreale</i> L.	Forb
Small-leaf angelica	<i>Angelica pinnata</i> S. Watson	Forb
Bladder campion	<i>Silene vulgaris</i> (Moench) Garcke	Forb
American vetch	<i>Vicia americana</i> Muhl. Ex Willd.	Forb
Virginia strawberry	<i>Fragaria virginiana</i> Duchesne	Forb
Spreading groundsmoke	<i>Gayophytum diffusum</i> Torr. & A. Gray	Forb
Rough false pennyroyal	<i>Hedeoma hispida</i> Pursh	Forb
Geyer larkspur	<i>Delphinium geyeri</i> Greene	Forb
Common yarrow	<i>Achillea millefolium</i> L.	Forb
Goldenpea	<i>Thermopsis rhombifolia</i> Nutt. Ex Richards. <i>Geranium viscosissimum</i> Fisch. &	Forb
Sticky purple geranium	C.A. Mey. ex C.A. Mey	Forb
Western salsify	<i>Tragopogon dubius</i> Scop.	Forb

6 months. The mean maximum temperature was 14.8°C, and the mean minimum temperature was -3.4°C. In the first 6 months of 2011, precipitation totaled 254 mm. Total precipitation for 2011 was 347 mm. Maximum temperature was 13.9°C, and the mean minimum temperature was -3.8°C. In the first 6 months of 2012, precipitation was 131 mm (NOAA, 2012). This was less than half of the precipitation of 2011. Site 1 was sprayed with AMCP alone and with CHLR, as well as picloram and MSM treatments and a nontreated control. Site 2 included all the treatments in the first site and the multiple rates of AMCP with MSM. The first block was excluded from analysis at Site 2 because of experimental implementation error.

Larkspur stalk reduction, vegetation cover, and species diversity were observed 30 days after treatment (DAT) and 1 yr after treatment (YAT). Data from 1 YAT are presented here. Larkspur stalks counted in a 1-m wide belt transect down the center of each plot. Stalks within this transect were classified as "dead" if they were completely desiccated or otherwise classified as "living." Larkspur stalk mortality was then calculated by dividing the number of dead stalks by the total number of stalks (living plus dead) and multiplying by 100. The number of living larkspur stalks counted 1 YAT was divided by the total larkspur stalks counted 30 DAT, subtracted from 1, then multiplied by 100 to obtain larkspur mortality 1 YAT. This method of evaluating stalk reduction accounted for natural senescence of stalks as observed in nontreated plots.

Species richness was evaluated by counting the total number of species rooted within each plot. Canopy cover was estimated using digital images. Three nadir-oriented images of the vegetation were taken 1.5 m apart along a central transect in each plot. Vegetation cover was classified into different categories using SamplePoint

<sup>2</sup> DPX-MAT28, Dupont, Wilmington, DE.

<sup>3</sup> Perspective, Dupont, Wilmington, DE.

<sup>4</sup> Streamline, Dupont, Wilmington, DE.

Measurement software (Booth et al., 2006) from the US Department of Agriculture's Agricultural Research Service (USDA, 2011). One hundred points per image were categorized as a specific plant species, litter, or bare ground. The Shannon-Weaver diversity index (Lehmann et al., 2002) was calculated (Equation [1]) on the basis of cover data. The Shannon-Weaver index ( $H$ ) is calculated using species richness ( $s$ ) and the proportional abundance of the species ( $p$ ). Individual species are represented by  $i$ .

$$H = \sum_{i=1}^s p_i \ln p_i \quad [1]$$

The effective number of species ( $ENS$ ) was then calculated using Equation 2 (Jost, 2006):

$$ENS = e^H \quad [2]$$

Shannon's equitability ( $E_H$ ) was used to estimate species evenness from the same cover data, by dividing  $H$  by the natural logarithm of  $s$  (Equation 3).

$$E_H = \frac{H}{\ln s} \quad [3]$$

A log-logistic model (Seefeldt et al., 1995) was fit to raw data to evaluate all variables measured in response to rates of AMCP excluding grass dry weights (see Equation 2). The  $b$  parameter in Equation 2 is the slope of the regression curve at the inflection point. The  $c$  parameter is the minimum asymptote of the regression curve. In this study the  $c$  parameter was constrained to 0 for some analyses, where minimum responses cannot reasonably be negative (as with species diversity).  $d$  is the maximum asymptote, which was constrained to a maximum of 100 for larkspur stalk reduction because injury or control > 100% is not meaningful.  $I_{50}$  is the dose of herbicide required to achieve 50% of the response. Results of picloram and MSM application were used as references for the success of AMCP, AMCP + CHLR, and AMCP + MSM.

