

## Research Note

# Cattle Grazing Toxic *Delphinium andersonii* in South-Central Idaho

James A. Pfister,<sup>1</sup> Daniel Cook,<sup>2</sup> and Dale R. Gardner<sup>3</sup>

Authors are <sup>1</sup>Rangeland Scientist, <sup>2</sup>Plant Physiologist, and <sup>3</sup>Chemist, USDA-ARS Poisonous Plant Research Laboratory, Logan, UT 84341, USA.

## Abstract

Anderson larkspur (*Delphinium andersonii* A. Gray) is a toxic plant responsible for cattle death losses in the western United States. The objectives of the present study were to determine when cattle consumed Anderson larkspur in relation to plant phenology and toxicity, and to determine if animal age influenced selection of Anderson larkspur. These grazing studies were conducted on semiarid sagebrush rangeland near Picabo, Idaho. Eight 6–7-yr-old mature Angus cows were used in 2008, whereas during 2009, 12 Angus cattle were used, including six yearling heifers and six 4-yr-old cows. The overall density of Anderson larkspur was 2.8 plants · m<sup>-2</sup> during 2008, and 4.9 plants · m<sup>-2</sup> during 2009. Total toxic alkaloid concentrations in Anderson larkspur plants were near or above 5 mg · g<sup>-1</sup> during both studies. During 2008 consumption peaked during the late flower and pod stage of growth. Overall in 2008 cows ate 3% of their bites as larkspur. During 2009 heifers ate about twice as much Anderson larkspur as did mature cows (5.1% of bites vs. 2.9%, respectively). Heifers repeatedly consumed sufficient larkspur that they collapsed; however, no animals were fatally intoxicated. Heifers appeared to become transiently averted to larkspur; however, heifers resumed consumption of *D. andersonii* after a period of one to several days of low or no consumption. Livestock management to reduce losses to Anderson larkspur should include timed grazing to avoid infested pastures during full flower to pod phenological stages, and grazing with older animals rather than yearling heifers.

## Resumen

La hierba Anderson larkspur (*Delphinium andersonii* A. Gray) es una planta toxica responsable de la perdida de ganado por muerte en el oeste de los Estados Unidos. Los objetivos de este estudio fueron determinar cuando el ganado consume Anderson larkspur en relación a la fenología y toxicidad de la planta, y determinar si la edad del animal influye en la selección de esta hierba. El estudio se realizo en un pastizal semiárido de artemisa cerca de Picabo, Idaho. Se usaron vacas Angus de ocho, seis a siete años de edad en 2008 mientras que en 2009 se usaron 12 vacas Angus incluyendo seis vaquillas de sobreaño y vacas de seis a cinco años de edad. La densidad total de Anderson larkspur fue de 2.8 plantas por m<sup>2</sup> durante el 2008 y de 4.9 plantas por m<sup>2</sup> durante 2009. La concentración total de alcaloides tóxicos en las plantas de Anderson larkspur fue cerca o arriba de 5 mg g<sup>-1</sup> durante ambos estudios. Durante 2008 el consumo alcanzo su máximo durante la fase tardía de la floración y el crecimiento de las vainas. En general, en 2008 la vacas comieron el 3% de sus mordiscos de la hierba. Durante el 2009 las vaquillas comieron más del doble de la hierba que las vacas adultas (5.1% de mordidas vs. 2.9%, respectivamente). Las vaquillas consumieron de manera consistente suficiente hierba larkspur que se colapsaron, sin embargo, ningún animal se murió por intoxicación. Aparentemente, las vaquillas evitan temporalmente la hierba larkspur pero, inician el consumo de *D. andersonii* después de un periodo de uno o varios días de bajo o nulo consumo. El manejo del ganado para reducir las pérdidas por Anderson larkspur deberán incluir pastoreo planeado para evitar los porteros infestados del periodo completo de floración al estado fenológico de vainas y pastorear con animales adultos en lugar de vaquillas de sobreaño.

