Late season toxic alkaloid concentrations in tall larkspur (Delphinium spp.)

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Abstract

Tall larkspurs [Delphinium barbeyi (L. Huth), D. occidentale (Wats.), D. glaucescens (Rydb.), D. glaucum (Wats.)] pose a serious poisoning threat to cattle on many summer ranges. Livestock producers often defer grazing until larkspur is mature, but specific information is lacking on toxic alkaloid concentrations in larkspur from pod stage to senescence. Tall larkspur leaves and seed pods were collected about every 2 weeks during the pod stage to senescence from marked plants in locations in Utah (Logan and Salina), Idaho (Ashton, Humphrey, and Oakley), Colorado (Yampa and Montrose), and California (Carson Pass) from 1995 to 1997. Toxic alkaloid concentions in pods ($\bar{x} = 2.9$ mg/g) exceeded leaf alkaloid concentrations ($\bar{x} = 1.5$ mg/g in all species, but the magnitude of the difference varied among the 4 species. Leaves showed a more rapid decrease in toxic alkaloid concentration with plant maturity compared to pods. Seed pods did not begin to lose substantial amounts of toxic alkaloid until larkspur matured and pods began to dessicate. At seed shatter, D. glaucescens pods retained more toxic alkaloid than the other species, and alkaloid concentration was sufficiently high after pods had shattered (3.5 mg/g) to pose a moderate grazing risk. After seed shatter, the toxic alkaloid concentrations in leaves and pods of D. barbeyi, D. occidentale, and D. glaucum were generally less than 2 mg/g; thus, risk of losing cattle would be low for the remainder of the grazing season.

Key Words: diterpenoid alkaloids, poisonous plants, grazing management

Tall larkspurs (*Delphinium barbeyi* (L. Huth), *D. occidentale* (Wats.), *D. glaucescens* (Rydb.), *D. glaucum* (Wats.)) are toxic plants that are often fatally ingested by cattle on western rangelands. The presence of larkspur often dictates summer grazing management of mountain rangeland as producers attempt to reduce or avoid livestock losses (Pfister et al. 1997). A longestablished management practice is to defer grazing until larkspur is mature, vaguely defined as the late flower or pod stage of growth (Marsh et al. 1916). Late-season grazing can provide 6 to 8 weeks of grazing depending on larkspur phenology, elevation, available forage, and weather (Pfister, personal observations).

Resumen

Las especies de "Tall larkspur" [Delphinium barbeyi (L. Huth), D. Occidentale (Wats.), D. glaucescens (Rydb), D. glaucum (Wats.)] son una seria amenaza de envenenamiento para el ganado que apacenta en pastizales de verano. Los ganaderos a menudo difieren el apacentamiento hasta que el "Larkspur" esta maduro, sin embargo, se carece de información especifica respecto a las concentraciones de alcaloides tóxicos que contiene el "larkspur" desde la producción de vainas hasta la senescencia de la planta. De 1995 a 1997, durante la etapa de producción de vainas a senescencia, se colectaron hojas y vainas "Tall larkspur" aproximadamente cada 2 semanas, la colecta se realizó en plantas marcada en localidades de Utah (Logan y Salina), Idaho (Asthon, Humphrey y Oakley), Colorado (Yampa y Montrose) y California (Carson Pass). Las concentraciones de alcaloides tóxicos de las vainas de todas las especies fueron mayores ($\bar{x} = 2.9$ mg/g) que las concentraciones de las hojas ($\bar{x} = 1.5 \text{ mg/g}$), pero la magnitud de la diferencia vario entre las 4 especies. Al madurar, la concentración de alcaloides tóxicos de las hojas descendió mas rápidamente que la concentración de las vainas. Las vainas no empezaron a perder cantidades substanciales de alcaloides tóxicos hasta que el "Larkspur" maduró y las vainas comenzaron a desecarse. En la etapa de caída de semilla, las vainas de D. glaucescens retuvieron mas alcaloides tóxicos que otras especies y la concentración de alcaloides en ellas después de haber liberados las semillas fue suficientemente alta (3.5 mg/g) para poseer un riesgo moderado durante el apacentamiento. Después de la caída de la semilla las concentraciones de alcaloides tóxicos en hojas y vainas de las especies D. barbeyi, D. occidentale y D. glaucum generalmente fueron menos de 2 mg/g; así, el riesgo de perder ganado sería bajo para el resto de la estación de apacentamiento.