$$f(x) = c + (d-c) / \left( 1 + (x/I_{50})^b \right) \quad [4]$$

Using Equation 4, the left-bound asymptote is an estimate of the response variable when no herbicide is applied. For response variables that increase in response to herbicide (like larkspur mortality and relative grass cover), the  $c$  parameter is the estimate of the nontreated, whereas the  $d$  parameter is the estimate of the nontreated for decreasing response variables (like relative forb cover and species diversity). The herbicide dose required to reduce larkspur stalk density by 90% ( $ED_{90}$ ) was estimated for each site. The  $ED_{90}$  (or effective dose) is expressed in g aminocyclopyrachlor  $ha^{-1}$  and provides an estimate of the amount of aminocyclopyrachlor required to provide an economically acceptable level of larkspur control. Herbicide rates less than the  $ED_{90}$  would be expected to provide < 90% reduction in larkspur stalk density.

## Results

### Larkspur Control

**Site 1.** AMCP rate ( $P < 0.0001$ ) and herbicide ( $P = 0.0003$ ) affected larkspur stalk reduction (Fig. 1A). The  $d$  parameter indicates that AMCP alone and AMCP + CHLR could potentially result in up to 97% stalk mortality (Table 2). The estimated  $ED_{90}$  for stalk mortality was 168 g ai  $\cdot$  ha $^{-1}$  of AMCP when applied alone and 102 g ai  $\cdot$  ha $^{-1}$  AMCP plus 41 g ai  $\cdot$  ha $^{-1}$  CHLR (see Table 2). This 102 + 41 g ai  $\cdot$  ha $^{-1}$  rate was predicted to result in 90% larkspur mortality and, therefore, is used as a standard of reference for additional response variables at Site 1. The recommended rate of picloram yielded 41% stalk reduction, and MSM applied alone at 63 g ai  $\cdot$  ha $^{-1}$  caused 100% stalk reduction.

**Site 2.** Larkspur stalk reduction increased with AMCP rate ( $P < 0.0001$ ) but was not changed by addition of CHLR or MSM. The log-logistic model shows increased control with increasing rate to an estimated maximum of 100% control for all AMCP-containing treatments (Fig. 1B). The estimated  $ED_{90}$  for stalk mortality was 303 g ai  $\cdot$  ha $^{-1}$  of AMCP when applied alone, 127 g ai  $\cdot$  ha $^{-1}$  AMCP plus 51 g ai  $\cdot$  ha $^{-1}$  CHLR, and only 47 g ai  $\cdot$  ha $^{-1}$  when mixed with 15 g ai  $\cdot$  ha $^{-1}$  MSM (see Table 2). Picloram and MSM applied alone at their recommended rates resulted in 81% and 82% stalk mortality, respectively.

### Total Vegetation

**Site 1.** Total vegetation cover declined with increasing AMCP rate ( $P < 0.0001$ ), litter increased with increasing AMCP rate ( $P < 0.001$ ), and bare ground was not affected by herbicide or rate. Mean vegetation cover in the nontreated control was 46%. If AMCP were applied at the larkspur  $ED_{90}$  rate of at 102 g ai  $\cdot$  ha $^{-1}$ , total vegetation cover was estimated to be 46% (data not shown). When CHLR was added, vegetation cover was reduced to 44% at the same rate of AMCP. Mean vegetation cover when MSM and picloram were applied at the recommended rates was 41% and 55%, respectively.

**Site 2.** Unlike at Site 1, we did not detect any effects of herbicide, rate, or a herbicide-by-rate interaction for total vegetation cover, litter, or bare ground ( $P > 0.05$ ). Mean vegetation cover of nontreated plots was 50%.

### Grass Responses

**Site 1.** Relative grass cover increased with AMCP rate ( $P = 0.0001$ ) and differed among herbicides ( $P = 0.004$ ; Fig. 1C). Relative grass cover in nontreated plots was 61–62%, as estimated by the  $c$  parameter (see Table 2). Estimated relative grass cover from the log-logistic model (Fig. 1C) was 85% at a rate of 102 g ai  $\cdot$  ha $^{-1}$  of AMCP alone and 99% at the equivalent rate with CHLR added at 41 g ai  $\cdot$  ha $^{-1}$ . MSM alone increased relative grass cover to 83%. Picloram increased relative grass cover to 91%. To further assess the change in grasses as a functional group, grass dry weight data were analyzed using a one-way analysis of variance (ANOVA), but no differences were observed among the treatments ( $P = 0.26$ ).