**Key Words:** alkaloids, diet, larkspur, livestock, poisonous plants

## INTRODUCTION

Anderson larkspur (*Delphinium andersonii* A. Gray) is a toxic range plant that causes cattle death losses in the western United States. *Delphinium andersonii* occurs in semiarid rangelands at 1 300–2 400-m elevation in California, Nevada, Oregon, Arizona, Utah, Idaho, and Montana. The toxins in Anderson larkspur are diterpenoid alkaloids, with most of the toxicity attributed to four specific alkaloids (Gardner and Pfister 2009; Welch et al. 2010). Cattle death losses to Anderson larkspur are often sporadic, although losses can be severe at times (Pfister et al. 2003).

There is no published information about cattle grazing of Anderson larkspur, and this information is important for ranchers with infested rangelands to make informed management decisions. Thus, the objectives of this study were 1) to determine when cattle consumed Anderson larkspur in relation to plant phenology and possible changes in alkaloid concentration, and 2) to determine if age influenced cattle selection of Anderson larkspur. Anecdotal accounts from ranchers suggested that young animals may consume more Anderson larkspur than older animals.

## MATERIALS AND METHODS

### General Site Description and Animals

The study site was located 14 km from Picabo, Idaho at lat 43°14'46"N, long 114°13'25"W at 1486 m elevation in a

Correspondence: James A. Pfister, USDA-ARS Poisonous Plant Research Laboratory, 1150 E 1400 N, Logan, UT 84341, USA. Email: jim.pfister@ars.usda.gov

Manuscript received 6 January 2011; manuscript accepted 6 June 2011.

sagebrush steppe. The study site was dominated by big sagebrush (*Artemisia tridentata* Nutt.) with large patches of Anderson larkspur.

Eight 6–7-yr-old Angus cows ( $616 \pm 45$  kg) were used in the 2008 study. Twelve Angus cattle were used during 2009, including six yearling heifers ( $343 \pm 16$  kg) and six 5-yr-old cows ( $528 \pm 40$  kg). Animals were not pregnant during the trials. None of the animals had previously been exposed to *D. andersonii*.

### Trial 1—21 May–18 June 2008

Anderson larkspur is ephemeral, and anecdotal accounts suggest that it is problematic only during spring. Anderson larkspur was in the vegetative and flower stage of growth at the beginning of the trial, and in the pod stage when the trial concluded. An 18-ha pasture was delineated with electric fence for the duration of the study. Density of Anderson larkspur was determined at the beginning of the trial with the use of 500, 0.5-m<sup>2</sup> plots placed every 10 m along three pace transects across the pasture.

Five individual larkspur plants were collected weekly, just outside the pastures, to determine alkaloid concentration; an additional five plants were collected randomly from within the pasture for nutrient analysis. All plants for alkaloid analysis were frozen, freeze-dried, and ground through a 1-mm screen. Larkspur samples were analyzed for four toxic alkaloids (methyllycaconitine, 16-deacetylgeyerline, 14-acetylbearline, and geyerline) and total toxic alkaloids by reverse-phase liquid chromatography/mass spectrometry as previously described (Gardner and Pfister 2009). Larkspur samples for nutrient analysis were oven dried at 40°C, ground to pass a 1-mm screen, and analyzed for crude protein (LECO FP-528 Nitrogen Analyzer, LECO Corp, St. Joseph, MI), NDF (ANKOM Fiber Analyzer system), and in vitro true digestibility (ANKOM Daisy II system).

Bite counts were used to determine animal diets, and categorized as Anderson larkspur, grasses, lupine, death camas, and other forbs. Cattle were placed in a corral overnight, and released each d about 0600 hours. Each cow was focally observed in a predetermined order for numerous 5 min periods; these observations continued during all active grazing periods until 1900 hours. Generally, we obtained 30–40 min of daily observation time for each animal.

### Trial 2—27 May–16 June 2009

The trial was conducted in the same location as previously described. In spring 2009, six 3.5-ha pastures were delineated with similar densities of Anderson larkspur. Anderson larkspur was generally in the early flower stage of growth when the trial began. Two heifers or adult cows were each allocated to three replicate pastures with heifers and cows in alternating and adjoining pastures. Density was determined with the use of 250 0.5-m<sup>2</sup> plots located in each pasture along pace transects. Bite counts were done as described in the previous trial. Collections of Anderson larkspur plants were made weekly for alkaloid analysis and nutritional analysis as described for Trial 1.