Major deficiencies in this recommendation include: (1) the concentration of toxic alkaloids (i.e., toxicity) in tall larkspurs often decreases with maturation, but this decrease is not constant or consistent (Pfister et al. 1994, Ralphs et al. 1997); (2) grazing during the late-flower period may expose cattle to a high risk of poisoning (Pfister et al. 1997); and (3) information specific to late summer and fall alkaloid concentrations and plant toxicity is insufficient to make definitive grazing recommendations.

Previously we evaluated seasonal changes in tall larkspur toxicity, but usually stopped sampling during the early pod stage (Pfister et al. 1994, Ralphs et al. 1997). Seed pods may take longer than 6 weeks to mature and shatter; furthermore, green leaves may remain after pods have shattered (Pfister, personal observations). Information on late-season toxicity is necessary for livestock pro-

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ducers to determine when the risk of poisoning is sufficiently low to allow grazing. In this study we provide information for toxic alkaloid concentrations in seed pods and leaves from the pod stage of growth to senescence.

Methods

Sample Collection

We collected tall larkspur leaves and seed pods about every 2 weeks from the early pod stage of growth to senescence from marked plants in each location (n = 8), with some exceptions (Table 1). Generally, sampling occurred from early August to late September or early October in each year, and where possible, was timed to represent 4 phenological stages: early pod, pod, late pod, and seed shatter. We marked 20 individual plants with a numbered fiberglass rod. Tall larkspurs generally have multiple stems, and sampling leaves and seed heads from a single stem provides a representative sample of the entire plant (Manners and Pfister 1996). Therefore, during each collection we selected an individual stem from each plant at random, and we harvested all the leaves and the seed head (i.e., entire seed pod including stem from upper 15 to 20 cm of the stem) from this stem. The harvested leaves and seed heads mimic the plant parts that cattle generally select (Pfister et al. 1988). All plant parts were maintained separately for alkaloid analysis. A sample size of 20 plants is usually sufficient to provide an alkaloid concentration within \pm 10% of the population mean with 95% confidence (Manners and Pfister 1996). We packed samples in ice for transport to the laboratory, where they were frozen at -20° C, freeze-dried, then ground to pass through a 1 mm screen for later analysis. We did not mark individual plants at Humphrey, Ida. because D. glaucescens seldom has sufficient stems for repeated sampling of the same plant. Instead, during 1995 and 1997, we selected 20 plants at random during each collection period, and collected leaves and seed heads. In 1996 at Ashton, Ida, we collected leaf and pod samples from 20 different plants on each date. We also collected seeds during 1996 from plant populations at Logan, Yampa, and Montrose; during 1997 we collected seeds from Oakley, Yampa and Montrose.

Alkaloid analysis

The primary toxins in tall larkspurs are 2 norditerpenoid alkaloids: methyllycaconitine (MLA) and 14-deacetylnudicauline (DAN). These alkaloids have nearly identical mammalian toxicity (mice LD₅₀ 4.0 to 4.7 mg/kg; Manners et al. 1995). The combined concentration of these 2 toxic alkaloids (i.e., MLA + DAN = toxic alkaloid concentration) was determined by Fourier transform infrared spectroscopy (FTIR, Gardner et al. 1997).

This methodology also provides a measurement of the total alkaloid (i.e., toxic + less-toxic alkaloids) concentration. Less-toxic alkaloids are 2 to 4 orders of magnitude less toxic than MLA (Manners et al. 1995) because they lack the correct structural specificity to impart significant toxicity. The total alkaloid concentration has relevance when producers use sheep as a management tool to reduce cattle losses (Ralphs and Olsen 1992), as higher concentrations of total alkaloids reduce lark-

spur consumption by sheep (Pfister et al. 1996). Because total alkaloid concentrations are less important than toxic alkaloids, only a brief summary is presented.