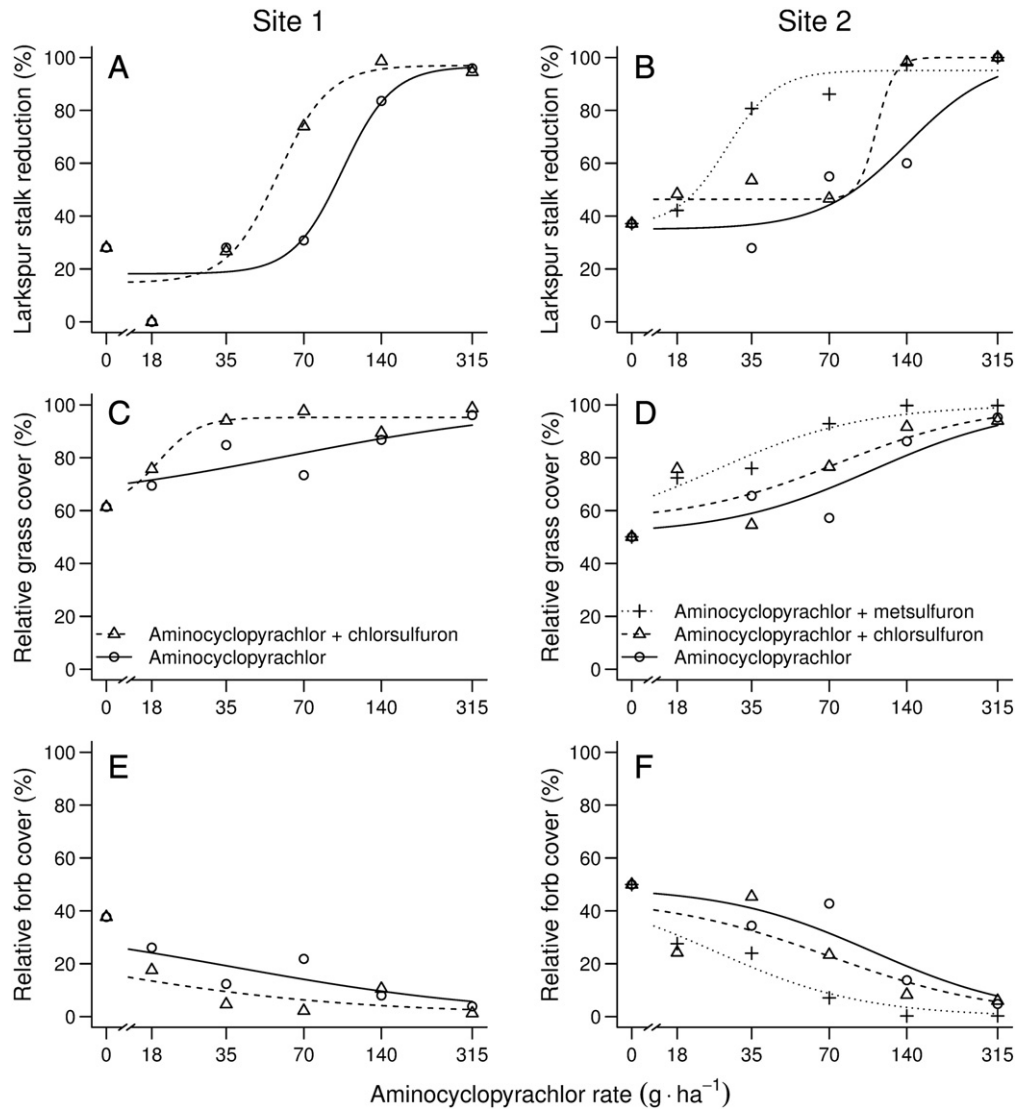
**Site 2.** Relative grass cover showed large increases, differing by AMCP rate ( $P < 0.0001$ ) and herbicide ( $P = 0.04$ ). Mean relative grass cover of nontreated plots was 51–56%, as estimated by the  $c$  parameter (see Table 2). On the basis of the log-logistic model, the  $ED_{90}$  rate increased relative grass cover to 93% when AMCP was applied alone. The  $ED_{90}$  rate for stalk mortality of AMCP + CHLR increased relative grass cover to 91%. AMCP + MSM applied at the  $ED_{90}$  rate further increased relative grass cover to 99%. The standards of picloram and MSM increased relative grass cover to 86% and 96%, respectively. As with Site 1, grass dry weight was analyzed using a one-way ANOVA, but no differences were observed among the treatments ( $P = 0.45$ ).