### Statistical Analysis

Only descriptive statistics were done on the 2008 bite-count data. For the 2009 bite-count data, a mixed linear model was

**Table 1.** Nutrient analysis<sup>1,2</sup> (g/100 g  $\pm$  SE) of *Delphinium andersonii* from south-central Idaho during spring 2008 and 2009.

Date	CP	NDF	IVTD
2008			
21 May	9.5 $\pm$ 0.1	34.6 $\pm$ 1.8	80.4 $\pm$ 1.7
28 May	9.1 $\pm$ 0.1	39.3 $\pm$ 1.2	74.5 $\pm$ 1.2
4 June	8.3 $\pm$ 0.3	37.1 $\pm$ 0.2	75.2 $\pm$ 0.7
10 June	6.8 $\pm$ 0.2	38.6 $\pm$ 0.8	74.3 $\pm$ 1.4
16 June	7.3 $\pm$ 0.2	41.6 $\pm$ 4.7	71.4 $\pm$ 3.2
2009			
27 May	8.7 $\pm$ 0.2	36.3 $\pm$ 0.5	76.7 $\pm$ 1.2
3 June	7.8 $\pm$ 0.3	43.6 $\pm$ 1.4	69.7 $\pm$ 1.5
10 June	7.7 $\pm$ 0.3	47.4 $\pm$ 1.4	66.8 $\pm$ 0.3
18 June	6.9 $\pm$ 0.4	51.5 $\pm$ 1.5	60.6 $\pm$ 1.6

<sup>1</sup>*n* = 5 plants. All concentrations are on a dry-matter basis.

<sup>2</sup>CP = crude protein; NDF = neutral detergent fiber; IVTD = in vitro true digestibility.

used (Proc Mixed) in SAS (SAS Institute, 2004). The model included treatment (heifers vs. mature cows), date, and the treatment  $\times$  date interaction, pasture nested within treatment, animals nested within treatment and pasture, and date  $\times$  pasture within treatment. Pastures (i.e., replicates) in this design were the experimental unit (*n* = 3). Least-square means were used for all comparisons, and the PDIF procedure in SAS (SAS Institute, 2004) was used with preplanned comparisons to evaluate the treatment  $\times$  date interaction. Only means and standard errors were calculated for data on nutritional analysis and alkaloid concentration.

## RESULTS

### Weather, Larkspur Density, and Forage Standing Crop

Precipitation in the Picabo area during April and May 2009 was two times greater than during the same period in 2008 (5.5 cm vs. 2.6 cm, respectively; National Climate Data Center [NCDC] 2010). That trend continued and intensified during June, as precipitation during the study period in June 2009 was 10.5 cm compared to 0.5 cm in 2008. The overall density of Anderson larkspur was 2.8 plants  $\cdot$  m<sup>-2</sup> during May 2008, and 4.9 plants  $\cdot$  m<sup>-2</sup> during May 2009.

### Nutritional Analysis

Anderson larkspur consistently contained about 7–9% crude protein during the study period in both 2008 and 2009 (Table 1). Larkspur was also relatively low in NDF (30–50%) and relatively high in digestibility (60–80%) during both yr.

### Toxic Alkaloid Concentration

Total toxic alkaloid concentrations were generally at or above 5 mg  $\cdot$  g<sup>-1</sup> during both yr (Table 2). The most dominant individual toxic alkaloid was geyerline (GEY), followed by methyllycaconitine (MLA). Alkaloid concentrations did not change greatly during maturation during either yr, although numerically toxic alkaloid concentration was about 25% higher in Anderson larkspur during 2009 (6.2 mg  $\cdot$  g<sup>-1</sup>) compared to 2008 (4.7 mg  $\cdot$  g<sup>-1</sup>).

**Table 2.** Diterpenoid alkaloid<sup>1</sup> concentration (mean mg · g<sup>-1</sup> ± SE) of Anderson larkspur (*Delphinium andersonii*) from south-central Idaho in spring, 2008 and 2009.