Statistical Analysis

The data were analyzed using the GLM procedure of SAS (1988) with the following effects: years, species, locations nested within species, plant part, phenological stage, and individual plants (subsampling term), and resulting interactions. Years, species, and the year x species interaction were tested using the sum of the location within species term and the year x location within species term as the single error term. Plant parts, year x part, species x part, and year x species x part were tested using the sum of the part x location within species and the part x year x location within species terms as the single error term. Phenological stage, year x stage, species x stage, year x species x stage, part x stage, year x part x stage, species x part x stage, species x part x stage, and the 4-way interaction were tested using the sum of the part x location within species x stage term and the part x year x location within species x stage term as the single error term. When significant F-tests were found, the Duncan's multiple range test was used to compare means. Differences in locations within each species (where applicable) were examined with the GLM procedure of SAS (1988) using a model with location, year within location, part, part x location, part x year within location, stage, stage x location, stage x year within location, stage x part, stage x part x location and stage x part x year within location. The year within location term was used to test for differences in location; part and

Table 1. Location of each tall larkspur collection, years of collection, elevation, associated vegetation type, and sampling methods.

Delphinium spp.		Location	Years	Elevation (m)	Vegetation Type	Sampling Method ¹
D. barbeyi	Montrose, Colo.	38°18'N 108°12'W	1995-97	2871	Conifer/Aspen	Marked plants
	Yampa, Colo.	40°12'N 107°04'W	1995-97	2580	Conifer/Aspen	Marked plants
	Salina, Ut. ²	38°44'N 111°39'W	1995-97	2895	Aspen	Marked plants
D. occidentale	Logan, Ut.	41°54'N 111°34'W	1996-97	2400	Conifer/Aspen	Marked plants
	Ashton, Ida.	44°05'N 111°08'W	1995-96	1815	Aspen	Marked plants ³
	Oakley, Ida.	42°10'N 114°11'W	1995-97	2247	Aspen	Marked plants
D. glaucescens	Humphrey, Ida.	44°32'N 112°10'W	1995,97	2396	Grass meadow	No marked plants
D. glaucum	Carson Pass, Calif.	38°40'N 119°59'W	1996-97	2700	Wet meadow	Marked plants ⁵

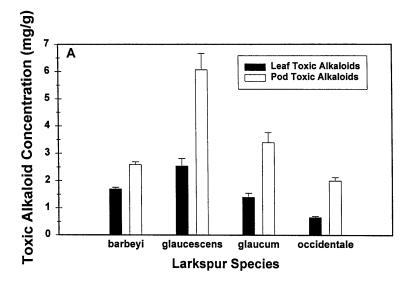
Unless noted otherwise, 20 individual plants were marked, and all leaves and the seed head were collected from 1 stem during each sample period; sampling recurred at approximately 2 week intervals during the pod stage until senescence.

This larkspur population includes some D. occidentale, but is primarily D. barbeyi.

Twenty different unmarked plants were sampled during each collection in 1995; 20 plants were marked and sampled during 1996.

Individual plants of *D. glaucescens* often do not have sufficient stems for multiple samples, therefore leaves and seed heads were collected from 20 different plants during each collection period during 1995 and 1997. Whole plants collected during 1996 were not included in the statistical analysis.

A composite sample from at least 20 plants was collected during each period in 1996; 20 plants were marked and sampled during 1997.



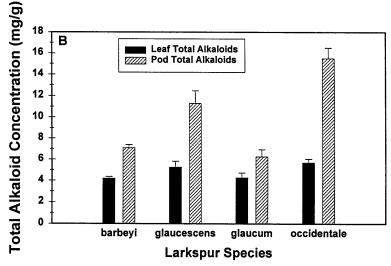


Fig. 1. Toxic (A) and total (B) alkaloid concentration (mg/g + SE) in leaves and pods of 4 *Delphinium* species during the later portion of the growing season.

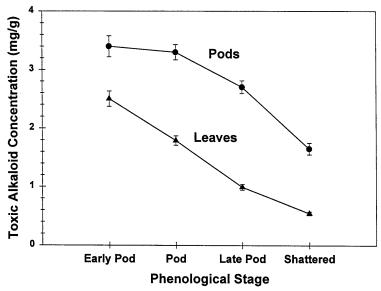


Fig. 2. Toxic alkaloid concentration (mg/g + SE) in *Delphinium* spp. pods and leaves in various growth stages during the later portion of the growing season.

the part x location interaction were tested using the part x year within location error term; stage and stage x location were tested using the stage x year within location term; stage x part and stage x part x location were tested using the stage x part x year within location error term. Correlation coefficients for alkaloid concentrations in plants were determined using mean concentrations for each plant for each location and year.

