Lupine Poisoning in Sheep on the USDA-ARS U.S. Sheep Experiment Station (USSES), Dubois, Idaho

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Abstract

The USDA-ARS United States Sheep Experiment Station (USSES), located north of Dubois, ID, manages approximately 13,759 hectares of rangeland in Idaho for range research, sheep breeding and nutrition research, and rangeland management. Over 210 sheep deaths occurred in 3 separate summer/fall grazing periods from 2004 to 2011. Death losses occurred in ram lambs (86; 2004), mature rams (12; 2010), and ewe lambs (112; 2011). Between 1978 and 1981, multiple losses were also reported; however, data representing exact numbers were not recorded. All reported death losses occurred on the Humphrey Ranch, and information recorded from 1985 to the present identified four specific pastures as problematic. Lupine samples were randomly collected throughout the headquarters and the Humphrey Ranch. Specimens were collected for chemical analysis and taxonomic identification. Five lupine species were identified: *Lupinus caudatus*, *L. leucophyllus*, *L. polyphyllus*, *L. sericeus*, and *L. argenteus*. Each lupine species contained a distinct chemical profile composed of quinolizidine and/or piperidine alkaloids.

Keywords: alkaloid, lupine, *Lupinus*, piperidine, poisoning, quinolizidine, rangelands, sheep

Introduction

The *Lupinus* genus contains more than 500 taxa of annual, perennial, or soft woody shrub-like species worldwide (Wink et al. 1995): 200-300 species in North and South America, 150 species in the Intermountain West of the United States (Cronquist et al. 1989), 95 species in California (Riggins and Sholars 1993), and 12 species in Europe and Africa (Wink et al. 1995).

Range lupines are found in a variety of habitats at all elevations from lowland deserts to the alpine crests (Kingsbury 1964). Most lupines in the continental United States grow in States and provinces west of the Cordilleras including the Rocky Mountains and Sierra Nevada extending northward to Alaska and southward into Chile in South America (Wink et al. 1995). Lupines are found eastward through the Great Plains to the Atlantic coast but consist of only relatively few annual and perennial species.

Stockmen in the western United States began to recognize the inherent danger of lupines late in the 1800s when large livestock losses, especially in sheep, were reported in Montana and other western States (Chesnut and Wilcox 1901). Most poisonings occurred in late summer or early fall or when sheep were fed “native” hay containing lupine in the winter. These losses were often sporadic and continued throughout the late 1800s and early 1900s (Sampson and Malmsten 1942, Stoddart and Smith 1955). Use of lupine hay for winter feed was greatly scrutinized following the winter of 1898-1899 when thousands of sheep died from consuming lupine hay.
containing large numbers of seed pods (Chesnut and Wilcox 1901). In one flock alone, 3,600 of 7,000 sheep died from eating lupine hay. Most ranchers that season lost over 50 percent of their flocks to lupine hay poisoning. Retrospective analysis determined that during the summer of 1898, most lupine hay was harvested early, between July 1 and July 20, and it was reported that the lupine had “formed an unusual quantity of seeds.” While alkaloids had not yet been described, it was clear that the seed pods were “rich in the poison” (Chesnut and Wilcox 1901). Due to research and changing grazing practices, large sheep losses from lupines are rare today. However, lupines continue to cause periodic sheep losses and large and even catastrophic losses to cattle producers from lupine-induced crooked calf syndrome in the western United States and Canada (Panter et al. 1997, 2013; Gay et al. 2007).

Lupines are very diverse, ranging from cultivated low-alkaloid species (sweet lupines) used for human and animal food to toxic wild species (bitter lupines) that have multiple alkaloids and are responsible for toxicoses and teratogenesis in livestock. Taxonomically, lupine species are difficult to classify because of extensive hybridization, lack of morphological uniformity, and absence of genetic barriers to interbreeding (Cronquist et al. 1989). Chemical profiles can support or contradict taxonomic identification but are essential for establishing risk of poisoning to livestock. For example, Cook and colleagues described seven chemotypes of *Lupinus sulphureus* alone and used alkaloid profiling (chemotaxonomy) to differentiate between three other lupine species and *L. sulphureus* (Cook et al. 2009, 2011). Alkaloid profiles in some lupines vary considerably within and between species, making risk assessment using taxonomy alone unreliable (Carey and Wink 1994, Wink and Carey 1994, Lee et al. 2007, Cook et al. 2009).