Year/date	16-deacetyl geyerline	Methyllycaconitine	Geyerline	14-acetylbearline	Total toxic alkaloids
2008					
28 May	0.52 ± 0.06	1.24 ± 0.25	2.71 ± 0.30	0.29 ± 0.05	4.88 ± 0.58
4 June	0.34 ± 0.10	1.30 ± 0.37	2.31 ± 0.26	0.19 ± 0.02	4.17 ± 0.67
10 June	0.54 ± 0.06	0.90 ± 0.20	2.85 ± 0.44	0.45 ± 0.08	4.80 ± 0.71
16 June	0.52 ± 0.07	1.23 ± 0.22	3.03 ± 0.63	0.18 ± 0.05	5.00 ± 0.84
2009					
27 May	0.97 ± 0.09	1.18 ± 0.11	3.12 ± 0.18	0.49 ± 0.12	5.91 ± 0.47
3 June	1.42 ± 0.21	1.79 ± 0.23	2.40 ± 0.29	0.57 ± 0.13	6.36 ± 0.78
10 June	1.35 ± 0.10	1.77 ± 0.005	3.10 ± 0.08	0.75 ± 0.035	7.20 ± 0.12
18 June	1.18 ± 0.09	1.16 ± 0.05	2.41 ± 0.19	0.45 ± 0.07	5.47 ± 0.35

<sup>1</sup>*n* = 5 plants. The alkaloids shown here are the major toxic ([methylsuccinimido] anthranoyllycoctonine; MSAL type) alkaloids found in Anderson larkspur; there are several minor toxic (MSAL type) alkaloids and numerous other less toxic (7,8-methylenedioxylycoctonine; MDL type) alkaloids that are not shown here. Total toxic alkaloid concentration indicates the total concentration of all toxic MSAL type alkaloids found in the plant.

## Diet Selection

During 2008, cows began to consume Anderson larkspur when flowers were just beginning to appear on the plants, but the largest consistent day-to-day consumption (Days 17–22) occurred during the late flower/pod stage of growth (Fig. 1A). Averaged over the entire trial, all the animals ate 3% of their bites as Anderson larkspur, whereas grasses constituted over 93% of cow bites. The largest spike in consumption occurred during a day-long rainstorm on 26 May 2008 (Day 6 in Fig. 1A). One animal showed periodic signs of larkspur intoxication (muscle tremors) after consuming a larger amount of plant on two occasions, but no animals died in 2008.

During 2009 there was a treatment × day interaction (*P* = 0.05) as heifers ate more Anderson larkspur on Days 3, 13, and 14 (Fig. 1B) than did mature cows. Further, there was a trend (*P* = 0.10) toward differences between the two age classes of animals on Days 15 and 16. Overall, heifers ate about twice as much Anderson larkspur as did mature cows (5.1% of bites vs. 2.9%, respectively). Several heifers repeatedly consumed sufficient Anderson larkspur that they showed violent tremors and collapsed into lateral recumbency on several occasions; however, no animals were fatally intoxicated. The consumption profiles of Anderson larkspur for two heifers over the study period are shown in Figures 1C and 1D. Individually, the heifers demonstrated an intoxication–detoxification cycle, often with periods of higher Anderson larkspur consumption followed by periods of reduced consumption.