Results and Discussion

Years

Overall, the toxic alkaloid concentration averaged 2.1 mg/g (SE \pm 0.05; range = 17.4). Years did not differ and year x species did not interact (P > 0.1). Ralphs et al. (1997) reported year differences in alkaloid concentrations for all species except D. glaucescens, but Pfister et al. (1994) found that concentrations in D. barbeyi in Colorado differed in only 1 of 3 years. We speculate that years did not differ in our study because sampling was confined to the late summer period when concentrations were relatively low, and therefore more stable, relative to vegetative and bud stages (Pfister et al. 1994, Ralphs et al. 1997). We caution that yearto-year variation in toxicity may be high, particularly at earlier stages of maturity.

Plant Parts

The plant part x species interaction was significant, as toxic alkaloid concentrations in pods exceeded leaf alkaloid concentrations in all species, but the magnitude of the difference varied among the 4 species (Fig. 1). We have noted in previous studies that pod toxicity generally exceeds that for leaves (Pfister et al. 1994, Ralphs et al. 1997). Seed pods apparently accumulate both toxic and less-toxic alkaloids early in the maturation process, although the degree of accumulation differs among species.

Phenological Stage and Species

Toxic alkaloid concentrations declined (P < 0.05) as tall larkspur matured. Leaves showed a more rapid decrease in toxic alkaloid concentration than pods (Fig. 2). Seed pods began to lose substantial amounts of toxic alkaloid beginning at the late pod stage (i.e., pods beginning to dessicate). In all species except for *D. bar-beyi* at Salina, Utah, we found no indication of the late-season increase in leaf toxic alkaloids that we previously reported

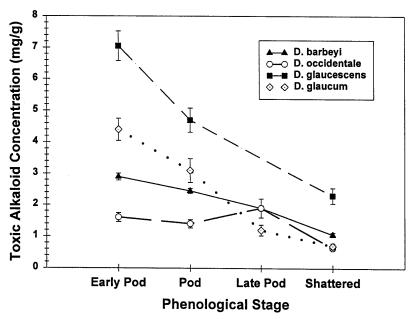


Fig. 3. Toxic alkaloid concentration (mg/g + SE) for 4 *Delphinium* species at various growth stages during the later portion of the growing season.

during one season in Colorado (Pfister et al. 1994). Pods apparently lose some alkaloids because of alkaloid-containing seeds dropping from the pods. Some alkaloids must be retained in the shattered pods because, as noted below, residual pods from D. glaucescens remained toxic after seed shatter. Seeds collected from D. bar beyi in Colorado (Yampa and Montrose) averaged 5.4 mg/g of toxic alkaloid, whereas D. occidentale seeds from Oakley, Ida. and Logan, Utah contained 7.1 and 0.25 mg/g of toxic alkaloid, respectively. No seeds were found in D. glaucescens plants at Humphrey, Ida. We speculate that the toxic alkaloid concentration remained high in the residual pods of D. glaucescens because seed formation and subsequent loss were incomplete.

D. glaucescens and glaucum exhibited higher initial toxic alkaloid concentrations than D. barbeyi and occidentale. At seed shatter, most tall larkspur species contained about the same concentrations of toxic alkaloid, except D. glaucescens retained more toxic alkaloid than the other species (Fig. 3). Previous work in our laboratory (Ralphs et al. 1997) determined that D. glaucum contained the highest overall concentration of toxic alkaloid, but the present study showed that toxic alkaloid concentration in D. glaucum decreased greatly during pod formation.

Same Species at Different Locations

D. barbeyi was sampled at 3 locations (Montrose, Salina, and Yampa). There were no location effects nor interactions (P > 0.1). We found a significant stage x part interaction for toxic alkaloids in D. barbeyi. Toxic alkaloid concentrations decreased more with maturation in D. barbeyi leaves than in pods (Fig. 4).