### Forb Responses

**Site 1.** Relative forb cover declined with increasing herbicide rate ( $P = 0.001$ ) and differed among herbicides ( $P = 0.004$ ). All herbicides reduced forb cover, and the magnitude of reduction depended on application rate for AMCP-containing herbicides. Relative forb cover in the nontreated control was 37–38%, as estimated by the  $d$  parameter. AMCP applied at 102 g ai  $\cdot$  ha $^{-1}$  was estimated to reduce relative forb cover to 12% (Fig. 1E, see Table 2). The  $ED_{90}$  dose for stalk reduction of AMCP + CHLR reduced relative forb cover to 4%. MSM and picloram reduced relative forb cover to 17% and 2%, respectively.

**Site 2.** Relative forb cover, as a percentage of total vegetation, was significantly affected by AMCP rate ( $P < 0.0001$ ) and herbicide ( $P = 0.04$ ). Nontreated plots had a mean relative forb cover of





**Figure 1.** Effect of aminocyclopyrachlor alone or in mixture with chlorsulfuron or metsulfuron on **A** and **B**, duncecap larkspur stalk numbers; **C** and **D**, relative grass cover; and **E** and **F**, relative forb cover 1 yr after treatment (YAT) at two high-elevation rangeland sites in the Bighorn Mountains of Wyoming, United States. **Panels A, C, and E** present data from Site 1. **Panels B, D, and F** present data from Site 2. Parameter estimates are in Table 2.

45–49%, as estimated by the  $d$  parameter. The log-logistic model for relative forb cover estimated a decrease to 4% relative forb cover at a rate of 284 g ai · ha<sup>-1</sup> of AMCP alone. By comparison, AMCP + CHLR and AMCP + MSM reduced relative forb cover to 6% and 0%, respectively, at similar rates. Picloram, by comparison, only reduced relative forb cover to 14%. MSM alone reduced relative forb cover to 4%.

#### Species Diversity

**Site 1.** Species richness in the absence of herbicide, as estimated by the  $d$  parameter, was estimated at 12.6 (95% confidence interval 11.6–13.6). Species richness decreased as herbicide rate increased ( $P = 0 < 001$ , see Fig. 2A) and was different depending on herbicide applied ( $P = 0 < 001$ ), but the rate by herbicide interaction ( $P = 0.051$ ) was marginally significant. Picloram and MSM reduced species richness to 8 and 9.8, respectively. At 102 g ai · ha<sup>-1</sup>, AMCP reduced species richness to 11.1. Adding 41 g ai · ha<sup>-1</sup> of CHLR further reduced species richness to 7.0; a reduction of 5 species at the rate of AMCP + CHLR was estimated to cause a 90% reduction in larkspur stalks.

To further investigate treatment effects on diversity of associated nontarget vegetation, the proportional contribution of each species to

the plant community, or the evenness component of diversity, was also included. The effective number of species ( $ENS$ ) and Shannon's equitability ( $E_H$ ) were calculated using cover data. Treatments containing chlorsulfuron reduced evenness compared with the nontreated control.

A decline in diversity was observed as AMCP rate increased ( $P = 0 < 001$ , see Fig. 2C). Herbicides differentially affected species diversity ( $P < 0.001$ ), but the herbicide by rate interaction was not significant ( $P = 0.25$ ). Mean  $ENS$  diversity in nontreated plots was 6.9. At 102 g ai · ha<sup>-1</sup> of AMCP alone,  $ENS$  was estimated to be 6.3. The  $ED_{90}$  dose for stalk mortality of AMCP + CHLR (102 plus 41 g ai · ha<sup>-1</sup>) reduced  $ENS$  to 3.8. MSM yielded  $ENS$  values of 6.1, and picloram yielded an  $ENS$  of 4.9.

**Site 2.** Species richness decreased in response to AMCP rate ( $P = 0.003$ ) but did not differ significantly depending on herbicide ( $P = 0.075$ ; see Fig. 2B). At the  $ED_{90}$  rate for larkspur stalk mortality, AMCP alone, AMCP + CHLR, and AMCP + MSM decreased species richness to 6.5, 6.3, and 6.8, respectively, from the nontreated control estimate of 11.2. Picloram resulted in a species richness of 6.3, and MSM resulted in a species richness of 6.8.