## DISCUSSION

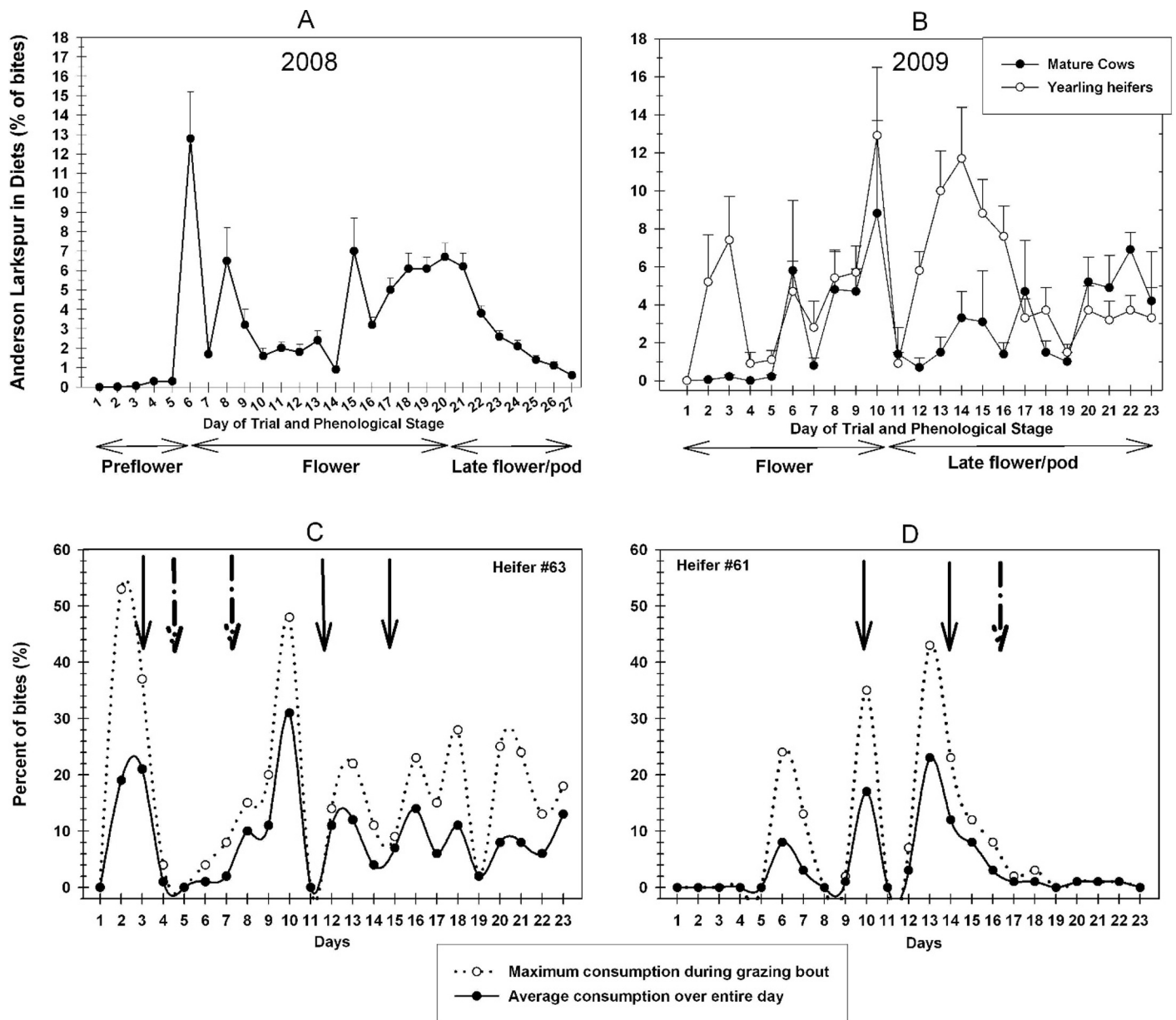
The toxic alkaloid concentrations in Anderson larkspur did not show major changes with plant maturity. The stability and relatively high concentrations of toxic alkaloids in Anderson larkspur increases the risk of cattle losses when the plant is abundant. The risk of poisoning from Anderson larkspur for cattle is also accentuated by the biomass of the plant compared to other low larkspurs (e.g., *Delphinium nuttallianum*). Anderson larkspur typically grows to 50 cm mature height compared to 20–30 cm for *D. nuttallianum*; thus there is more alkaloid per plant. The relatively high concentration of one of

the major alkaloids, geyerline, would also contribute to the toxic risk in conjunction with the other toxic alkaloids. The lethal dose (LD<sub>50</sub>) of geyerline in mice is 6.2 mg · kg<sup>-1</sup> body weight and the LD<sub>50</sub> for MLA is 4.5 mg · kg<sup>-1</sup>. Lethality in mice correlates with toxicity in cattle (Welch et al. 2010), indicating the highly toxic nature of the major alkaloids in Anderson larkspur.

During 2008 cattle began eating Anderson larkspur in the flower stage of growth, with variable consumption during the flowering and pod stages of growth. Studies with tall larkspur (*D. barbeyi*) have shown that consumption by cattle is not related to alkaloid concentration in the plants (Pfister et al. 1996). The lack of major fluctuations in toxic alkaloid concentration in Anderson larkspur precludes making definitive statements about alkaloid concentrations related to selection by cattle. In other work with *D. nuttallianum* (Pfister and Gardner 1999), consumption by mature cattle occurred in proportion to availability. Our observations during 2008 suggest that mature cows ate Anderson larkspur as they encountered the plant, neither seeking nor avoiding the plants as they grazed.