For *D. occidentale* the location x plant part interaction was significant; stage of

growth was also a significant factor. The interaction with location occurred because the Logan tall larkspur population had much lower toxic alkaloid concentrations over the late summer compared to either Ashton or Oakley, and concentrations at Logan were largely unaffected by stage of growth (Fig. 5). Furthermore, leaves and pods at the Logan location had almost identical, but very low, concentrations of toxic alkaloids which changed little over time. Conversely, at both Ashton and Oakley, toxic alkaloid concentrations declined as plants matured and were lowest at seed shatter (Fig. 5).

We offer no explanation for the much lower concentration of toxic alkaloids in D. occidentale collected near Logan compared to collections from Oakley and Ashton. Ralphs et al. (1997) similarly reported that D. occidentale collected near Bozeman, Mont. and Jackson, Wyo. had much lower concentrations than the Oakley population. In the Ralphs et al. (1997) study, collections from Jackson and Bozeman were from a single year; nonetheless, concentrations were similar to our results from Logan. The Logan population was also unusual in that total alkaloid concentrations were high (pod $\bar{x} =$ 18.4 mg/g; leaf $\bar{x} = 5.6$ mg/g).

Correlation coefficients relating toxic and total alkaloid concentrations in leaves and pods from individual plants were low (r=0.2 to 0.5), indicating that generally toxic and total alkaloid concentrations

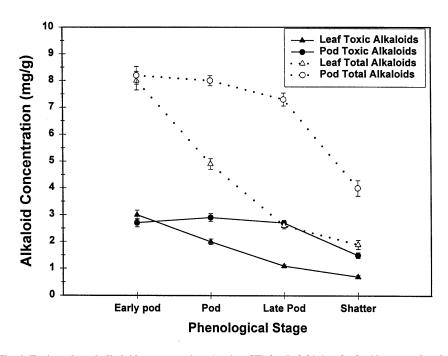


Fig. 4. Toxic and total alkaloid concentrations (mg/g + SE) for Delphinium barbeyi leaves and pods at various growth stages during the later portion of the growing season.

vary independently in plant parts. Because it seems unlikely that environmental conditions are sufficiently different in these mountain habitats to produce such large differences in toxicity, we speculate that genetic differences in populations are responsible. Current work in our laboratory is exploring genetic differences in populations and related alkaloid concentrations (Li, Gardner, Wang, and Ralphs, unpublished data).

Total Alkaloid Concentrations

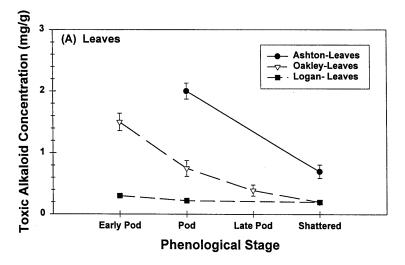
Overall, total alkaloid concentration averaged 7.0 mg/g (SE \pm 0.12; range = 29.9). Total alkaloid concentrations in pods and leaves averaged 9.4 (SE ± 0.18) and 4.7 mg/g (SE \pm 0.12), respectively. Pod concentrations of total alkaloids in D. glaucescens and occidentale were 2 to 3 times greater than found in leaves (Fig. 1), but smaller differences were found in D. barbeyi and glaucum. Total alkaloid concentrations averaged 10.3, 7.8, 5.0, and 4.4 mg/g for the early pod, pod, late pod, and shattered stages, respectively. Pod and leaf concentrations at various phenological stages are shown in Fig. 4. In general, the leaves and pods of most tall larkspur species contained insufficient total alkaloids to be potentially deterrent to sheep grazing. We found earlier that total alkaloid concentrations above 12 mg/g deter consumption by sheep (Pfister et al. 1996); most concentrations in this study were below this threshold except for pods from D. occidentale.

Management Implications

Risk, the potential to poison livestock from larkspurs, is a continuum from low to high, and degree of risk depends largely on the amount that cattle eat and the concentration of toxic alkaloids in the ingested larkspur (Pfister et al. 1997). Previous studies have determined that at toxic alkaloid concentrations below 3 mg/g, toxicity and the resultant likelihood of poisoning is low, at 3 to 6 mg/g toxicity is moderate, and above 6 mg/g toxicity is high (Pfister et al. 1997). In assessing risk to cattle from eating seed pods, we assumed that all of the alkaloids in the seed pods are extracted in the gut and that bioavailability is complete (i.e., worst case scenario), but we have not tested this assumption.