The objectives of this report were to (1) identify lupine species and associated sheep losses on the USSES, (2) determine taxonomic identification and chemical profiles for lupine species, and (3) provide basic management recommendations to prevent future sheep losses from lupines.

**Materials and Methods**

**History and Site Description**

The USDA-ARS United States Sheep Experiment Station (USSES) is located north of Dubois, ID (figure 1), and consists of approximately 13,759 hectares of rangeland in Idaho for range research, sheep breeding and nutrition research, and rangeland management. On two of the USSES properties, the headquarters (11,303 hectares) and the adjoining Humphrey Ranch (1,052 hectares), *Lupinus* spp. are abundant. Over 210 sheep deaths occurred in 3 separate summer/fall grazing periods from 2004 to 2011. Death losses occurred in ram lambs (86; 2004), mature rams (12; 2010), and ewe lambs (112; 2011). Between 1978 and 1981, multiple losses were also reported; however, data representing exact numbers were not recorded. All reported death losses occurred on the Humphrey Ranch, and information recorded from 1985 to the present identified four specific pastures as problematic. Lupine samples were collected throughout the headquarters and the Humphrey Ranch. Specimens were collected for chemical analysis and taxonomic identification. Five lupine species were identified: *Lupinus caudatus*, *L. leucophyllus*, *L. polyphyllus*, *L. sericeus*, and *L. argenteus*; each contained a distinct alkaloid profile represented by multiple quinolizidine alkaloids and/or piperidine alkaloids.

**Plant Collections**

Lupine samples were collected randomly throughout the USSES headquarters and the Humphrey Ranch (figure 1) from June through August of 2009-2012 when plants were in full flower and early seed stage. Whole aboveground plant parts, including vegetative and reproductive parts, were collected at several locations for taxonomic evaluation and chemical analysis. Paired specimens were collected, one assigned for taxonomic evaluation and the other for chemical analysis. The taxonomic specimen was pressed, mounted, and submitted to the S.L. Welsh Herbarium (BRY; S. Welsh) at Brigham Young University, Provo, UT, for morphological comparison and taxonomic identification. Once classified, the specimen was assigned a voucher number and permanently filed in the Poisonous Plant Research Laboratory (PPRL) Herbarium, Logan, UT. Subsequently, a small amount of leaf material was removed from the voucher specimen for chemical analysis at the PPRL, and the chemical profile was then compared with the paired sample chemically analyzed for verification.

**Alkaloid Analysis**

The plant material sampled from the pressed specimens and plant material from field samples were ground to pass through a 2 mm screen, stored at room temperature, and submitted for chemical
Figure 1. Map of the USDA-ARS U.S. Sheep Experiment Station Humphrey Ranch, with lupine sample collection sites, which are identified by a diamond symbol. Pastures where past lupine poisoning events with sheep have occurred (see table 1) are Dam Field (2004), Center Field (2010), Corral Field (1985 and 2011), and Lupine Field (estimated 1978-1981).

extraction and analysis (Lee et al. 2007). Briefly, a measured quantity (50 mg herbarium sample, 100 mg field collection) of the ground plant material was weighed into a 16 mL screw-top glass test tube. The plant material was extracted by mechanical rotation using the Rugged Rotator (Glas Col, LLC) with a mixture of 1 N HCl (4.0 mL) and CHCl₃ (4.0 mL) for 15 min. The samples were centrifuged (5 min) and the aqueous layer removed. An additional 2.0 mL of 1 N HCl was added to the test tube containing plant material and CHCl₃ and extracted again by mechanical rotation (15 min), centrifuged, and the aqueous layer removed. The aqueous portions were combined into a clean 16 mL screw-top glass test tube. The pH of the aqueous layer was adjusted to 9.0-9.5 with concentrated NH₄OH. The basic solution was extracted twice with CHCl₃, first with 4.0 mL and then with 2.0 mL. The CHCl₃ solutions were combined and filtered through anhydrous Na₂SO₄ into a clean 16 mL screw-top glass test tube, and the solvent evaporated under N₂ at 60 °C. The alkaloid fraction extracted was reconstituted in 2 mL (herbarium samples) or 4 mL of methanol (field collections) containing 1.3 µg/mL caffeine (internal standard). A portion (~1 mL) was transferred to 1.5 mL gas chromatography (GC) autosampler vials for GC/mass spectrometry (MS) analysis.