The 2009 trial showed that heifers selected a higher proportion of their diet as Anderson larkspur compared to mature cows. Overall, heifers selected about twice as much Anderson larkspur as did mature cows. Differences between age classes in diet selection have been explained by differences in body size and allometric relationships with intake capacity, digestibility, and selectivity (Demment and Greenwood 1988; Cazcarra et al. 1995; Rook et al. 2004). Other literature has shown inconsistent results comparing diet selection of adult vs. juvenile livestock (Walker et al. 1981; Mellado et al. 2004).

Heifers on numerous days consumed numerically more *D. andersonii* than did mature cows, and consumption of the toxic alkaloids provoked muscular tremors and often also caused both sternal and lateral recumbence. We speculate that the daily mean differences between heifers and mature cows in *D. andersonii* consumption would have been more pronounced, however, intoxicated heifers typically were negatively affected for 1 or 2 d after a visible poisoning episode. Behaviorally they appeared to be less vigorous and to reduce grazing time. We have shown previously that animals intoxicated by larkspur



**Figure 1.** A and B, consumption (% of bites  $\pm$  SE) of Anderson larkspur (*Delphinium andersonii*) by cattle during grazing trials near Picabo, Idaho from 21 May to 16 June 2008 and 27 May to 18 June 2009. Preflower, flower, and late flower/pod refer to the dominant phenological stage of Anderson larkspur. C and D, consumption (% of bites) of Anderson larkspur by two yearling heifers during 2009. The dotted line represents the maximum consumption during a grazing bout on that specific day. The solid line represents the average consumption over all grazing bouts during the entire day. The solid arrow above represents a time period during which the animal collapsed into lateral recumbence. The broken arrow above indicates a time period during which the animal showed visible muscular tremors but no collapse.

alkaloids have reduced total feed intake, and concomitantly reduced larkspur intake for several days after becoming intoxicated (Pfister et al. 1997). Heifers were often in different phases of this intoxication–detoxification cycle; thus this lack of synchrony reduced the average daily peaks of consumption for heifers as a group, and diminished the differences between heifers and adult cows. Further, heifers eating amounts of *D. andersonii* sufficient to cause sternal and lateral recumbency may have been transiently averted to larkspur; however, in each case the heifers resumed consumption of *D. andersonii* after a period of one to several days of low or no consumption, as we have found in other larkspur studies (Pfister et al. 1997). Other

studies have shown that younger animals form weaker aversions to foods than adult animals (Ralphs and Cheney 1993); in this 2009 trial age and increased larkspur consumption are confounded and no definitive statement is possible.

Mature cows occasionally selected sufficient Anderson larkspur that they showed muscular tremors and short-term sternal recumbency. Those episodes typically occurred when cattle were running while playing, and the brief muscular exertion caused them to collapse temporarily. We have noted this pattern in previous larkspur studies (Pfister et al. 2002), as cattle often consume larkspur in amounts just below that of a critical threshold (Pfister et al. 1999); the exertion and subsequent



collapse highlights how close cattle are to a critical threshold of intoxication (Pfister et al. 1997). Adult cattle also showed cyclic consumption for several days as noted for heifers, but both larkspur consumption and the toxic outcome (i.e., severity and duration of recumbency) were reduced compared to heifers.

## IMPLICATIONS

Consumption of *D. andersonii* by cattle during spring and early summer causes substantial death losses when the plant is abundant. Cattle consumed most *D. andersonii* during the flower and pod stages of growth. Further, yearling heifers consumed more *D. andersonii* than did mature cattle, and typically resumed eating the plant after intoxication episodes resulted in collapse. Livestock managers with infested rangelands should be aware of the time period during which the plant is in flower or pod, and avoid grazing cattle, particularly young animals, during those periods to reduce risk of losses. This suggestion is particularly important during years when cool, wet winter and spring weather result in high plant densities. The toxicity of *D. andersonii* is relatively high and stable during the growing season; thus grazing management and risk assessment must be based on larkspur density and phenology.

## ACKNOWLEDGMENTS

We thank Kermit Price, Clint Stonecipher, Ed Knoppel, Danny Hansen, and Rex Probst for assistance with the study. We also thank Bud and Nick Purdy, Picabo Livestock, Picabo, Idaho, for their cooperation and support during the studies.