Species evenness was not significantly affected by any herbicide treatment or rate at this site ( $P > 0.303$ ). Shannon's equitability ( $E_H$ )

**Table 2**  
Parameter estimates, observable effect doses, and ED<sub>90</sub> values (with standard errors in parentheses) for reduction in duncecap larkspur stalks, 1 yr after treatment with aminocyclopyrachlor applied alone or in mixture with chlorsulfuron or metsulfuron. Data fit to a 4-parameter log-logistic model (Equation 2)

	Parameter (std error) <sup>1,2</sup>				ED <sub>90</sub> <sup>3</sup>
	<i>b</i>	<i>c</i>	<i>d</i>	<i>I</i> <sub>50</sub>	
<i>Site 1: larkspur reduction</i>					
Aminocyclopyrachlor	−4.5 (3.2)	18 (8.1)	97 (14.1)	99 (23.7)	168 (78)
Aminocyclopyrachlor + chlorsulfuron	−3.3 (1.7)	0 (18.5)	97 (10.7)	48 (12.0)	102 (42)
<i>Site 2: larkspur reduction</i>					
Aminocyclopyrachlor	−1.8 (1.6)	22 (42.5)	100 (23.1)	107 (109.1)	303 (255)
Aminocyclopyrachlor + chlorsulfuron	−19 (205.7)	49 (7.0)	100 (12.1)	118 (225.9)	127 (148)
Aminocyclopyrachlor + metsulfuron	−4.5 (4.8)	36 (12.0)	95 (7.7)	28 (7.2)	47 (26)
<i>Site 1: relative grass cover</i>					
Aminocyclopyrachlor	−0.9 (0.55)	62 (6.3)	100 (17.8)	62 (87.2)	—
Aminocyclopyrachlor + chlorsulfuron	−5.6 (9.2)	61 (6.0)	95 (3.5)	19 (3.4)	—
<i>Site 2: relative grass cover</i>					
Aminocyclopyrachlor	−1.5 <sup>4</sup>	51 (5.4)	100 (10.0)	104 (44.7)	—
Aminocyclopyrachlor + chlorsulfuron	−1.5 <sup>4</sup>	56 (9.3)	100 (15.4)	76 (66.2)	—
Aminocyclopyrachlor + metsulfuron	−1.5 (0.64)	51 (5.9)	100 (5.6)	25 (8.7)	—
<i>Site 1: relative forb cover</i>					
Aminocyclopyrachlor	0.8 (0.76)	0 (19.3)	37 (6.6)	37 (59.3)	—
Aminocyclopyrachlor + chlorsulfuron	0.8 (5.94)	0.8 (41.9)	38 (6.5)	8 (33.4)	—
<i>Site 2: relative forb cover</i>					
Aminocyclopyrachlor	1.5 (0.67)	0 <sup>5</sup>	49 (6.3)	104 (31.8)	—
Aminocyclopyrachlor + chlorsulfuron	1.3 (2.27)	0 <sup>5</sup>	45 (19.7)	71 (79.8)	—
Aminocyclopyrachlor + metsulfuron	1.5 (0.51)	0 <sup>5</sup>	49 (5.7)	25 (7.3)	—

<sup>1</sup> Parameters are described in Equation [1].

<sup>2</sup> Observable effect dose (OED) is the rate of aminocyclopyrachlor at which the estimated response exceeds the 95% confidence interval of the left-bound asymptote.

<sup>3</sup> ED<sub>90</sub> is the dose of aminocyclopyrachlor required to reduce larkspur stalk density 90%.

<sup>4</sup> The *b* parameter was fixed at the same value as aminocyclopyrachlor plus metsulfuron to aid in model fitting; the 4-parameter log-logistic model would not converge.

<sup>5</sup> The *c* parameter in the 4-parameter log-logistic model was equal to 0, so a 3-parameter model was fit, setting *c* = 0.

for herbicides ranged from 0.71 to 0.81, while the nontreated control had an  $E_H$  value of 0.74. As measured by the effective number of species, though, diversity decreased in response to herbicide rate ( $P = 0.011$ ), but decreases were similar with respect to herbicide ( $P = 0.22$ ) and the rate by herbicide interaction ( $P = 0.505$ ). Not applying herbicides resulted in an average *ENS* of 6.5. On the basis of the log-logistic model, the ED<sub>90</sub> rate for stalk mortality of 303 g ai · ha<sup>−1</sup> reduced *ENS* to 4.1 when using AMCP alone. ED<sub>90</sub> rates of AMCP + CHLR and AMCP + MSM reduced *ENS* to 4.0 and 4.5, respectively. Picloram and MSM yielded *ENS* values of 4.5 and 4.0, respectively.