GC/MS analysis was performed (Lee et al. 2007). In brief, representative samples (2 µL) of each plant sample were analyzed by GC/MS using a Polaris Q mass spectrometer and Trace GC Ultra gas chromatograph (Thermo Electron Corp.) equipped with a split/splitless injector and a DB-5MS (30 m x 0.25 mm; J&W Scientific) column. Injection port temperature was 250 °C and operated in the splitless mode. Split vent flow rate was 50 mL/min and purged after 0.80 min. Oven temperature was 100 °C for 1 min, 100-200 °C at 40 °C/min, 200-275 °C at 5 °C/min; and held at 275 °C for 1.5 min. Electron impact ionization (EI) at 70 eV was used with an ion source temperature of 200 °C. The detector scanned the mass range m/z 50-650.
Alkaloid identification was performed (Lee et al. 2007). Four individual alkaloids were identified from commercially obtained standards [sparteine and lupanine and authenticated (MS, NMR) samples of ammodendrine and anagyrine from the PPRL alkaloid collection]. The remaining alkaloids were determined from correlation of measured retention times to retention indices (RI) calculated by linear extrapolation from RI values generated from known standards and assigned RI numbers from the literature and their electron ionization (EI) and chemical ionization (CI) mass spectra (Wink et al. 1995). Alkaloids were also determined by correlation of measured relative retention times (RRt) to lupanine and EI mass spectra to those reported in the literature (Kinghorn and Balandrin 1984).

Results and Discussion

Five lupine species were identified on the two properties of the USSES: *L. argenteus* (PPRL Accession #3678 and #3801), *L. caudatus* (PPRL Accession #4499 and #4503), *L. leucophyllus* (PPRL Accession #3800, #4497, and #4498), *L. polyphyllus* (PPRL Accession #3802, #4494, and #4496), and *L. sericeus* (PPRL Accession #4500, #4501, #4502, and #4504). Each lupine contained a diagnostic alkaloid profile composed of quinolizidine and piperidine alkaloids (figures 2-6). The alkaloid profile of *L. sericeus* was quite simple and contained one major quinolizidine alkaloid, lamprolobine. The alkaloid profiles of *L. argenteus, L. caudatus, L. leucophyllus*, and *L. polyphyllus* were more complex and contained three or more major quinolizidine alkaloids. All five *Lupinus* species could pose a toxic risk to grazing livestock.

More than 150 quinolizidine alkaloids and several piperidine alkaloids have been identified from the *Lupinus* genera (Keeler and Gross 1980, Schmeller et al. 1994). Lupine alkaloids are toxic; however, toxicity varies depending on structural features of individual alkaloids, and toxicity in animals depends on alkaloid profiles, total alkaloid concentration, and rate of plant ingestion. Typically, plant alkaloid content is elevated during early phenological stages, decreasing through the flower stage, and increases in pods and seeds through translocation (Keeler et al. 1976, Lee et al. 2006). The clinical signs of lupine poisoning begin with nervousness, depression, grinding of the teeth, frothing round the mouth, relaxation of the nictitating membrane of the eye, frequent urination and defecation, and lethargy (Panter et al. 1999). These signs progress to muscular weakness and fasciculations, ataxia, collapse, sternal recumbency leading to lateral recumbency, respiratory failure,

![Gas chromatogram of the alkaloid profile representative of *L. argenteus*. Peaks annotated on the chromatogram: internal standard, caffeine (Int Std); ammodendrine (1); 5,6 dehydrolupanine isomer (2); 5,6 dehydrolupanine (3); and lamprolobine (4). All other peaks are unknown.](image1)

![Gas chromatogram of the alkaloid profile representative of *L. caudatus*. Peaks annotated on the chromatogram: internal standard, caffeine (Int Std); 5,6 dehydrolupanine isomer (2); 5,6 dehydrolupanine (3); (2R)-hydroxyaphyllidine (6); (2S)-hydroxyaphyllidine (6); (2R,9R)-dihydroxyaphyllidine (7); (2S,9R)-dihydroxyaphyllidine (7); and anagyrine (8). All other peaks are unknown.](image2)
Figure 4. Gas chromatogram of the alkaloid profile representative of *L. leucophyllus*. Peaks annotated on the chromatogram: internal standard, caffeine (Int Std) and ammodendrine (1). All other peaks are unknown.