D. glaucescens

The average toxic alkaloid concentration for *D. glaucescens* was sufficiently high



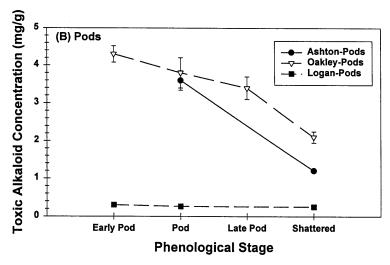


Fig. 5. Toxic alkaloid concentrations (mg/g + SE) for *Delphinium occidentale* leaves (A) and pods (B) at 3 locations (Ashton and Oakley, Ida.; Logan, Ut.) at various growth stages during the later portion of the growing season.

(4.4 mg/g) that grazing risk would be moderate on rangelands with abundant populations of this species, even after seed shatter. More than 70% of the D. glaucescens pod samples that we collected had toxic alkaloid concentrations above 3 mg/g ($\bar{x} = 6.1 \text{ mg/g}$), whereas about 4% of the leaf samples contained more than 3 mg/g. D. glaucescens leaves showed a large decrease in toxic alkaloids from 2.5 to 0.6 mg/g from the pod to seed-shatter stage. Thus, cattle eating only leaves would not be at risk during the later part of the grazing season. Pods averaged almost 9 mg/g of toxic alkaloid in the early pod stage, and only declined to 3.5 mg/g when shattered. Thus, cattle eating pods before seed shatter would be at high risk. Even after seed shatter, cattle could potentially become intoxicated because of the residual toxic alkaloids remaining in the plant. Ranchers with cattle grazing on rangelands with populations of *D. glaucescens* must exercise caution throughout the entire grazing season. Unlike *D. occiden-tale* and *barbeyi*, we have not determined when cattle eat *D. glaucescens* in relation to plant phenology, although Ralphs and Olsen (1992) observed that cattle readily ate flowering *D. glaucescens*. On ranges with dense patches of *D. glaucescens*, grazing sheep ahead of cattle may be the only viable grazing management option to reduce risk to cattle (Ralphs and Olsen 1992).

D. occidentale

In some locations (e.g., near Logan, Ut.), *D. occidentale* would not be a threat to poison livestock because of low toxicity in both pods and leaves. At other locations (e.g., Ashton, Ida. and Oakley, Ida.) pods were sufficiently toxic (near 4 mg/g)

before maturation to pose significant risk to grazing cattle. Moderate risk persisted until seed shatter and toxicity declined to 1 to 2 mg/g. Generally, D. occidentale leaves were less toxic than pods and therefore pose less of a risk to cattle. Previous grazing studies have determined that cattle eat substantial amounts of *D. occidentale* pods during the late summer (Pfister and Manners 1991). At toxic alkaloid concentrations near 4 mg/g, cattle could be poisoned by eating 30% of their daily diet as pods (Pfister et al. 1997). Once D. occiden tale pods have shattered, cattle would have to eat >60% of their diets as pods in order to be poisoned. This level of consumption only rarely occurs (Pfister et al. 1988).

D. barbeyi

Leaves and pods of D. barbeyi during the pod stage at the various locations contained 2 to 3 mg/g, indicating that poisoning risk is generally low once pods have lost their green color. At Salina, Utah, the average toxic alkaloid concentration in leaves actually increased from 1.3 mg/g at the pod stage to 2.5 mg/g at seed shatter. This increase in late-season leaf toxicity has been noted at other times (Pfister et al. 1994). We speculate that this increase occurred because of late-season environmental conditions allowing leaves to translocate or synthesize toxic alkaloids. Even with this increase, leaves were not highly toxic at Salina at the end of the season, and cattle would need to eat >50% of their diets as tall larkspur leaves to be poisoned. In the other locations where D. bar bevi was sampled, leaf toxicity decreased to very low levels (0.4 to 0.9 mg/g) when pods shattered, and risk would have been correspondingly low if cattle ate only leaves. Toxic alkaloid concentrations in pods at all 3 locations were < 2 mg/g when pods shattered, thus risk of losing cattle would be low for the remainder of the grazing season.