#### Individual Species Responses

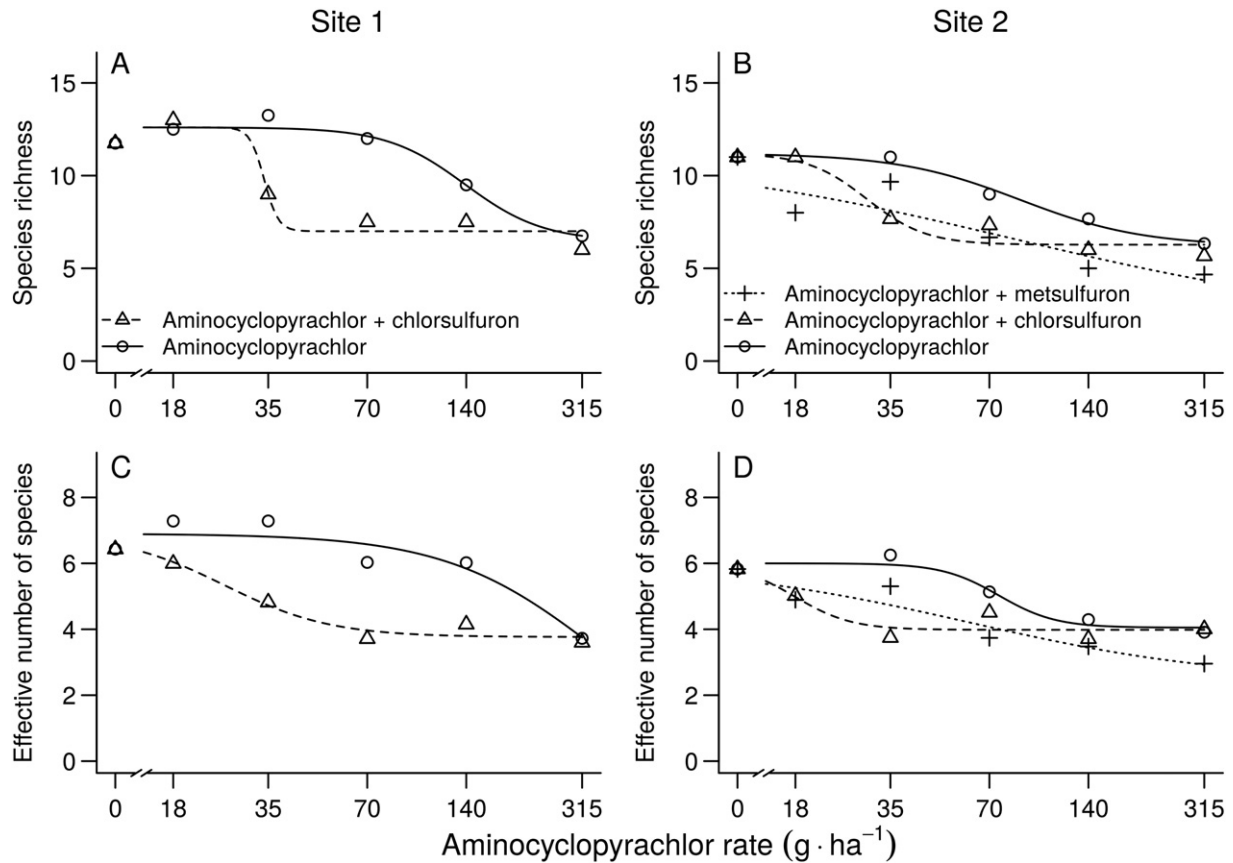
Although relative cover was evaluated by individual species, herbicide and rate effects on graminoid cover varied by species and site,

with few consistently identifiable responses in this study. Responses of *Achnatherum nelsonii*, *Bromus inermis*, *Bromus marginatus*, *Festuca idahoensis*, and *Carex* spp. to herbicide treatments can be found in supplementary materials (Fig. S1). In general, few responses were consistent between sites. For example, sedges decreased in response to AMCP at Site 1 but increased at Site 2. The most likely explanation for these inconsistencies is that AMCP did not have strong direct effects on these species. Rather, changes in the plant community may have been primarily driven by environmental conditions and competitive interactions, which were indirectly influenced by herbicide applications. Interactions appeared to depend on the initial composition of the plant community, which differed between sites. For example, sedges accounted for more than 12% of vegetation cover in the untreated control plots at Site 1, compared with less than 4% at Site 2. Such differences between sites made generalizations about direct species-level impacts