Figure 5. Gas chromatogram of the alkaloid profile representative of *L. polyphyllus*. Peaks annotated on the chromatogram: internal standard, caffeine (Int Std); lupanine (5); spartiene (9); 13β-hydroxylupanine (10); and 13α-hydroxylupanine (11). All other peaks are unknown.

Figure 6. Gas chromatogram of the alkaloid profile representative of *L. sericeus*. Peaks annotated on the chromatogram: internal standard, caffeine (Int Std) and lamprolobine (4). All other peaks are unknown.

graze on USSES. Crooked calf syndrome is a condition in which calves are born with a variety of skeletal deformities such as arthrogryposis, scoliosis, kyphosis, torticollis, and cleft palate (Shupe et al. 1967a, 1967b, 1968). The principal time of insult was identified as the 40-70th days of gestation and may extend to as late as day 100. Anagyrine (Keeler et al. 1976) and some piperidine alkaloids (Keeler and Panter 1989, Panter et al. 1998) were shown to reduce fetal movement during this critical period of gestation (Panter et al. 1990, Panter and Keeler 1992), causing the spine and limbs to develop in contracted or misaligned positions and occasional cleft palate. Cattle losses in several western U.S. States due to lupine-induced CCS are still prevalent today (Lee et al. 2009, Panter et al. 2013). *L. caudatus* on the USSES contained the teratogenic alkaloid anagyrine while *L. leucophyllus* and *L. argenteus* contained the suspected teratogen ammodendrine. If pregnant cattle grazed these pastures during days 40-100 of gestation, there would be a significant risk of CCS because of the presence and concentrations of anagyrine and ammodendrine found in these species (Panter et al. 1999). While death losses occur more frequently in sheep than in cattle, cattle deaths are occasionally reported (Panter et al. 2001). However, the greater impact in cattle is CCS, which continues to cause large economic losses to cattle producers in western United States and Canada (Panter et al. 2013).

Documented sheep death losses on the USSES from lupine have occurred on the Humphrey Ranch (table 1). Based on this limited survey, three of the
Table 1. Historical account of lupine-induced sheep deaths at the USDA-ARS U.S. Sheep Experiment Station Humphrey Ranch research location

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Animal type</th>
<th>Death toll</th>
<th>Pasture</th>
<th>Lupine prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>September</td>
<td>ewe lambs</td>
<td>112</td>
<td>Corral Field</td>
<td>Concentrated under sagebrush</td>
</tr>
<tr>
<td>2010</td>
<td>July</td>
<td>mature rams</td>
<td>12</td>
<td>Center Field</td>
<td>Concentrated along ridge tops</td>
</tr>
<tr>
<td>2004</td>
<td>September</td>
<td>ram lambs</td>
<td>86</td>
<td>Dam Field</td>
<td>Concentrated around a secondary water source</td>
</tr>
<tr>
<td>1985</td>
<td>September</td>
<td>ram lambs</td>
<td>multiple</td>
<td>Corral Field</td>
<td>Concentrated under sagebrush</td>
</tr>
<tr>
<td>1978-1981</td>
<td>Fall</td>
<td>sheep</td>
<td>multiple</td>
<td>Lupine Field</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

1Refer to figure 1 for pasture location.
2Specific location characteristics of high-density lupine infestations that were grazed by sheep.
3Specific dates were not recorded but were determined by historical notes and personal communications with U.S. Sheep Experiment Station employees.

five lupine species identified on the USSES were found on the Humphrey Ranch pastures (*L. caudatus, L. polyphyllus, and L. sericeus*) while four lupine species were found on the headquarter pastures (*L. argenteus, L. caudatus, L. polyphyllus, and L. leucophyllus*). *Lupinus polyphyllus* and *L. caudatus* were both collected from the Corral Field where 112 sheep died in 2011; however, *L. sericeus* was the most prevalent species collected at the Humphrey Ranch. No plant samples were collected from the Lupine Field (figure 1) as this pasture had undergone recent rangeland improvements following multiple herbicide applications in past years to mitigate the historic sheep losses that occurred from 1978-1981. In contrast, *L. leucophyllus* and *L. polyphyllus* were the most prevalent species collected at headquarters.