D. glaucum

Although *D. glaucum* is the most toxic tall larkspur early in the growing season (Ralphs et al. 1997), toxicity declined rapidly once pods began to develop. Toxic alkaloid concentrations in leaves decreased from 3.4 mg/g in the early pod stage to 1.3 mg/g in the pod stage and below 0.5 mg/g thereafter until seed shatter. Developing pods had toxic alkaloid concentrations of 5.4 mg/g, and fully ripe pods also contained 4.9 mg/g of toxic alkaloids. Thus, if cattle eat substantial amounts of pods (> 30 % of their daily diet), risk of poisoning is moderate until

seed shatter. After shattering, toxic alkaloid concentration decreased to 1.2 mg/g in seed heads, and risk to cattle would be very low.

In summary, livestock producers with tall larkspur on their summer ranges need to determine which species of tall larkspur is present. Once tall larkspur is identified, these results can guide grazing management decisions from the pod stage until pods shatter. Current research in our laboratory is partially focused on development of methods suitable for field estimation of toxic alkaloid concentrations by extension personnel or livestock producers (Pfister et al. 1999). In the meantime, for specific determination of toxic alkaloid concentrations on a site-by-site basis, livestock producers may contact the authors regarding sampling and analysis of tall larkspur.

Literature Cited

- Gardner, D.R., G.D. Manners, M.H. Ralphs, and J.A. Pfister. 1997. Quantitative analysis of diterpenoid alkaloids in larkspur (*Delphinium* spp.) by Fourier Transform Infrared Spectroscopy. Phytochem. Anal. 8:55-62.
- Manners, G.D. and J.A. Pfister. 1996. Sampling a poisonous plant population: quantifying toxic alkaloids in tall larkspur (*Delphinium barbeyi*) leaves. Weed Sci. 44:782–788.
- Manners, G.D., K.E. Panter, and S.W. Pelletier, 1995. Structure-activity relationships of norditerpenoid alkaloids occurring in toxic larkspur (*Delphinium*) species. J. Nat. Prod. 58:863–869.
- Marsh, C.D., A.B. Clawson, and H. Marsh. 1916. Larkspur poisoning of live stock. USDA Bureau of Animal Industry Bull. 365.
- Pfister, J.A. and Manners, G.D. 1991. Mineral supplementation of cattle grazing larkspur-infested rangeland during drought. J. Range Manage. 44:105–111.
- Pfister, J.A., Manners, G.D., Gardner, D.R., and Ralphs, M.H. 1994. Toxic alkaloid levels in tall larkspur (*Delphinium barbeyi*) in western Colorado. J. Range Manage. 47:355–358.
- Pfister, J.A., G.D. Manners, M.H. Ralphs, Z.X. Hong, and M.A. Lane. 1988. Effects of phenology, site and rumen fill on tall lark-spur consumption by cattle. J. Range Manage. 41:509–514.
- 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. J. Chem. Ecol. 22:1147–1168.
- Pfister, J.A., M.H. Ralphs, G.D. Manners, D.R. Gardner, K.W. Price, and L.F. James. 1997. Early season grazing of tall larkspur- (*Delphinium* spp.) infested rangelands. J. Range Manage. 50:391–398.

- Pfister, J.A., D.R. Gardner, K.E. Panter, M.H. Ralphs, G.D. Manners, B.L. Stegelmeier, and T.K. Schoch. 1999. Larkspur (*Delphinium* spp.) poisoning in livestock. J. Nat. Tox. 8:81-94.
- Ralphs, M.H., G.D. Manners, J.A. Pfister, D.R. Gardner, and L.F. James. 1997. Toxic alkaloid concentration in tall larkspur species in the western U.S. J. Range Manage. 50:497–502.
- Ralphs, M.H. and Olsen, J.D. 1992. Prior grazing by sheep reduces waxy larkspur consumption by cattle. J. Range Manage. 45:136–139.
- **SAS. 1988.** SAS/STAT user's guide, version 6 edition, SAS Institute Inc., Cary, N. C.