**Table 3**  
Parameter estimates for species richness and diversity 1 yr after application of aminocyclopyrachlor alone or in mixture with chlorsulfuron or metsulfuron. Data fit to a log-logistic model (Equation 2)

	Parameter (std error) <sup>1</sup>			
	<i>b</i>	<i>c</i>	<i>D</i>	<i>I</i> <sub>50</sub>
<i>Site 1: Species richness</i>				
Aminocyclopyrachlor	3.6 (3.1)	6.5 (1.5)	12.6 (0.48)	139 (33)
Aminocyclopyrachlor + chlorsulfuron	20.0 (612)	7.0 (0.54)	12.6 (0.48)	34 (31)
<i>Site 2: Species richness</i>				
Aminocyclopyrachlor	2.3 (2.8)	6.2 (2.4)	11.2 (1.3)	86 (66)
Aminocyclopyrachlor + chlorsulfuron	4.7 (10.0)	6.3 (0.96)	11.2 (1.3)	30 (9)
Aminocyclopyrachlor + metsulfuron	0.79	1.9 (9.5)	11.2 (1.3)	83 (238)
<i>Site 1: Effective number of species</i>				
Aminocyclopyrachlor	1.9 (1.5)	0 (12.9)	6.9 (0.36)	345 (643)
Aminocyclopyrachlor + chlorsulfuron	2.7 (1.7)	3.8 (0.39)	6.9 (0.36)	26 (7)
<i>Site 2: Effective number of species</i>				
Aminocyclopyrachlor	5.0 (22)	4.0 (0.96)	6.0 (0.66)	75 (59)
Aminocyclopyrachlor + chlorsulfuron	5.0 (11.3)	4.0 (0.50)	6.0 (0.66)	18 (7)
Aminocyclopyrachlor + metsulfuron	1.1 (1.3)	2.4 (2.4)	6.0 (0.66)	61 (94)

<sup>1</sup> Parameters are described in Equation [1].



**Figure 2.** Effect of aminocyclopyrachlor alone or in mixture with chlorsulfuron or metsulfuron on **A** and **B**, species richness and effective number of species (Jost, 2006) **C** and **D**, 1 yr after treatment (YAT) at two high-elevation rangeland sites in the Bighorn Mountains of Wyoming, United States. **Panels A** and **C** present data from Site 1. **Panels B** and **D** present data from Site 2. Parameter estimates are in Table 3.

on nontarget plants difficult. However, the larger impacts on diversity, richness, and total vegetation cover remain sound because similar species were present and the trends in these response variables were similar between sites. Future research should be conducted to determine the direct effect of these herbicides on desirable species.

## Discussion

Overall, AMCP-containing herbicides showed the ability to control larkspur with limited damage to grasses. As expected with a relatively broad-spectrum broadleaf herbicide, vegetation in treated plots was more dominated by grasses than forbs, which were greatly reduced at the highest application rates. Both sites exhibited short-term changes in plant community composition with herbicide application including reductions in species richness and diversity and changes in vegetation cover with increasing herbicide rates. Although we maintained consistent experimental approaches at both sites, and in both years of implementation, vegetation response was not as consistent between sites as we expected *a priori*. Such interannual differences emphasize some of the challenges faced by rangeland managers as they implement weed management actions across large spatial scales for multiple years.

The rates required to obtain acceptable larkspur control, as well as the accompanying impacts on nontarget vegetation, differed between the two sites, but some response patterns remained consistent. Reduced herbicide activity in 2012 (Site 2) could potentially be explained by very low precipitation in the months following treatment. Although foliar herbicide uptake is no doubt important for the herbicides used in this study, long-term control with AMCP may be strongly influenced by soil activity (Kniss and Lyon, 2011; Lindenmayer, 2012; Conklin and Lym, 2013). Kniss and Lyon (2011) showed injury to wheat even when AMCP was applied 6 months before planting, suggesting

strong soil activity. AMCP applied directly to soil provided similar Canada thistle (*Cirsium arvense*) control as foliar plus soil applications (Lindenmayer, 2012). Therefore, reduced soil moisture in the months following herbicide application could affect efficacy on target weeds by limiting soil availability. Site 1 received > 100 mm of precipitation in the 3 months following herbicide application, compared with only 43 mm at Site 2.