Although *Lupinus* spp. can be found on all USSES properties, historic records indicate that under the current management practices, the greatest risk for sheep loss is on the Humphrey Ranch. *Lupinus* spp. were well distributed across the Humphrey Ranch in 1981. To fully evaluate lupine populations, long-term vegetation monitoring transects should be established and surveys conducted with taxonomic identification and chemical verification.

In the mid- to late 1980s, various attempts (herbicide application, pasture renovation, etc.) were used to control lupine infestations on the Lupine Field, and this resulted in a substantial reduction of lupine on the property west of Interstate Highway I-15 (figure 1). Subsequent to this, all the sheep losses occurred on pastures east of I-15 (figure 1). Some mitigation efforts (herbicide) were attempted east of I-15 in the late 1980s and early 1990s. Although the success of these efforts was not quantified, one could speculate that the lack of poisoning incidences from 1985 to 2004 could suggest some degree of lupine control.

Beginning in 2004 and repeated in 2010 and 2011, significant lupine poisoning events were documented (table 1) at the Humphrey Ranch. Most losses occurred within a 400 acre area, which is divided among three adjoining pastures: Center Field, Corral Field, and Dam Field (figure 1). In the spring of 2004, a pre-grazing inspection was conducted prior to moving sheep into the pastures on the Humphrey Ranch location. According to USSES records, visible *Lupinus* spp. were in the vegetative stage with very few flowers present, and it was determined that risk of poisoning was low or at least presumed acceptable for grazing. However, poisoning occurred, and 86 ram lambs died. Three lupine species were identified in these areas: *L. caudatus, L. polyphyllus,* and *L. sericeus.* Immediately after the poisoning event, isolated, dense populations of lupine plants with pods were found in the areas where sheep had grazed. These isolated “patches” of lupine were reported to be remote and somewhat obstructed from view of the road, such as along ridge tops, concentrated under sagebrush, or along secondary watering sources that appear in wet years (table 1).

In this report, we identified five lupine species and documented five distinct alkaloid profiles within these species. This report suggests that *L. sericeus, L. polyphyllus,* and/or *L. caudatus* were most likely responsible for the sheep losses that have occurred on the USSES rangelands. However, more investigation and research needs to be done to determine if one of these species is primarily
responsible for the majority of the losses. With the knowledge that lupine was responsible for the sheep deaths, appropriate mitigation efforts should be implemented to reduce sheep losses due to lupine grazing on the USSES pastures in the future.

Conclusions and Management Recommendations

These results provide important information to rangeland and sheep managers on the USSES to mitigate further sheep losses and to implement future lupine control measures. There are multiple management approaches that will reduce or eliminate sheep losses including one or more of the following recommendations: (1) Evaluate rangelands and identify poisonous plants and elucidate potential risk. This report provides a good example for livestock producers to evaluate risk from poisonous plants. (2) Targeted herbicide control of concentrated patches of lupines or broad generalized control. Recommended herbicides include 2,4-D (2 lb ae/acre), 2,4-D plus dicamba (1 + 0.5 lb ae/acre), or triclopyr (0.5 to 1.5 lb ae/acre) (Ralphs et al. 1991). Spray actively growing plants after they are 5 inches high but before bloom (Panter et al. 2011). Retreatment may be necessary every 4 to 5 years because viable seed reserves in the soil persist for many years. (3) Utilize high-risk pastures early in the growing season when other lush forage is available. Generally, livestock will avoid poisonous plants when adequate good-quality forage is available. Also, early grazing may reduce flowering success of lupines and inhibit pod development, further reducing risk of poisoning. (4) Graze high-risk pastures late in the growing season after lupine pods have shattered. Once lupine pods have shattered, alkaloid levels are very low, and risk of poisoning is substantially reduced. (5) Avoid driving animals through lupine patches or unloading hungry animals near poisonous plant populations. (6) Do not bed sheep near patches of poisonous plants, and do not place salt or water near populations of poisonous plants. Avoid creating situations where sheep travel through poisonous plants to get access to salt, water, or supplements. This report can serve as a template for investigating other cases of sheep or cattle losses associated with lupines or other poisonous plants.

While some lupine species are readily grazed by livestock and contain substantial nutritional qualities, the risk of poisoning can only be determined by alkaloid analysis and risk assessment (Panter et al. 2001, 2013). It is recommended that lupine plants (preferably while in flower) be submitted to the Poisonous Plant Research Laboratory, Logan, UT, for taxonomic identification and chemical analysis before animals are turned out for grazing.

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