## LITERATURE CITED

CAZCARRA, R. F., M. PETIT, AND P. D'HOOR. 1995. The effect of sward height on grazing behavior and herbage intake of 3 sizes of Charolais cattle grazing cocksfoot (*Dactylis glomerata*) swards. *Animal Science* 61:511–518.

DEMMENT, M. W., AND G. B. GREENWOOD. 1988. Forage ingestion: effects of sward characteristics and body size. *Journal of Animal Science* 66:2380–2392.

GARDNER, D. R., AND J. A. PFISTER. 2009. HPLC/MS analysis of toxic norditerpenoid alkaloids: refinement of toxicity assessment of low larkspurs (*Delphinium* spp.). *Phytochemical Analysis* 20:104–113.

MELLADO, M., A. RODRÍGUEZ, A. OLVERA, J. A. VILLARREAL, AND R. LOPEZ. 2004. Age and body condition score and diets of grazing goats. *Journal of Range Management* 57:517–523.

NATIONAL CLIMATE DATA CENTER (NCDC). 2010. Available at: <http://www.ncdc.noaa.gov/oa/ncdc.html>. Accessed 8 July 2010.

PFISTER, J. A., AND D. R. GARDNER. 1999. Consumption of low larkspur (*Delphinium nuttallianum*) by cattle. *Journal of Range Management* 52:378–383.

PFISTER, J. A., D. R. GARDNER, K. E. PANTER, G. D. MANNERS, M. H. RALPHS, B. L. STEGELMEIER, AND T. K. SCHOCH. 1999. Larkspur (*Delphinium* spp.) poisoning in livestock. *Journal of Natural Toxins* 8:81–94.

PFISTER, J. A., D. R. GARDNER, B. L. STEGELMEIER, K. HACKETT, AND G. SECRIST. 2003. Catastrophic cattle loss to low larkspur (*Delphinium nuttallianum*) in Idaho. *Veterinary and Human Toxicology* 45:137–139.

PFISTER, J. A., G. D. MANNERS, D. R. GARDNER, K. W. PRICE, AND M. H. RALPHS. 1996. Influence of alkaloid concentration on acceptability of tall larkspur (*Delphinium* spp.) to cattle and sheep. *Journal of Chemical Ecology* 22:1147–1168.

PFISTER, J. A., F. D. PROVENZA, G. D. MANNERS, D. R. GARDNER, AND M. H. RALPHS. 1997. Tall larkspur ingestion: can cattle regulate intake below toxic levels? *Journal of Chemical Ecology* 23:759–777.

PFISTER, J. A., M. H. RALPHS, D. R. GARDNER, B. L. STEGELMEIER, G. D. MANNERS, K. E. PANTER, AND S. T. LEE. 2002. Management of three toxic *Delphinium* species based on alkaloid concentrations. *Biochemical Systematics and Ecology* 30:129–138.

RALPHS, M. H., AND C. D. CHENEY. 1993. Influence of cattle age, lithium chloride dose, and food type in the retention of food aversions. *Journal of Animal Science* 71:373–379.

ROOK, A. J., B. DUMONT, J. ISSELSTEIN, K. OSORO, M. F. WALLIS DE VRIES, G. PARENTE, AND J. MILLS. 2004. Matching type of livestock to desired biodiversity outcomes in pastures—a review. *Biological Conservation* 119:137–150.

SAS INSTITUTE. 2004. SAS/STAT User's Guide. Version 9.1. Cary, NC, USA: SAS Institute, Inc. 5180 p.

WALKER, J. W., R. M. HANSEN, AND L. R. RITTENHOUSE. 1981. Diet selection of Hereford, Angus × Hereford and Charolais × Hereford cows and calves. *Journal of Range Management* 34:243–245.

WELCH, K. D., B. T. GREEN, D. R. GARDNER, D. COOK, J. A. PFISTER, B. L. STEGELMEIER, K. E. PANTER, AND T. Z. DAVIS. 2010. Influence of 7,8-methylenedioxycocatonine-type alkaloids on the toxic effects associated with ingestion of tall larkspur (*Delphinium* spp.) in cattle. *American Journal of Veterinary Research* 71:487–492.