Higher AMCP rates were required to achieve larkspur control when applied alone compared with when it was mixed with an additional active ingredient (either CHL or MSM). Concurrently, AMCP alone had less impact on species richness and diversity than did the mixed herbicides at similar rates of AMCP. MSM alone provided excellent larkspur control at both sites with little impact on grass cover, but with similar impacts as AMCP on species richness and forb cover. These patterns suggest several potential alternatives for land managers. If AMCP alone were used for larkspur control, higher rates would be necessary to achieve satisfactory results but nontarget impacts may be diminished compared with mixed herbicides. If land management goals primarily focus on grass production for cattle consumption, then AMCP + CHL or AMCP + MSM, or the standard comparator of MSM alone, may prove good options from a weed control standpoint.

In all cases, the herbicide rates required to provide 90% larkspur control (ED<sub>90</sub>) increased relative grass cover and decreased relative forb cover. This result provides a reasonable degree of certainty that if AMCP-containing herbicides are applied for larkspur control, a shift in the vegetation cover toward graminoids and away from forbs will be observed. The results for species richness and diversity were not as consistent, however. Our results suggest that at least in some circumstances (like for AMCP applied alone), larkspur may be controlled effectively without necessarily observing a concurrent decrease in species richness and diversity. However, addition of MSM or CHL resulted in greater impacts on richness and diversity.

We encourage readers to interpret the patterns observed in this study for what they represent—*short-term*, but relatively rapid, vegetation responses to a suite of herbicides with residual soil activity. We were not able to document the longer-term vegetation impacts of these herbicides within the scope of this research. Because AMCP is a relatively new herbicide, prolonged vegetation responses are not yet available for rangeland ecosystems. However, previous research indicates that long-term larkspur control with a single herbicide application is probable. Whitson et al. (1993) observed 95% and 67% control of duncecap larkspur 3 years after MSM (70 g ai · ha<sup>-1</sup>) and picloram (1120 g ai · ha<sup>-1</sup>) application, respectively, when larkspur was at the 4- to 6-leaf growth stage. MSM at 70 g ai · ha<sup>-1</sup> applied in the vegetative stage yielded 99% duncecap larkspur control 4 yr after application at a site in Idaho, but control reduced significantly over the same period at a site in Utah, largely due to larkspur recruitment from seed (Ralphs et al. 1995). In the same study, total grass cover nearly doubled 1 yr after treatment but declined by 4 yr after treatment, although not to pretreatment levels. Given these prior observations, we may predict patterns similar to what we observed to last for 3–5 yr given optimum conditions, but we cannot be certain of such predictions until longer-term data are collected.

Land managers should determine if shifts in grass and forb species within vegetation communities are acceptable before using AMCP or other herbicides for larkspur control. Results from this study indicate that expected composition changes would likely include relative increases in Idaho fescue, mountain brome, and subalpine needlegrass cover at rates needed to control larkspur. These three species are palatable to cattle (Dittberner and Olson, 1983), and increases in their presence would potentially benefit a livestock grazing operation.

Simply stated, this research indicates that application of AMCP-containing herbicides can control duncecap larkspur to a level that should provide a meaningful reduction in cattle deaths from poisoning. This result could also likely be achieved using MSM applied at the same larkspur phenological stage. However, application of these herbicides should be weighed against impacts on species diversity and shifts in community composition. We did not explore economic feasibility in this publication, but it is an important consideration for land managers. Further research is necessary to investigate long-term effects of herbicides for controlling poisonous plants, how application timing affects vegetation response, and true reductions in cattle losses due to duncecap larkspur management.

### Management implications

A relatively new herbicide active ingredient, AMCP, is anticipated for range and pasture registration soon and may provide a new tool for chemical control of poisonous plants. AMCP alone or mixed with chlorsulfuron (CHLR) or metsulfuron (MSM) resulted in good larkspur control across a range of rates. At rates needed to provide sufficient larkspur control, managers can expect to observe a reduction in plant species richness, primarily driven by a reduction in forbs species, and an increase in relative grass cover. Although our results clearly indicate a shift in species composition from forbs to grasses, we did not observe an accompanying increase in grass biomass production. The general pattern of vegetation response was consistent across sites, but the magnitude of responses varied—illustrating the sometimes variable responses to weed management activities. Land managers should

evaluate the potential benefits of controlling larkspur against the potential impacts of decreased forb cover and species richness through the lens of their land use goals.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.rama.2016.06.004>.

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